OCCUPATIONAL HAZARDS IN A PUBLIC AMBULANCE SERVICE

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OCCUPATIONAL HAZARDS IN A PUBLIC AMBULANCE SERVICE (Abstract): **Aim:** To identify the occupational risks in an ambulance service and to assess their impact on the health of employees. **Material and methods**: Two marched groups (number, sex, age, length of exposure) from two different work sectors were selected. A 60 item questionnaire was used. The 60 items were grouped into four categories related to work organization, work environment, neuropsychosensorial risk factors and health system and occupational safety-related risk factors and hazards. The data were statistically processed, significant correlations between the risk factors and the associated symptoms being found. **Results and discussion:** Compared to the control group, significantly higher values ($p<0.001$) for work complexity, work environment factors, and organizational risk factors (walking on uneven ground, carrying weight, vicious postures) were found in ambulance service staff; cervical and lumbar spine problems were the most common complaints of ambulance service staff. **Conclusion**: The specific risk factors for ambulance service employees directly related to musculoskeletal disorders have been established. **Keywords**: OCCUPATIONAL HEALTH, AMBULANCE SERVICE, RISK FACTORS, OCCUPATIONAL MUSCULOSKELETAL DISORDERS.

From the total employees of the European Union, 10% work in the health and social protection system. Healthcare is a field characterized by many occupational hazards, the most specific ones being ranked as follows: biological hazards, musculoskeletal constraints, psycho sensory and chemical risks. The public ambulance system, by the wide range of services (prehospital emergency care, outpatient care, transport of biological and non-medical products, 24 hours a day) and increasing number of accepted claims, is a job characterized by multiple occupational health hazards for its staff (1, 2, 3).

MATERIAL AND METHODS

Two groups were selected: group I included 50 emergency medical service staff members (24 females and 26 males), average age and exposure time of 49 years and 26 years, respectively: stretcher bearers (25), nurses (21) and physicians (4); group II (control group) included 50 school employees (8 primary school teachers, 37 middle school and high school teachers, 2 nurses, 3 janitors). The groups were marched for sex, age and average exposure time.

A 60-item questionnaire was selected following a recent and extensive review of

the literature relating to occupational hazards faced by health staff in general and the ambulance services staff in particular (2, 4, 5). The questions were related to occupational constraints imposed by: work environment (noise, microclimate, vibration, toxic substances etc.), work organization (exertion level, work demands, posture and position during work), psycho sensory constraints and other symptoms arising from the practice of profession, as well as assessments of occupational health and security system. The statistical analysis (chi^2, odds ratio, confidence interval) made possible obtaining highly significant correlations between risk factors and associated symptoms.

RESULTS AND DISCUSSION

By analyzing the data on work organization, most respondents (87% and 90%, respectively) have a fixed work schedule (8 h/day schedule established by the management with 48 consecutive hours off work in each week for ambulance service staff working night shifts). Thirty-eight percent of ambulance service employees occasionally work on Sundays, public holidays and/or night shifts compared to 5% in education.

There were no significant differences between the responses regarding the work skills and human relations, the responses of both groups being favorable. Differences were recorded between the two groups with regard to the complexity of work (fast decision making, working under time pressure, responsibility in conducting maneuvers with vital implications, neuropsycho-sensory constraints (auditory, visual, psycho-emotional (tab. I).

TABLE I

Constraints resulting from work complexity

Constraints	Group I (n=50)	Group II (n=50)	p Value
Fast decisions and work under time pressure	46	10	< 0.001
High responsibility	48	12	< 0.001
Emotional nature	19	27	>0.05
Marked by events	38	19	< 0.001
Occupational stress	46	39	0.02

Interpretation: Significantly increased exposure ($p<0.001$) of the ambulance service staff compared to education personnel in terms of work complexity.

Occupational stress is recognized by most employees, the difference in perceived occupational stress being insignificant between the two groups ($p = 0.002$).

Interpretation: Significantly increased exposure ($p<0.001$) of ambulance staff compared to education personnel for noise levels over 87 dB, ambiental noise, low temperature, humidity and air currents (tab. II).

Interpretation: Significantly increased exposure ($p<0.001$) of ambulance staff in comparison to the education personnel regarding walking on uneven/inclined ground, carrying weights exceeding the prescribed limits (stretcher bearers often carrying obese patients), maneuvers overexerting the cervical spine that require keeping the head and neck in a fixed position; vicious postures (anterior trunk flexion, nonphysiological angulations, squatting position during resuscitation) that overexert the dorsolumbar spine (2, 3).

For the education staff, exposure is sig-

nificantly higher (p<0.001) in case of extended arm positions (keeping the arms raised) required for writing on the blackboard (table III).

TABLE II

Work environment-related risk factors

Risk factors	Group I (n=50)	Group II (n=50)	p Value
Noise > 87 dB	47	11	< 0.001
Ambiental noise	47	6	< 0.001
High temperature	32	29	>0.05
Low temperature	40	24	< 0.001
Humidity	40	9	< 0.001
Air currents	47	8	< 0.001

TABLE III

Work organization-related risk factors

Risk factors	Group I (n=50)	Group II (n=50)	p value	Odd ratio (OR)	Confidence interval (CI 95%)
Orthostatism	36	41	>0.05		
Wearing weights	39	5	< 0.001	16.53	4.31-62.5
Vicious postures	47	15	< 0.001	9.06	2.65-30.95
Walking on uneven, inclined ground	45	11	< 0.001		
Overload of neck, lumbar spine and peri-scapular-humeral joint	41	10	< 0.001		
Keeping arms in the air	10	39	< 0.001	6.75	2.04-22.25

Interpretation: Markedly high frequency (p <0.001) in group I of the damage to cervical spine, neck and dorsolumbar spine and significantly high frequency (p <0.05) of hearing problems and skin lesions (due to latex gloves, antiseptics); in group II there was a significantly increased frequency (p<0.001) of the symptoms characteristic to vocal strain (dysphonia/hoarseness, irritative cough) (table IV).

TABLE IV

Symptoms resulting from exposure to risk factors in the two occupational groups

Symptoms	Group I (n=50)	Group II (n=50)	p Value
Neck and cervical spine pain	41	10	< 0.001
Low back pain	47	37	< 0.001
Hearing problems	29	17	< 0.008
Dry, itching skin	33	21	< 0.008
Tired eyes	43	39	< 0.12
Visual disturbances	39	42	< 0.12
Dysphonia, irritative cough	5	27	< 0.001

As to work security, 50% of the ambulance service respondents assert that the minimum endowment and work safety and health requirements are met compared to 60% of the teaching staff who believe this is formal and theoretical.

CONCLUSIONS

Of all respondents, 94% in group I and 84% in group II stated that there are occupational hazards at the workplace. The occupational particularities of the public ambulance service reflected in specific risk factors had a statistically significant increased relevance for musculoskeletal pathology.

The identification of risk factors is part of an extensive technical-administrative and educational prevention program; occupational safety and health training for workers must aim to prevent the specific hazards and risks.

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NEWS

EXERCISE AND SLEEP A BIDIRECTIONAL RELATIONSHIP

Numerous studies conducted with individuals without sleep disorders have shown that exercise improves sleep quality but only a few studies evaluates relationships between exercise and sleep in people with sleep disturbances. In a study included women with insomnia, Baron and colleges were demonstrated that exercise improved total sleep time, sleep efficiency and sleep quality but the exercise during the day improved not the sleep on same night. Furthermore, lack of sleep decreased willingness to exercise. The bidirectional relationship between exercise and sleep showed that regular long-term exercise is a healthier way to improve sleep and encourage exercise participation. (Baron KG; Reid KJ; Zee PC. Exercise to improve sleep in insomnia: exploration of the bidirectional effects. *J Clin Sleep Med* 2013; 9(8): 819-824).

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AN INDIRECT EVALUATION OF THE NATIONAL PROGRAM OF DIABETES MELLITUS– STUDY CASE OF ROMANIA

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AN INDIRECT EVALUATION OF THE NATIONAL PROGRAM OF DIABETES MELLITUS– STUDY CASE OF ROMANIA (Abstract)Diabetes mellitus (DM) represents an important problem of public health according to the worldwide statistical data. In Romania, the national program is facing the same situation: increasing incidence (both for children and adults) and prevalence of this disease. **Aim:** To evaluate the results of the national program for DM through some indirect indicators. **Material and methods:** This study represents a descriptive analysis of hospitalized DM cases between 1.01.2009 and 31.03.2013, in Romania. The lack of statistical data available from the Ministry of Public Health (the latest update on the web page for DM is from 2007) makes the evaluation of the outcomes of this program quite difficult. An indirect evaluation is possible using the national hospital registrations for DM complications, recorded by the National School of Management and Public Health. For diagnostic registration was used the classification for Diagnostic Related Group (DRG), version HCFA DRG v. 18.**Results:** Despite the facilities offered to diabetic patients through the program, the burden of DM over the health system is quite large due to the large number of patients having complications. **Conclusions:** Even the objectives of the program appear to be accomplished; the indirect outcomes of the evaluation suggest that could be a gap between the hospital specialist and family doctors and a lack of health education for patients with DM. **Keywords:** DIABETES MELLITUS, NATIONAL PROGRAM, EVALUATION, HOSPITAL, FAMILY DOCTOR.

Diabetes mellitus (DM) represents an important problem of public health according to the worldwide statistical data, the World Health Organization (WHO) describing this as the chronic disease epidemic of this century.

According to Diabetes Commission from Ministry of Public Health, in Romania are actually registered 800,000 diabetic patients, more than 600,000 having neuropathy due to the disease. Incidence and prevalence of diabetes mellitus (DM) are increasing in Europe and, as well, in Romania. The 5th edition of IDF Diabetes Atlas estimates that the prevalence rate in 2011 was 9.2% of the adult population (almost 1,506,300 people) and will rise up to 11.1% in 2030 (1). The latest WHO data published in April 2011 shows that DM deaths in Romania reached 2,203 or 1.02% of total deaths. The age adjusted death rate is 6.29 per 100,000 of population, ranking

Romania on the 179th place in the world (2). The incidence of childhood onset type 1 diabetes is increasing in many countries in the world, with geographic differences in trends within Europe with an overall annual increase estimated around 3%. There is also good evidence to suggest that, in relative terms, increases are greatest in young children (1).

The treatment for diabetic patients is free of charge, due to the National program for DM. According to the latest legislation, the cost for the treatment will be supported by the Insurance Houses at the national and local levels. The national health programs represent, according to Health Reform Law no. 95/2006, a set of multi-annual actions, in order to evaluate, prevent, treat and control the diseases having a population' health status major impact (3). The program for DM is one of them. The aim of this program is to improve the health status of diabetic patients and to increase life span through the access to specific treatment.

The objectives are as follows: prevention and control for diabetes and other nutrition diseases; treatment with insulin for diabetic patients; treatment with oral antihyperglycemic agents. The activities included are referring at: information, education, communication and training for the personnel involved in the program; periodically and annually evaluation for damage blood vessels and glycosylated hemoglobin HbA1c; insuring the self-monitoring for insulin-treated patients; facilitating the access to some special treatments, such as insulin pumps or special shoes, in certain cases; implementing the National Register for Diabetes.

The coordination of the program is ensured by the General department for policies, strategies and health quality management. The Institute for Diabetes, Nutrition and Metabolic Diseases (located in Bucharest) is responsible for technical coordination.

MATERIAL AND METHODS

The evaluation of the DM national health program results through some indirect indicators is a descriptive analysis of hospitalized DM cases between 1.01.2009 and 31.03.2013 in Romania. The lack of statistical data available from the Ministry of Public Health (the latest update on the web page for DM is from 2007) makes the evaluation of the outcomes of this program quite difficult. An indirect evaluation is possible using the hospital registrations for DM complications, recorded by the National School of Management and Public Health, with some certain limitations. The records are referring to the hospitals indicators using the classification for Diagnostic Related Group (DRG), the United States version HCFA DRG v. 18. The limitations of the study are referring to some aspects: these counts may include multiple hospitalizations by the same individuals, which are usually for patients with diabetes; the unavailability of data for hospitalization costs; we cannot separate the cases between type 1 and type 2 diabetes or by age-groups (4).

RESULTS

Despite the facilities assured through the national health program, the burden of DM over the health system is quite heavy due to the large number of patients having complications. The hospitals are facing with problems of acute episodes of DM, which is a chronically condition. According to the database of The National School of Public Health and Management, the number of diabetic cases with severe complications and co-morbidities (CC) is increasing

between 2010 and 2012. The cases without severe CC are slightly decreasing during 2009 - 2012. The total amount of days in hospitals is more than one million between 01.01.2009 and 31.03.2013 for 185,260 cases (tab. I).

TABLE I

Number of hospitalized diabetic cases by year, type of complications, and number of days in hospital

Year	Diagnostic	No. of cases	Days in hospital
2009	Diabetes with severe CC	0	0
	Diabetes without severe CC	24,161	152,282
	Diabetic foot	0	0
2010	Diabetes with severe CC	23,413	160,571
	Diabetes without severe CC	21,440	133,383
	Diabetic foot	1,919	21,652
2011	Diabetes with severe CC	24,678	165,390
	Diabetes without severe CC	19,957	122,675
	Diabetic foot	1,878	21,099
2012	Diabetes with severe CC	26,145	175,229
	Diabetes without severe CC	15,522	95,082
	Diabetic foot	2,142	23,086
2013- first trim.	Diabetes with severe CC	7,178	47,648
	Diabetes without severe CC	4,353	25,719
	Diabetic foot	0	0
Total			1,143,816

TABLE II

Number of hospitalized diabetic cases by year, and type of care

Year	Diagnostic	No. of cases in acute care units	No. of cases in chronic care units
2009	Diabetes with severe CC	0	0
	Diabetes without severe CC	23,453	726
	Diabetic foot	0	0
2010	Diabetes with severe CC	22,666	747
	Diabetes without severe CC	20,562	878
	Diabetic foot	1,919	0
2011	Diabetes with severe CC	23,920	758
	Diabetes without severe CC	19,109	848
	Diabetic foot	1,878	0
2012	Diabetes with severe CC	25,396	749
	Diabetes without severe CC	14,671	851
	Diabetic foot	2,141	1
2013- first trim.	Diabetes with severe CC	23,920	758
	Diabetes without severe CC	0	0
	Diabetic foot	0	0

The inpatients with complications were hospitalized in acute care units or chronic care units. It is evident that patients, or more correctly, the number of cases receiving health care for DM acute episodes are far greater than those receiving health care in chronic units: in average, 32 acute episodes to 1 in chronic care units (tab. II).

The average length of stay (ALOS) is varying due to complications and type of received care (acute or chronic) (tab. III).

TABLE III.

Average length of stay for diabetic cases by year, and type of care

Year	Diagnostic	Acute care ALOS (days)	Chronic care ALOS (days)
2009	Diabetes with severe CC	0	0
	Diabetes without severe CC	6.15	11.30
	Diabetic foot	0	0
2010	Diabetes with severe CC	6.69	12.00
	Diabetes without severe CC	5.98	11.88
	Diabetic foot	11.28	0
2011	Diabetes with severe CC	6.54	11.80
	Diabetes without severe CC	5.91	11.53
	Diabetic foot	11.23	0
2012	Diabetes with severe CC	6.69	12.00
	Diabetes without severe CC	5.98	11.88
	Diabetic foot	10.78	15.00
2013- first trim.	Diabetes with severe CC	6.54	11.80
	Diabetes without severe CC	0	0
	Diabetic foot	0	0

DISCUSSION

Despite the facilities offered by the national health program, the hospital burden due to DM is large. In Romania, funds allocated in 2010 to the National Program for Diabetes and other Metabolic Diseases amount to approximately EUR 57,731,000, representing 1.47% of total health expenditure (5). The amount of money is directly increasing with the age of the diabetic patient, because the duration of diabetes is the main factor determining complications prevalence, the most important factor associated with an increased risk of complications being the glycemic control (5, 6). The ALOS is higher than other values registered in other countries. For example, in US, in 2004, the mean length of stay for hospitalizations among patients with diabetes was 5.5 days, which was one day longer compared to non-diabetes stays (7). According to Centers for Disease Control and Prevention, the value for ALOS of hospital discharge with diabetes as first-listed diagnosis decreased from 8.2 days in 1988 to 5.0 days in 2009 (8). In Singapore, admission for diabetes with complications varies within the limits 4.9 to 10.1 days (9).

This study reveals some of the indirect outcomes of the national program for diabetes, these indicators having more possible explanations. The level of socio-economic development of Romania could be one of them. The treatment for the DM

is not only a medical one. It implies, as a first step, a changing in life style, a dietary regimen. These changes require special attention, considerable expenses, and psychological efforts from patients and their families. The lack of money could be, for many patients, an obstacle to keep the blood sugar under control, so most of them are not able to have a proper diet. On the other hand, many diabetic patients consider that the medical doctor specialist in diabetes is the only „in charge" with their health condition. Or it is known that DM, as the other chronic diseases, requires ongoing adjustments by the affected person and interactions with the health care system (10). So we want to underline the importance of family doctor, who is not, unfortunately, part of this equation in many cases. His role is to help patients to achieve the self-management of the diabetes.

CONCLUSIONS

Even all people have access to periodic checkups for diabetes carried out by specialists, and injectable insulin and pens, insulin pumps and accessories, blood glucose monitoring strips and meters, lipid testing, retinopathy screening are fully reimbursed, the indirect evaluation of the National health program for DM indicated some deficiencies not at the central level, but at the local one. There is a lack of care coordination between specialist in hospital / ambulatory units and family doctors, the last ones being the most indicated to follow-up continuously the diabetic patients. As a consequence, patients are inadequately trained to manage their illnesses in the specific socio-economic conditions of their life.

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DEMODEX: COMMENSAL OR PATHOGEN?

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DEMODEX: COMMENSAL OR PATHOGEN? (Abstract): *Demodex* mites are considered commensals of human pilosebaceous units. Their role in skin disease is controversial. The **aim** of our study was to investigate the *Demodex* infection in patients with characteristic skin lesions and to correlate the level of infestation with epidemiological and clinical features. **Material and methods**: we have studied 120 symptomatic patients, diagnosed between January 2012 and December 2012; the detection of mites was realized by microscopic examination of potassium hydroxide preparations from biological samples taken by scraping and squeezing method**. Results**: The total prevalence rate of infection in our study group was 40.83%. We did not find statistical significant correlation with gender or age or with the type of lesions. **Conclusions**: Prevalence of *Demodex* infestation in facial dermatitis is high and its detection must be taken into consideration in routine diagnostic of facial suggestive lesions. Keywords: *DEMODEX*, FACIAL DERMATITIS, RISK FACTORS.

The microorganism was first reported in 1842 by Gustav Simon, after the microscopic examination of the sebaceous content of acne vulgaris lesions. He describes "an animal" with head, legs and body (1). The adult parasite has a worm-like appearance, the body consisting of cephalothorax and abdomen. The cephalothorax has fine lines, irregularly distributed, while the abdomen has horizontal, visible lines.

According to current taxonomy, *Demodex* is a genus of parasitic *mites* belonging to the family *Demodicidae*, superfamily *Cheyletoidea*, subclass *Acar*i, phylum *Arthropoda.* Demodex species found on humans are *Demodex folliculorum* and *Demodex brevis*, *D. folliculorum* species being the most common.

Although it was discovered and described over 170 years ago, its role in pathogenesis remains unclear. It is considered by many scientists as a commensal microorganism that inhabits the sebaceous follicle and the normal hair follicles. Although this mite can be found all over the skin, it prevails on the face, especially in areas with a high density of sebaceous follicles such as nasal wings, nasogenian groove, cheeks, forehead or chin. *D. folliculorum* can be found in hair follicles as well as sebaceous ducts and meibomian glands, while *D. brevis* was only detected in the deep part of sebaceous follicles (1).

Other researchers consider it an obligate parasite. *Demodex* is believed to be a risk factor associated with lesions of acne rosacea (2, 3, 4), seborrheic dermatitis, perioral dermatitis, pigmentations, blepharitis

(5,6) and even with sebaceous adenoma lesions (7) or epithelioma (8). Some studies report a higher frequency in patients with diabetes or severe immune deficiency (9).

At the moment, it is considered that transition from comensalism to parasitism is related to alterations in host immune response. The increase in the number of parasites (more than 5 mites/field or more than 5 mites/cm^2 of skin surface or skin biopsy) or their intradermal penetration is accompanied by nonspecific inflammatory and immune responses that lead to irritation and various skin lesions. Demodicidosis is a term that defines the skin lesions secondary to *Demodex* infestation.

The mode of transmission is direct, usually by close contact between individuals. Regarding the moment of contamination, it is considered to be an early acquisition, children being infected relatively quickly after birth, through direct contact with their carers. Some studies report a high infection rate, of over 90% in student communities (10). Another study reported a contamination level of 37% in asymptomatic persons handling biological waste and in health workers (11). Other studies show significant associations between *Demodex* infestation and gender, certain occupations, but especially with age (10, 11).

In terms of treatment, there is no standard regimen. Therapeutic options include topical medication (there is documented effectiveness for permethrin, crotamiton, lindane, salicylic acid, retinoids, metronidazole) as well as systemic therapy (currently used agents are retinoids, ivermectin and metronidazole) (1).

MATERIAL AND METHODS

We conducted a descriptive, retrospective study on a group of 120 patients, diagnosed between January 2012 and December 2012 with various facial skin lesions. Detection of *Demodex* was performed in patients with facial dermatitis for which the parasite is considered a risk factor (rosacea, seborrheic dermatitis, acne vulgaris). Suggestive lesions from which the samples were taken were papular, papulopustular or granulomatous lesions, predominantly localized on the face, forehead and scalp. Investigations were performed in a private medical laboratory on five dermatologists' recommendation. The control group consisted of 20 asymptomatic individuals from the same age group as those in the study group.

Facial sampling for the detection of *Demodex spp* can be done by manual extraction (squeezing method) or with the extractor of comedones and pustular lesions. In the case of squamous lesions, samples can be taken by scraping method. Cellophane tape method (CTM) is recommended for the asymptomatic parasite detection (10). In our study harvesting was done by the same laboratory doctor. We used extraction for follicular lesions and scraping for squamous lesions. For asymptomatic control group we used CTM.

Wet mount preparations with 20% KOH were examined under a microscope with 10X and 40X objectives. The number of parasites was semi quantitatively assessed. We considered the presence of 1 parasites/sample or mite fragments as low contamination and a number of more than 5 parasites as an important contamination (fig. 1).

We analyzed the prevalence of infection in symptomatic patients from the study group compared with asymptomatic controls. We searched for correlations between infestation and age or gender of patients, type of clinical lesions and sample.

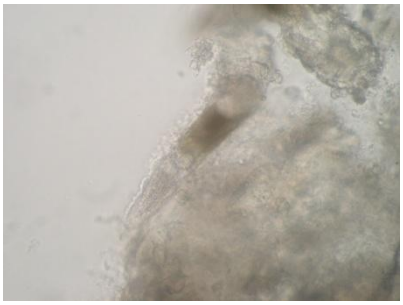

Fig.1. *Demodex folliculorum* (wet mount preparation with KOH, 100X magnification).

Data were examined with Epi Info program. Statistical analysis was performed by t-Student test. A value of $p < 0.05$ was considered significant.

Informed consent was obtained for each patient enrolled in the study.

RESULTS

In our study group, the total prevalence rate of *Demodex spp* infection was 40.83% (49/120). In the control group the prevalence of infection was 15%. We found a significant association between infestation and the presence of skin lesions ($p = 0,026$) (tab. I).

TABLE I

Epidemiological and clinical characteristics of the group with demodicidosis

Investigated characteristics	**Number of patients (ratio)**		
Prevalence	Positive *Demodex* detection	49(40.83)	
	Negative	71(59.16)	
	Total 120		
Gender	Males	18(36,73)	
	Females	31(63.26)	
	Total - 49		
Age group	<30	Males	6(42.85)
		Females	8(57.14)
		Total - 14	
	31-45	Males	8(36.36)
		Females	14(63.63)
		Total - 22	
	>46	Males	4(30.76)
		Females	9(69.23)
		Total - 13	
Lesions type	Rosaceea-like lesions	20(40.81)	
	Seborrheic lesions	15(30.61)	
	Acneform lesions	14(28.57)	
Sample type	Facial flakiness	28(57.14)	
	Scalp flakiness	5(10.20)	
	Folicular content	16(32.65)	
Degree of infestation (number of parasites / sample)	1, fragments	8(16,32)	
	2 - 4	35(71.42)	
	>5	5(10.20)	

The individuals enrolled in our study were aged between 19 and 85 years. Prevalence of infestation was 33.33% for the age group under 30 years, 42.30% in age group 31-45 years and 52.00% in the group with age over 46 years. There is an increased level of infestation in patients with advanced age but no statistically significant correlation was found, probably due to the small number of patients in the study group.

Regarding the type of clinical lesions or the type of sample taken, there are not statistically significant differences between groups.

All patients were infected with *D. folliculorum* species. In one patient, we found microscopic features suggestive for the *D. brevis* species.

DISCUSSION

This is the first study performed to Romanian patients related to the prevalence of *Demodex* infection in patients with facial skin lesions. There are only two articles published in Rev Med Chir journal, reporting two clinical cases of *Demodex* dermatitis, late in the 1960' (12).

In our study we found a significant association of infestation in symptomatic patients compared with the control group. Parasitism rate between the control and study group is similar to that from previously published data, typically up to 12% of the population showing contamination of follicles with *D. folliculorum* (13). Studies on larger groups indicate values between 20 and 80% (10, 11). Large differences are closely related to the study group selection criteria and especially to the method of sampling.

Gender ratio (male/female) was 1/1.7 but the prevalence rate is higher in males (47.36%) than females (37.80%); however, this difference was not statistically significant. Usually, infestation is stronger in men than in women and in younger populations than in adults, probably due to different distribution of seborrheic glands. Others studies report a growth rate of parasitism level with age (11), but there are studies that show a relatively uniform distribution of cases in the three age groups considered (1).

While most scientists agree that there is a tendency to develop skin lesions with age, especially in men, but that was not the case in our study. This is probably due to reduced addressability to the dermatologist of male patients, regardless of age, perhaps due to different mentality in our country.

Significant association of mites with different facial dermatitis are described in many studies (2, 3, 4, 10, 11). We did not find significant differences in relation to the type of lesion or sample type, probably because of the small number of samples examined. The most common association is described in published clinical trials with papulopustular lesions rosacea, and perioral dermatitis lesions and the degree of parasitisation is directly related to the frequency and dosage of corticosteroids used (13).

Regarding the intensity of infestation we did not find any statistically significant differences related to age or type of biological samples. Number of parasites detected/sample is lower when compared to data from other studies and this is very likely influenced by sampling technique used. We mainly used the extraction and scrapping techniques but studies revealed that the most effective detection is made by surface biopsy, a relatively laborious technique described by Klingman, but difficult to put in clinical in practice (14).

CONCLUSIONS

Demodex mite is a commensal that can become pathogenic under certain conditions. Demodicidosis lesions are highly polymorphic, differential diagnosis is sometimes difficult when relying solely on clinical appearance. Laboratory diagnosis is relatively easy and rapid but given the high rate of infection in asymptomatic subjects, it is very important to monitor not only the presence of parasite and their evolution, especially in high patients group. The prevalence in the study group was high (40.83% respectively), and future studies are necessary to improve the rate of positive diagnosis, using different methods of sampling on larger groups of patients The quantitative detection can be correlated with other risk factors that can cause an immune response from the host and can ultimately lead to development of lesions. In any case, commensal or pathogenic, *Demodex* must be taken in consideration and its detection should be a routine investigation for patients with facial dermatitis.

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COLON CANCER AT MOLECULAR LEVEL- USEFULNESS OF EPITHELIAL- MESENCHYMAL TRANSITION ANALYSIS

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COLON CANCER AT MOLECULAR LEVEL - USEFULNESS OF EPITHELIAL- MESENCHYMAL TRANSITION ANALYSIS (Abstract): Colorectal cancer (CRC) is the third form of cancer in both men and women. In Romania, the incidence of CRC in 2000 is 17.74 ‰, in 2002 becoming the second cause of death. We reviewed a series of studies that are related to colon cancer and studied the epithelial-mesenchymal transition at the front of tumor invasion (EMT). Cellular phenotypic changes characteristic of EMT can be induced by the absence of transition cofactor (p300) involved in cellular regulation. Loss of syndecan-1 marker is associated with local tumor stage and metastasis. Modulators of protein kinase resistance was associated with changes in genes involved in EMT (including vimentin hyperexpression) and genes involved in invasion (N-cadherin) with a decrease expression of genes involved in epithelial cell adhesion (E-cadherin). Progression in colon cancer is characterized by activating mutations in Ras genes and tumor growth factor action. Vimentin expression associated with EMT initiates molecular program. One of the characteristics of EMT is the loss of E-cadherin. TGF-β (transforming growth factor beta) induces epithelial-mesenchymal transition in colon cancer cell lines with the microsatellite stability, inducing cell invasion and migration. EMT is a critical early event involved in invasion and metastasis of colorectal cancer, characterized by the presence of markers specific to each phenotype, epithelial or mesenchymal. Multiple biomarkers involved in the induction of EMT may represent future therapeutic target in the treatment of colonic neoplasia. **Keywords:** COLON CANCER, EPITHELIAL-MESENCHYMAL TRANSITION, BIOMARKERS

Colorectal cancer (CRC) is the third most frequent cancer in women, after breast and uterine cancers, and in men after lung and stomach cancers. In 2006 there were 412,900 new cases of colorectal cancer in Europe, accounting for 12.9% of all cancers. Colorectal cancer was responsible for 217,400 deaths in Europe in 2006. This accounts for 12.2% of all death from cancer (1). In Romania, the frequency of CRC is increasing rapidly, with a twofold increase in incidence and mortality in the last 20 years. Our country belongs to the countries with a mean CRC incidence due to the yearly incidence of 17.74 ‰ reached in 2000. In 2002, CRC became the second leading cause of death after lung cancer, exceeding gastric cancer by 4150 deaths (19.05 ‰ inhabitants) (2).

Epithelial-mesenchymal transition (EMT)

is supposed to be an event in carcinogenesis in general. Through EMT, epithelial tumor cells can achieve transdifferentiation ability in myofibroblasts which lose their malignant phenotype but constitute the desmoplastic stroma essential for tumor growth, invasion, and metastasis, thus providing tumor cells an additional survival advantage. By defining the morpho-functional aspects of epithelial-mesenchymal transition in colon neoplasms, the identification of some of their relationships with a number of prognostic markers becomes possible. Colon cancer diagnosis requires histopathological confirmation, and thus a protocol for interpreting EMT as a component of tumor microenvironment with impact on prognosis and therapeutic choices could be recommended. Clonal expansion and acquisition of tumor cells malignant characteristics is favored by the presence of microenvironment, very often ignored (3).

Epithelial-mesenchymal transition is characterized by loss of epithelial markers simultaneously with the acquisition of mesenchymal components. E-cadherin is low while N-cadherin, vimentin, fibronectin are increased. E-cadherin is an epithelial marker that plays an important role in maintaining epithelial integrity. Vimentin is a marker for detecting epithelial transition and acquisition of fibroblastoid-like phenotype (13).

Literature review by accessing several databases and selecting the studies on colon cancer and the phenomenon studied at the level of tumor invasion front represented by EMT

DISCUSSION

In literature there are numerous studies on epithelial-mesenchymal transition (EMT) aimed at identifying markers that characterize this phenomenon. Colon cancer has been classically described by its clinicopathological features that allow prognosis to be predicted only after surgical resection and staging. A study by Loboda demonstrated that EMT is the dominant phenomenon in colon cancer. Microenvironment analysis in a series of 326 patients with colon cancer has identified the first main component (PC1) of the most variable set of genes through which adhesion and extracellular matrix were significantly affected. Also, it revealed that cell line derived EMT is significantly associated with PC1 gene expression. Expression of this gene was able to differentiate favorable from poor prognosis and was strongly correlated with recurrence and disease progression even in intermediate stages II and III. It was also correlated with neoplasm progression and the poorly differentiated nature of the disease. PC1 had a role in predicting metastasis-free interval to the onset of metastases and recurrence. PC1 gene expression and epithelial versus mesenchymal differentiation may be useful in the characterization of cell lines. Understanding which cell lines best represent the epithelial versus mesenchymal phenotype will be useful in determining the best models for subsequent treatment and will allow tumor description with the help of cell lines (4).

EMT characteristic cell phenotypic changes can be induced by the absence of a transition cofactor (p300) involved in regulating many cellular processes. In a study in which the absence of p300 was induced it was noticed that cells with this deficiency have a phenotype corresponding to an aggressive cancer, with loss of intercellular adhesion and cell-matrix adhesion defects. Beta-cadherin was found to increase p300 nuclear fraction that might contribute to EMT inducing intercellular signals (5).

Another studied factor was LIM protein 2 (Four-and-a-half LIM protein 2 - FHL2). This protein acts as a mediator that induces EMT. Expression profile of FHL2 in normal cells and colon cancer was inversely correlated with the expression profile of E-cadherin. FHL2 was expressed only by cancer cells and E-cadherin was expressed predominantly by normal epithelial cells. This marker was expressed both by primary and metastatic colon cancer cells. FHL2 can be regarded as a marker of progression and an important therapeutic target in colon cancer (6).

In colorectal adenocarcinomas, loss of syndecan-1 marker is associated with tumor stage and local metastasis. Syndecan-1 is a transmembrane proteoglycan with important roles in intercellular adhesion and cell adhesion to extracellular matrix, and acts as a growth factor co-receptor. In a study analyzing the expression of this biomarker in the basement membrane it was found that the presence of syndecan-1 correlated with advanced stage and lymph node metastases, which is more common in men ($p = 0.042$), but was not associated with age and tumor location (7).

Protein kinase Cs (PKC) plays an essential role in signal translation at cell level (8). It includes at least 12 isoforms with variations in tissue expression, with substrate specificity and cell locations related to cell function: cell differentiation, proliferation, apoptosis, motility, adhesion, invasion and metastasis (9). Resistance to PKC modulators was associated with changes in the genes involved in EMT (including vimentin overexpression) and genes involved in invasion (N-cadherin), together with decreased expression of messenger RNA (mRNA) by several genes involved in epithelial cell adhesion (E-cadherin), all involved in cancer progression (10).

Colon cancer progression is characterized by the activation of mutations in Ras genes and response of tumor growth factor (transforming growth factor-β-TGF-β). Ras and TGF-β act synergetically and increase expression and stability of messanger RNA for vascular endothelial growth factor (VEGF), an angiogenesis regulator both in intestinal epithelial cells (iRAS) and in cell culture models. TGF-β has been shown to have both a role of tumor suppressor and tumor promoter (11). Oncogenic Ras and TGF-β acting synergistically regulate genes at posttranscription level, mechanism involved in cell transformation and tumor progression of colorectal cancers (12).

To analyze cell phenotype in colorectal cancer and their invasive capacity, a study was conducted to assess the importance of reducing the expression of PTEN (Phosphatase and tensin homolog deleted on chromosome ten) coupled with TNF-α response. The result revealed cell changes consisting in EMT and increased invasive capacity of colorectal cancer cell. It was found that PTEN inhibition associated with tumor microenvironment factors favor the invasive phenotype with invasion and metastasis role in colorectal cancer (13).

Neuropilins (NRPs) are transmembrane non tyrosine-kinase glycoproteins normally described in the nervous system. Recently new roles of NRPs involved in oncogenesis have been identified. Besides their association with tumor vessels, NRPs were expressed in a variety of tumors, suggesting that they have an important role in cancer progression. A study has shown that neuropilin-2 confers a fibroblast-like phenotype to cancer cells, suggesting that it is involved in epithelial-mesenchymal transition. The presence of neuropilin-2 in colorec-

tal carcinomas was correlated with loss of such epithelial markers as cytokeratin-20 and E-cadherin and acquisition of some mesenchymal molecules such as vimentin (14).

In a study analyzing the phenotype of colon adenocarcinoma cells it has been shown that BRAF and RAS oncogenes (Ras homolog gene family) provide properties that intervene in neoplastic migration and invasion conferring cells a more aggressive phenotype (15).

Blood vessel epicardial substance (BVES) was analyzed in a study that found that its expression is suppressed in all stages of colorectal carcinoma and adenomatous polyps, indicating that inhibition occurs in the rapid stages of malignant transformation. BVES in CRC cell lines induces an epithelial phenotype, reducing proliferation, migration, and invasion. Thus, BVES determines EMT, and its suppression may be an important step in colonic carcinogenesis (16). Epithelial-mesenchymal transition is also present in such pathological processes as parietal tumor progression. Colorectal cancer tumor cells at the invasion front have mesenchymal features characterized by hypermigratory capacity, poor differentiation, and are hyperproliferative and unable to establish intercellular connections (15).

Expression of vimentin, a classical mesenchymal marker present in tumor cells of colonic epithelium initiates the molecular program associated with EMT. One of the features of EMT is the loss of E-cadherin. In a study that analyzed the expression level of the two molecules it was found that E-cadherin was significantly lower in regions where vimentin was strongly expressed, whereas vimentin could be barely detected in most regions which maintained an increased E-cadherin expression. This mutual pattern was noticed in all examined tumors, although only a few cancer cells maintained a moderate expression of both genes. These results further support the idea that some neoplastic cells of early intestinal adenomas initiate the epithelial-mesenchymal transition (17).

Another study conducted at the Masachusett General Hospital Boston aimed to determining whether dysfunctional signaling of transforming growth factor-beta (TGF-β) impairs the induction of EMT, the functional and morphological effects of TGF-β on microsatellite stability and instability in colon cancer cells was examined. Two groups of patients with colon cancer, one with microsatellite stability and the other with microsatellite instability identified at the cell level were analyzed retrospectively from 2001 to 2008. Study results showed that TGF-β induces epithelial-mesenchymal transition in colon cancer in the cell lines with the microsatellite stability. In the same cell lines, TGF-β induces cell invasiveness and migration (18).

Tumor-host interactions at the invasion front in colorectal cancer represent a critical interface occurs where tumor progression and tumor cell dissemination occur. Evidence suggests that the presence of infiltrating growth pattern is inversely correlated with the presence of immune or inflammatory responses at the invasive tumor front. On the one hand, the infiltrating tumor border promotes progression and dissemination of tumor cells by lymphatic and vascular vessels penetration. On the other hand, the host attempts to reject this attack by mounting an immune response, especially cytotoxic T lymphocytes to protect lymphatic and vascular channels from tumor invasion. Several tumor associated antigens, such as CD3, CD4, CD8, CD20 and other types of inflammatory cells, were

identified as potential prognostic factors. Together they suggest that the balance between pro- and anti-tumor factors (immune response and various types' inflammatory cells) at the invasive front in colon cancer may be critical in tumor progression and clinical outcome in patients with colon cancer (19).

CONCLUSIONS

The molecular mechanisms leading to malignant transformation and metastasis in colon cancer are incompletely understood. Knowledge of these mechanisms is of great importance if we hope to treat patients who seek a reduction in cancer-related mortality. Epithelial-mesenchymal transition is a critical early events involved in the invasion and metastasis of colorectal cancer, characterized by the presence of markers specific to each phenotype, epithelial or mesenchymal. The multiple identified markers, with a role in EMT induction, may be subsequent therapeutic targets in the treatment of colonic neoplasm.

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NEWS

SERUM HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS - PROGNOSTIC FACTOR IN GASTRIC CANCER

Gastric cancer is an important cause of morbidity and mortality. The 5-year survival rate after complete gastrectomy for gastric cancer is approximately 60% and the rate of recurrence after surgery is 30%. Low levels of high-density lipoprotein cholesterol (HDL-C) are associated with a higher risk for gastrointestinal tract cancers. A retrospective study by Tamura and colleagues examined the association between preoperative serum HDL-C levels and gastric cancer. They compared patients with normal levels of HDL-C with patients with low levels of HDL-C, for 5 years after the surgery. Results showed that the lymphatic and vascular invasion was significantly increased in the low HDL-C group. The patients in the normal HDL-C group had a significantly better prognosis than those in the low HDL-C group, which presented an increased rate of mortality from gastric cancer. In conclusion preoperative serum HDL-C level may be a clinical prognostic factor for gastric cancer patients (Takafumi Tamura, Satoshi Inagawa, Katsuji Hisakura et al. Evaluation of serum high-density lipoprotein cholesterol levels as a prognostic factor in gastric cancer patients. *J Gastroenterol Hepatol*. 2012; 27:1635-1640).

Cătălina Luncă

THE ROLE OF STATINS IN DEEP VEIN THROMBOSIS

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THE ROLE OF STATINS IN DEEP VEIN THROMBOSIS (Abstract): Deep vein thrombosis (DVT) represents the formation of a blood clot in one of the deep veins of our body, predominantly occurring in the legs. According to international guidelines, the goal of the treatment in deep vein thrombosis is to prevent the progression and the recurrences of thrombotic process. The aim of this article is to explain the pathogenetic mechanism of deep vein thrombosis and the benefits of statins in such a disease. The vasoprotective effects of statins are related to their anti-thrombotic and anti-inflammatory properties. The administration of statins is associated with a lower incidence of venous thromboembolism (VTE) in the general population. It also helps in reducing the frequency of recurrences, being prescribed after an optimal 6 to12 month anticoagulation. The study of these properties creates new opportunities for the use of statins in the prevention of post-thrombotic syndrome and recurrent venous thromboembolism (VTE).
Key words: STATINS, VENOUS THROMBOSIS, THROMBOEMBOLISM.

About 1 in a 1000 adults develops annually a *Deep Vein Thrombosis* (DVT). The incidence increases from 0.01% in young adults to 1% in people over 60. More than half of these events involve deep vein thrombosis in the inferior vena cava basin. In 25% of untreated cases, the thrombi extend towards proximal veins. Consequently, in the case of proximal DVT the risk of asymptomatic or symptomatic pulmonary thrombo-embolism increases to 5% and that is more likely to occur if the emboli are caused by proximal thrombi. In order to decrease the risk of fatal pulmonary emboli, the early diagnosis and prompt treatment of DVT are essential.

DVT pathogenesis mentions three broad categories of factors also known as Virchow's triad: endothelial injury, venous stasis and hypercoagulability. The vessel wall injury interferes with the coagulation inhibition function and prevents the local fibrinolytic process of the endothelium. Venous stasis, caused by patient immobilization or vein obstruction blocks the clearance and dilution of activated coagulation factors and finally the congenital or acquired thrombophilia, leading to hypercoagulation.

The venous thromboembolism pathogenesis is multifactorial, often resulting from a combination of several risk factors. The most common risk factors for secondary venous thrombosis include cancer,

surgery, immobilization for long periods of time, fractures, paralysis, pregnancy, birth and use of estrogens or of selective estrogen receptor modulators. Still, for approximately 30% of the patients the cause of DVT occurrence remain inexplicable, the same as in the case of spontaneous or idiopathic thrombosis.

THE CORRELATION BETWEEN DEEP VEIN THROMBOSIS AND ATHEROSCLEROSIS PROCESS

The existence of an association between atherosclerosis and idiopathic venous thrombosis was demonstrated for the first time in 2003 by Professor Prandoni and his research group. In a clinical case-control study about half of the patients diagnosed with idiopathic acute venous thrombosis developed signs of asymptomatic atherosclerosis (at least one carotid atherosclerotic plague, estimated by scanning the carotid arteries) (1). The essence of this association is still unknown: could venous thrombosis be induced by atherosclerosis or do the two conditions have a parallel development, in the presence of common risk factors? Certain factors correlated to the development of both pathologies have been identified: old age, obesity, diabetes mellitus, the metabolic syndrome (2). On the one hand the prothrombotic state in atherosclerosis can favor venous thrombotic events. In the case of atherosclerosis (ASVD) the evaluated elements are: the plaque aggregation activation, the blood coagulation activation and the increase of fibrogenesis. All these elements can lead to thrombotic complications both at the arterial level and in the venous system. On the other hand, both clinical conditions can be simultaneously triggered by certain biological stimuli, responsible for the activation of coagulation and swelling in the vascular system. Thus, the excess of fibrinogen, von Willebrand factor antigen, plasminogen tissue activator, D-dimers, coagulation factor VII, C-reactive protein (CRP), tumor necrosis factor- α (TNF-α) and interleukin, that circulate in the blood, cause a procoagulant and pro-inflammatory state both in the venous and the arterial system (3). We cannot exclude the probability that both hypothesis are true, representing thus two pathophysiologic scenarios for the development of venous thrombosis. These hypotheses are confirmed by vast authentic evidence, resulted from important clinical studies. The Cardiovascular Health Study (2009) proved that asymptomatic atherosclerosis represents a risk factor for VTE (venous thromboembolism) (4), while Atherosclerosis Risk in Communities Study (ARIC) showed that that the anti-plaque treatment and/or other antithrombotic treatments plays an important role in the prevention of venous thromboembolism dysfunctions in patients with clinically manifest atherosclerosis (5). Another study assessed the relapse rates for DVT, post-thrombotic damage severity and mortality 10 years after the first VTE episode, in patients previously included in the Duration of Anticoagulation (DURAC) study. After ten years the mortality rate due to acute myocardial infarction (AMI) or fatal *ictus cerebri* was significantly higher (approximately 28%) in patients with spontaneous VTE, if compared with patients with secondary VTE and with the general population (6). Other randomized control trials (P. Becattini; P. Prandoni; N. Bova) demonstrate a higher incidence of symptomatic atherosclerosis (ischemic cardiomyopathy, acute myocardial infarction, cerebral vascular accident, atherosclerosis of the peripheral arteries, ischemic cardiac failure, sudden death) in patients with idio-

pathic venous thrombosis if compared with patients with secondary VTE and with the general population.

THE ROLE OF STATINS IN PRIMARY AND SECONDARY PREVENTION OF VTE (VENOUS THROMBOEMBOLISM)

The objectives of DVT treatment, according to international guidelines, consist in the prevention of the thrombotic process progression as well as the prevention of relapses. Treatment is initiated with unfractionated heparin or low molecular weight heparin, followed by an indirect anticoagulant. The administration of direct anticoagulants will be ceased within 7-10 days while indirect anticoagulants will be administered for at least another six month, if there are no contraindications. The risk of hemorrhage occurrence represents the main criteria in the limitation of oral anticoagulant treatment duration. In most patients, the benefits of long-term prophylactic treatment of recurrent VTE can be compromised by an increased risk of bleeding, especially after 12 months of continuous administration. This fact has consequently demanded the research of alternative strategies for the secondary prevention of VTE, which have recently led to discussions about HMG-CoA reductase inhibitors (7). HMG-CoA reductase inhibitors represent one of the most commonly used groups of drugs. The special attention they are granted is justified not only by their high efficiency as hypolipidemic agents. Numerous experimental in vitro, in vivo and clinical studies have confirmed in the past years several benefic effects resulted through mechanisms that have no connection with the changes in lipid metabolism and are commonly named pleiotropic effects. The vasoprotective effect of statins is ensured by these properties, the anti-thrombotic and anti-inflammatory activity being the most important. These characteristics may lead to new perspectives regarding the post-thrombotic syndrome and VTE recurrences prevention.

The discovery of connections between atherosclerosis and venous thrombosis lead to the initiation of clinical and experimental studies meant to estimate the role of statins in diminishing the VTE risk. A randomized, placebo-controlled, multicenter trial, *Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER)*, included 17,800 practically healthy subjects that received 20 mg of rosuvastatinum or placebo, its main purpose being the study of statin efficiency in the decrease of major cardiovascular events rate. The assessment of pulmonary embolism or VTE primary episodes occurrence constituted one of the secondary purposes of the trial (8). After an average surveillance period of 1.9 years (up to a maximum of 5 years) it was concluded that rosuvastatinum administration reduces the symptomatic VTE risk with 43% for the practically healthy subjects participating in the study, when compared to the administration of placebo, no matter if the episode was idiopathic or was caused by a well-known factor.

Specialized literature (9) mentions a series of clinical trials that explore the alternative strategy hypothesis in the VTE prevention and provide scientific evidence of the influence of statins on VTE risk. The multicenter, observational, prospective randomized controlled clinical trial, *Heart and Estrogen/Progestin Replacement Study*, that included 2,763 female subjects provided data that proved a significant decrease of 50% of VTE risk in patients receiving statins. Statins determined a decrease of 22% of the relative risk of DVT

occurrence in patients over 65, in an observational retrospective trial that included a total of 125,000 patients with no history of atherosclerosis, venous thrombosis or cancer (10). Certain authors of case-control clinical trials mention a venous thrombosis risk decrease with approximately 26%-58% following statins therapy (11). In the population-based case-control study (MEGA study) that assessed 10,500 subjects, including 4,500 patients with primary episode of venous thrombosis in the lower limbs or pulmonary embolism, the use of statins, regardless of the active substance or the duration of the treatment, was associated with a significant 55% decrease of primary and recurrent DVT, proving to have a better effect in combination with the main treatment (13). An estimation of the venous thrombotic risk (by means of vascular Doppler) in 2,427 post-menopausal female patients aged between 30 and 89, of which 465 had a first episode of venous thrombosis, showed a significantly lower risk for the subjects who received statins (12). It was at the same time noticed that the risk rate was influenced by simvastatin, regardless of the dose, but remained unchanged in the case of pravastatin administration, probably due to differences in action at the level of coagulation activation internal mechanisms, blocked by lipophilic simvastatin and uninfluenced by hydrophilic pravastatin (13). Another such study assessed 5,824 VTE patients and a control group consisting of 58,000 subjects without a history of venous thrombosis. The outcomes lead to two important conclusions: patients with a history of cardiovascular events are exposed to a certainly higher VTE risk, especially within the first 3 month following an acute myocardial infarction or stroke; constant administration of statins is associated with a lower relative risk of VTE (approximately 26%) (14). The outcomes of a retrospective study that assessed 593 patients with myocardial infarction or ischemic cerebrovascular accident (CVA) showed that statins can be efficient for VTE prevention in atherosclerosis patients, the DVT risk being three times higher in patients who do not receive statin therapy (15). The effect depends on the dosage, being more significant in case of high doses (50% lower risk if compared with the standard 20 mg statin doses).

The use of statins in the case of patients with solid tumors (colon, pancreas, breast, ovarian, renal and cerebral cancer) was associated with a relatively low probability of VTE occurrence (8% as opposed to 21% in the group without statins), including VTE and pulmonary embolism. Regression analysis provided an identical result, regardless of other factors involved, such as smoking habits, the presence of metastases, chemotherapy administration, immobilization, aspirin administration (16). In that particular context, there were some attempts to compare the effect of statins to the effect of fibrates, in a randomized control trial assessing 1,354 patients hospitalized for idiopathic venous thrombosis (17). The use of statins resulted in a significantly lower risk of recurrent VTE episodes, while fibrate administration was associated with a higher risk (0.53 *vs.* 1.88) (18). In another study – the MEGA study – other groups of hypolipemiants (non-statins) caused an increase of 22% in primary or recurrent venous thrombosis risk, compared to patients who did not received hypolipemiant drugs (19). In the above mentioned observational case-control study the female patients were administered statins, fibrates, bile acid sequestrants or niacin; a lower risk of venous thrombosis was signaled only in the group that received statins (20).

The specialized literature does not produce any evidence regarding VTE aggravation or risk increase due to statins therapy. The benefic action of HMG-CoA reductase inhibitors, in the venous thrombosis pathology, is ensured by the anti-plaquetary, anticoagulant, profibrynolitic, anti-inflammatory, anti-cytokine, anti-oxidant and hypolipemiant effects that ensure all of them, the proper functioning of the vascular endothelium and finally contribute to maintaining an optimal balance between pro-thrombotic and fibrinolytic mechanisms. Manifestations of anti-thrombotic effects of statins consist in the inhibition of plaquetary aggregation, a decrease in thrombin production, the blockage of pro-thrombin activation, of the factor V and factor XVIII, the inhibition of the tissue factor with an important part in the maintenance of extrinsic coagulation cascade, the decrease of D-dimers seric level (marker of the prothrombotic state and independent risk factor for VTE recurrences), the stimulation of fibrinolytic activity through the intensification of tissue plasminogen activator (tPA) and the diminishing of plasminogen activator inhibator-1 (PAI-1) (21). Since inflammation is known to be one of the lesion mechanisms of the vascular wall and consequently an important factor in the destabilization of coagulation-fibrinolysis processes, we cannot neglect the role of the anti-inflammatory pleiotropic effect of statins in preventing recurrent primary venous thrombosis and the development of the post-thrombotic syndrome. Statins reduce the level of inflammation markers: C-reactive protein (in the CARE, PRINCE, AFCAPS/TexCAPS studies), tumoral necrosis factor-α, -1β and -2 interleukin, fibrinogen, CD40L ligand. There exists an association between the high risk of VTE, the increase of plasmatic values of triglycerides (over 1.05 mmol/l) and the decrease of HDL cholesterol (under 1.79 mmol/1) (22). In this context, statins are benefic due to their well-known effect on the serum lipid spectrum.

CONCLUSIONS

VTE is associated with high morbidity and mortality. The optimal remedy for the prevention and treatment of VTE should combine the advantages of efficiency, minimal bleeding risk and ease of administration. Statins seem to meet the last two criteria, yet their efficiency has not yet been fully demonstrated. They cannot be suggested as alternative anti-thrombotic remedies instead of oral anti-coagulants in the treatment of acute of sub-acute venous thrombosis. Yet, clinical studies demonstrate that the use of statins is rational as far as VTE prophylaxis is concerned, long-term secondary prophylaxis included. Statin therapy is associated with a decrease of VTE incidence in the general population as well as with a decrease of the incidence of recurrences, when administered after the recommended 6-12 month anticoagulant treatment. Still, more studies are needed in order to prove the efficiency of this drug for such a condition and create the opportunity to extend the indications for this group of drugs for certain population categories.

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THE DEGREE OF AGREEMENT BETWEEN HPV TESTING, PAP SMEAR AND COLPOSCOPY IN CERVICAL DYSPLASIA DIAGNOSIS

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THE DEGREE OF AGREEMENT BETWEEN HPV TESTING, PAP SMEAR AND COLPOSCOPY IN CERVICAL DYSPLASIA DIAGNOSIS (Abstract): **Material and methods:** The current study analyzed the degree of agreement between HPV testing, Pap smear, and colposcopic directed cervical biopsies. The study was performed on a group of 332 patients diagnosed and treated for cervical dysplasia at Cuza-Vodă Obstetrics-Gynecology Clinic Hospital and Suceava County Hospital between 2006 and 2011. **Results:** 190 patients (57.23%) were positive for HPV, 56 (35%) were positive for two HPV types and 42 (22.10%) for three or more HPV types. High grade cervical squamous intraepithelial lesion (HSIL) accounted for 88 (26.5%), low grade cervical squamous intraepithelial lesion (LSIL) for 92 (27.71%), atypical squamous cells of undetermined significance (ASC-US) for 69 (20.78%) and squamous cell carcinoma accounted for 5 (1.5%) of referral Pap smears. Colposcopic directed cervical biopsies reported no pathological abnormality (negative) in 64 (19.28%), HSIL in 105 (31.62%), LSIL in 83 (25%) and other lesions in 80 (24.1%) women. Exact degree of agreement between Pap smear and cervical biopsy was fair (k = 0.5) when analyzing for high grade cervical squamous intraepithelial lesion. The high-risk HPV types 16, 18, 66 and 68 and the low-risk HPV types 11, 54, 83, and 61 were the most frequently detected HPV types. **Conclusions:** The current study showed the fair agreement between Pap smear and colposcopic biopsy. Incorporation of HPV testing into the present Pap screening program has the potential to make screening for cervical cancer more effective, and a necessary prelude to assessing this is determining the prevalence of the high-risk types. **Key words:** CERVICAL CANCER, PAP SMEAR, COLPOSCOPY, CERVICAL BIOPSY, HPV TESTING

According to the American Congress of Obstetricians and Gynaecologist Practice Bulletin, it is estimated to be the second or third most common cause of cancer in women worldwide, despite the fact that a screening test, Pap smear, is available and has been demonstrated to reduce the incidence of the disease by at least seventy percent (1).

Several tests are required to screen and diagnose cervical dysplastic lesions: cervical cytology, colposcopy, colposcopic-guided cervical biopsy histopathology, HPV DNA.

Currently, there is great interest in the possible application of *Human Papilloma virus* (HPV) testing to supplement Pap screening for cervical cancer. Many epide-

miological studies provide evidence that persistent infection with specific types of HPV is a strong independent risk factor for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. Additionally, it appears that the predictive value of a negative HPV result together with a repeat Pap smear in women with an initial low grade Pap result is greater than 96 % (82.8-99.4%) (2).

Despite the improvement in detection, there is wide variation in clinician practice in relation to the types and sensitivity of tests used for the diagnosis of cervical dysplasia, and also in regards to the timing, schedule and duration of follow up for women after cervical dysplasia detection and treatment.

The current study analyzed the degree of agreement between HPV testing, Pap smear, and colposcopic directed cervical biopsies.

MATERIAL AND METHODS

The study was performed on a group of 332 patients diagnosed and treated for cervical dysplasia at Cuza-Vodă Obstetrics-Gynecology Clinic Hospital and Suceava County Hospital between 2006 and 2011.

The patient selection was done according to the following inclusion criteria: women 18 years or older who underwent Pap smear, HPV testing and colposcopic biopsy. The exclusion criteria included patients that underwent total hysterectomy or followed previous treatment for CIN.

RESULTS

The cohort for this study consisted of 332 women who underwent HPV testing, Pap smear and final colposcopic directed cervical biopsy procedure for cervical dysplasia during the study period (tab. I).

The mean age for all participants was 36.9 years of age. The respondents ranged in age from 21 to 68 years old. In this study, the largest group of women were within ages 31-40 (fig. 1).

TABLE I
Characteristics of studied patients

Age (mean, SD)	36.9±10.5years
Nulligravida	134 (40.36%)
Nulliparous	149 (44.87%)
Non-smoker	194 (58.43%)
Smoker	123 (37.05%)
No smoker information	15 (4.52%)
No contraception	105 (31.62%)
Oral contraception	123 (37.04%)
Other contraceptive methods	104 (31.32%)
Hormonal replacement therapy	4 (1.2%)

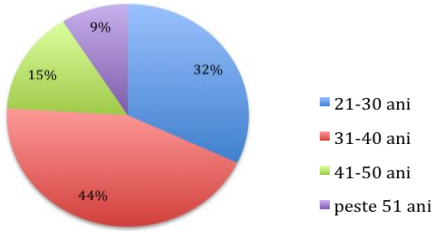

Fig. 1. Distribution of studied patients according to age group

HSIL accounted for 88 (26.5%), LSIL for 92 (27.71%), atypical squamous cells of undetermined significance (ASC-US) for 69 (20.78%) and squamous cell carcinoma (SCC) accounted for 5 (1.5%) of referral Pap smears in those women with cervical dysplasia (table II). Pap smear was negative in 78 cases (23.49%).

Colposcopic reported no pathological abnormality (negative) in 64 (19.28%), HSIL in 105 (31.62%), LSIL in 83 (25%) and other lesions in 80 (24.1%) women.

The degree of agreement between Pap smear and cervical biopsy histopathology

was examined. The overall kappa value was 0.3 and it represented poor agreement. When the degree of agreement was compared for HSIL only, the kappa value was 0.5, which represented fair agreement. The sensitivity and specificity was not calculated, because these parameters were beyond the objectives of the current study.

From the total of 332 patients, 190 (57.23%) were positive for HPV. The high-risk HPV types 16, 18, 66 and 68 and the low-risk HPV types 11, 54, 83, and 61 were the most frequently detected HPV types (fig. 2). 56 patients (35%) were positive for two HPV types and 42 (22.10%) for three or more HPV types (fig. 3).

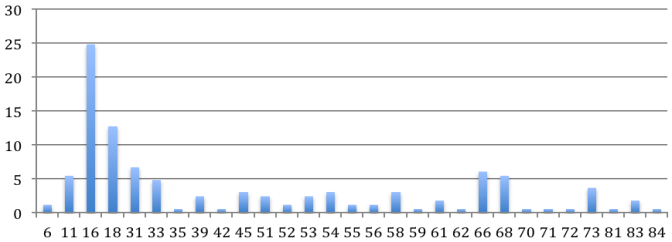

Fig. 3. Frequencies of HPV types in the study group

Table II shows the OR's and 95% CI for the selected variables and cervical dysplasia adjusted first for age and then for all the other variables.

TABLE II

Odds ratios (OR) and 95% confidence intervals (95% CI) for cervical dysplasia and age, adjusting for the other variables and then additionally adjusting for HPV

Age group	Basal OR	Basal 95% CI	Fully adjusted OR	Fully adjusted 95% CI	HPV adjusted OR	HPV adjusted 95% CI
21-30	1.0	Referent	1.0	Referent	1.0	Referent
31-40	1.02	0.6-1.7	1.03	0.6-1.7	1.5	0.8-2.7
41-50	0.45	0.2-0.9	0.43	0.2-0.9	1.3	0.6-2.8
>51	0.33	0.1-0.7	0.32	0.1-0.8	0.66	0.3-2.2

Women aged 30 and younger had the highest OR for cervical dysplasia and it was less than half for the women over 30. This OR according the age became insignificant in the fully adjusted model and in fact it was HPV that most influenced this change The age-adjusted odds of having cervical dysplasia increased significantly and dramatically with the increasing viral load. The p-value for this trend is <0.0001.

DISCUSSION

Using histopathology, the sensitivity of colposcopy for cervical abnormalities is high at 96.0 to 97.0%, but specificity is lower, at about 48 to 54.8% (12). Gage et al. (2006) reported that the combined sensitivity of colposcopy for diagnosing HSIL increased to 83.3% from 68.3% when more than one cervical biopsy was taken (7).

This study found a strong association (OR: 28) between high-risk HPV types and cervical dysplasia. Comparison with other studies has been difficult because of the different techniques used for detection and the different combinations of strains being tested for, nonetheless, OR's of over 15 have been reported in a review of case-control studies that used reliable methods for HPV DNA

A series of studies have revealed that a

unique feature of HPV testing is the ability to predict the subsequent development of cervical cancer precursors among cytological negative women (9).

The most important determinant of HPV infection is age, with the highest prevalence (60-80%) between the ages 21 and 40. Infection in these individuals, however, appears to be transient and prevalence decreases to about 10% by age 30 and 3% by age 50 (10).

CONCLUSIONS

Cancer of the cervix is a preventable disease and, theoretically, with regular screening dysplasia can be detected early and invasive disease can be prevented. . The protective effect of cervical cytological screening (relative risks of 0.25-0.37) among women who have been screened cytologically compared to those who have never been screened increases with an increased number of screening smears and decreases with the increased length of interval since the last smear. Although these results establish that the cytological screening program is successful, an unacceptable number of women who have been cytologically screened develop invasive cervical cancer because of too many false negatives. The current study showed the fair agreement between Pap smear and colposcopic biopsy. Incorporation of HPV testing into the present Pap screening program has the potential to make screening for cervical cancer more effective, and a necessary prelude to assessing this is determining the prevalence of the high-risk types. To summarize, in our opinion, the approach of investigation and management of women with cervical dysplasia is a stepped process. Initially Pap smear is used to screen women followed by colposcopic examination and colposcopic biopsy of those women whose Pap smear is reported as abnormal. Based on histopathology of colposcopic guided cervical punch biopsy women will be advised to undergo final cervical excision procedure.

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PARTICULARITIES OF ORAL HEALTH PROBLEMS IN PATIENTS WITH ALZHEIMER`S DEMENTIA

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PARTICULARITIES OF ORAL HEALTH PROBLEMS IN PATIENTS WITH ALZHEIMER`S DEMENTIA (Abstract) **Aim:** Dementia and especially Alzheimer`s dementia represent one of the most important disorder of this century with a lot of negative consequences for the patient himself, but also for family and community. The essential features of dementia are represented by multiple cognitive deficits, at least one of the following: aphasia, apraxia, agnosia, or a disturbance in executive, which diminished the quality of life of these patients. Unfortunately, in majority of the cases, the oral health care of these patients does not represent a priority. The aim of our study is to establish a series of general coordinates on the pattern of development of oral diseases in patients with dementia. **Material and methods:** We focused on the clinical evolution of 50 patients from "Socola" Institute of Psychiatry Iasi, with the diagnosis of Alzheimer`s dementia, according to ICD 10 criteria, from the point of view of the general and dental status, the prognosis and the therapeutic possibilities. **Results:** Our results reveal that 78% of patients with moderate dementia had partial and total edentation and 56% of patients with severe dementia had partial edentation, while 21% total edentation. **Conclusions:** Thus, in this context, increasing the quality of life of these patients means to improve the general condition of the elderly, which is closely related to the maintenance of natural teeth and the development of well-adapted prostheses.
Keywords: ORAL HEALTH, DEMENTIA, QUALITY OF LIFE.

Dementia is a progressive and irreversible neurodegenerative disorder characterized by a variety of symptoms: cognitive impairment, memory disorders, progressive deterioration of daily exercise capacity, and a range of neuropsychiatric symptoms. According to the World Alzheimer Report from 2015, regarding "The Global Impact of Dementia", the number of people diagnosed with dementia will increase from 46 million, in the present, to 131,500,000 by 2050 (1). Statistical data show that about 9.9 million new cases of dementia will be diagnosed this year around the world. Thus, a new case is diagnosed every three seconds. According to the report, about 94% of people living with dementia in low-income and middle-income countries are

home-grown. In many of these countries there is no developed nursing and specialized care network for people with dementia (1, 2).

The world population is experiencing an aging process, the number of people over 60`s years old suffered an increase of 450 million in only 40 years. The structure of the elderly population has changed to more than 50% in developed countries, the most aging being from the European area. Dementia caused by Alzheimer's disease is the most common type, representing approximately 60% of all dementia patients. As for the link between age and illness, it is estimated that 5% of persons of 65 years old have dementia in Alzheimer's disease, and at the age of 85, the rate goes up to 25%(1, 3, 4, 5).

Oral health problems may adversely affect the quality of life of patients with Alzheimer's dementia. However, as the patient suffering from Alzheimer's dementia requires a wide range of care, often oral health care is not a priority. Increasing the quality of life and improving the general condition of the elderly is closely related to the maintenance of natural teeth and the development of well-adapted prostheses (6, 7, 8). Given that cognitive functions and motor skills are progressively altered in patients with Alzheimer's dementia, this circumstance can lead to a level inadequate control of biofilm and oral hygiene. Alzheimer's dementia is a complex pathology that, beyond the loss of cognitive function, leads to alteration of the ability to perform common daily activities (7, 9, 10).

The spectrum of adverse effects attributed to the medication used to control the symptomatology specific to dementia also reaches the oral sphere, emphasizing the issues already exposed. On the one hand, anti-dementia medication (anti-acetylcholinesterase) causes hypersalivation, which makes it more difficult to maintain a dry field during dental treatment and causes difficulties in maintaining a mobilizable prosthesis (10, 11, 12, 13). On the other hand, xerostomia is a common side effect associated with adjuvant medication used in dementia, such as antidepressants and benzodiazepines. Insufficient salivation reduces the lubrication of mucosal tissues, increasing the potential for mucosal ulceration, and increasing the difficulty of maintaining a mobilizable prosthesis. At the same time, xerostomia leads to alteration of normal oral flora, causing gingival plaque formation, predisposing to periodontal disease, dental caries and increased risk of infection (14, 15, 16).

MATERIAL AND METHODS

The study included 50 patients admitted to acute department III, V and VII in “Socola” Psychiatry Institute Iasi, during the year 2017. The study group consists of roughly equal numbers of males and females with a slight superiority of female gender (56% female and 44% male). Clinical and anamnestic data were extracted from the observation sheets of these patients. The dental evaluation was conducted within the dental office of the Institute. The criteria for inclusion in the study were: confirmed diagnosis of Alzheimer's dementia, both with early onset and with late onset and edentation.

Alzheimer's dementia diagnosis is established according to ICD 10 criteria (17). Dementia in Alzheimer's disease with early onset: The criteria for dementia in Alzheimer's disease must be met, and the age at onset being under 65 years; In addition, at least one of the following requirements

must be met:(a) evidence of a relatively rapid onset and progression; (b) in addition to memory impairment, there is aphasia (amnesic or sensory), agraphia, alexia, acalculia, or apraxia (indicating the presence of temporal, parietal and/or frontal lobe involvement).

Dementia in Alzheimer's disease with late onset: The criteria for dementia in Alzheimer's disease must be met and the age at onset must be 65 or more. In addition, at least one of the following requirements must be met:(a) evidence of a very slow, gradual onset and progression (the rate of the latter may be known only retrospectively after a course of three years or more); (b) predominance of memory impairment over intellectual impairment (see general criteria for dementia).

Once the patient is diagnosed, the progression of the disease is clinically and anamnestically assessed, also with the help of the Mini Mental State Examination, a nonspecific instrument that has no diagnostic but merely indicative role. At the same time, scores on the Hamilton Depression Rating Scale of patients with Alzheimer's dementia showing negative hypertimic symptoms are also recorded.

In terms of edentation, patients were in one of the following classes: partial edentation, when at least three consecutive teeth in the lateral area or four front teeth are missing; subtotal edentation, when the arcade remains at most three teeth; total edentation, lack of all teeth.

Oral Health Indicators used in this study are the OHI index and the CAO-D index, both of which are internationally recognized by dental scientific organizations. The OHI is the sum of the soft deposit index and the tartar index.

The soft deposit index: 0-absence of soft deposits; 1-soft deposits covering up to 1/3 of the surface of the teeth; 2-soft deposits covering 1/3-2/3 of the surface of the teeth; 3-soft deposits covering more than 2/3 of the surface of the teeth.

*Tartar index:*0-the absence of tartar; 1-supragingival tartar covering not more than one-third of the surface of the tooth; 2-supragingival tartar that covers between 1/3 and 2/3 of the exposed tooth surface; 3-surgingival tartar covering more than 2/3 of the tooth surface.

RESULTS

The results obtained by correlating the degree of dental affection with the degree of psycho-cognitive impairment and the presence of depression symptoms, apathy and serious difficulty in targeting, indicate the existence of a direct relationship between these variables. Thus, most patients with dementia have associated oral pathology, the most significant, with an increased impact on the patient's outstanding function being the edentation of different degrees. It is noticeable that in most cases we face subtotal edentation, followed by the total edentation, present in a significant percentage.

Distribution of cases by age reveals a predisposition for the eighth decade. It should be noted that patients are not newly diagnosed cases and clinical and anamnestic information in the observation sheets reveals that most of them were originally dementia Alzheimer type, with early onset (tab. I, fig. 1).

Patients with dementia develop depressive symptoms of varying degrees of intensity, the group of those with overweight depressed mood associated with 92% of total and subtotal edentation (fig. 2).

The distribution of the CAO-D index in patients in the study group is the following:

number of caries-1,12 teeth; number of absent teeth - 4.48; number of teeth closed - 0.32. Regarding the oral hygiene index, 84% of the patients obtained a weak index, 14% moderate and only 2% of the patients a good hygiene index.

TABLE I

The distribution of cases by age and sex

Age	Male		Female		Total	
	Number of patients	%	Number of patients	%	Number of patients	%
50-59	1	2	2	4	3	6
60-69	4	8	6	12	10	20
70-79	11	22	15	30	26	52
80-89	6	12	5	10	11	22
Total	22	44	28	56	50	100

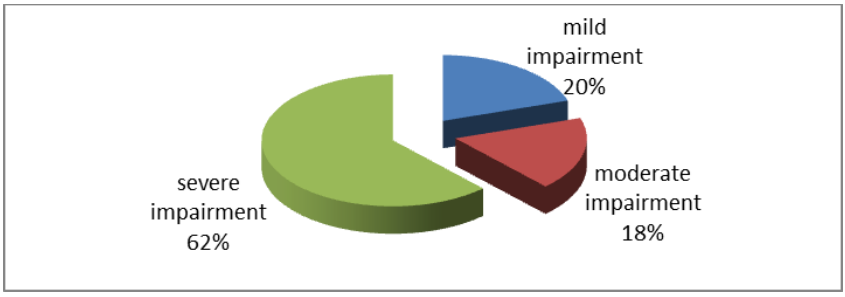

Fig. 1. Degree of cognitive impairment

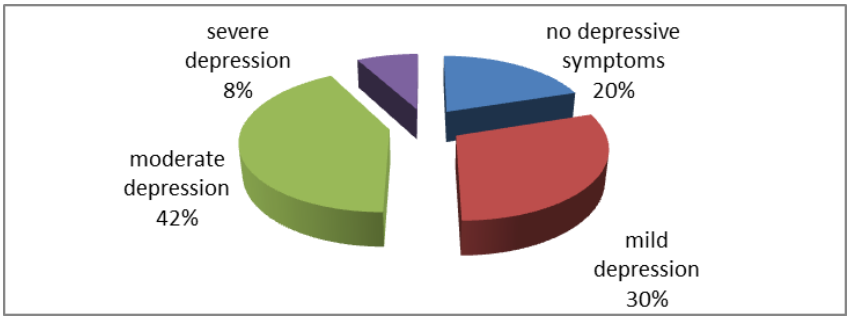

Fig. 2. Depressive symptoms in patients with Alzheimer`s disease

Of all patients included in the present research, it is noted that there are none that do not show a degree of edentation (fig. 3).

In addition, 78% of patients with moderate dementia had partial and total edentation and 56% of patients with severe dementia had partial edentation and 21% had total edentation.

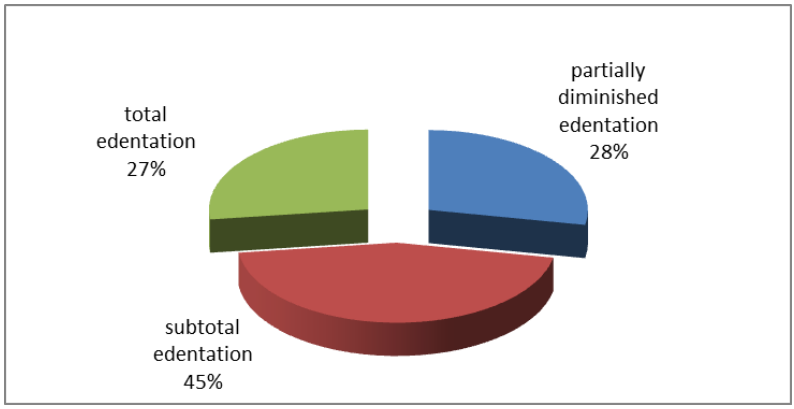

Fig. 3. Edentation in patients with Alzheimer`s disease

DISCUSSION

In 2014, the General Assembly of the World Federation of Public Health Associations issued a resolution on the importance of oral health of patients with dementia (18, 19). The prevalence of dementia is growing globally. Patients with dementia gradually install serious communication difficulties with those responsible for their care. Recent studies show that the precarious oral condition of people with dementia is directly correlated with socio-economic status, but also with demographic variables (20). Thus, the results of this research are linked to the results obtained in the literature, emphasizing the direct relationship of a precarious socio-economic status and a greater severity of the pathology in the oral sphere in the patients diagnosed with dementia (21).

In addition, as the present research shows, patients with dementia receive psychotropic treatment, especially for controlling behavioral and psychotic symptoms, but also for controlling depressive symptoms that can cause xerostomia and dry mouth syndrome, favoring candida infections, but also the appearance of dental caries and, on the other hand, sialorrhea. In addition to this, patients with dementia either forget or lose their ability to ensure daily oral hygiene. Thus, the persons responsible for these patients (family members or medical staff) must ensure this process (22).

Depending on the evolutionary state of dementia, oral health becomes a growing problem, being totally dependent on the care of others. Multiple studies have directly correlated the degree of cognitive impairment with the precariousness of oral health (23). In the same vein, the present research shows that most cases of severe edentation (subtotal and even total) occur in patients with moderate to severe dementia, who are, moreover, frequently hospitalized in psychiatric or institutionalized services.

A study conducted in Sweden between the years 2007 and 2015 (24) shows that as dementia progresses, addressability for dental services decreases significantly. At the same time, the MMSE score has been shown to be a remarkable predictor of edentation, in the context of increasingly poor dental hygiene and the appearance and evolution of dental caries. These elements are also found in the results of this research. In addition,

the same study is the first longitudinal research that examines oral health in premorbid stages and after diagnosis of dementia. Unlike our study, it reports the results to the population with cognitive impairment before dementia. This study only indicates the prevalence of oral pathology among patients with already diagnosed dementia and especially among those with moderate-severe dementia (24, 25).

In 2017, a study realized by Campos et al. (26) shows that the masticatory efficacy in Alzheimer's dementia remains below the control level, as indicated by our study, which highlights the severity of the masticatory and swallowing disorders in direct proportion to the evolutionary stage of dementia.

Cognitive decline, apathy and apraxia, symptoms that characterize the average progression of dementia are responsible for the lack of interest and inability to perform oral hygiene techniques (24). In advanced stages, Alzheimer's disease leads to total disability, augmented by the associated general health problems and by the diverse oral pathology resulting in even greater dysfunctionality. Loss of teeth reduces masticatory capacity, increasing the risk for serious swallowing and denaturing disorders (25).

The results of this research relate to the results of international general statistics, revealing a remarkable share of depressive symptoms in dementia, which correlates with specific progressive deterioration, aggravates apato-abulic phenomena, as well as apraxia itself, that correlates directly with the appearance and evolution of oral pathology, reaching to the edentation.

CONCLUSIONS

This study could be the premise of a program designed to increase the quality of life of patients with dementia, including oral health care among all the other fundamental problem of these patients. The results of our study show once more that beyond the psychiatric symptomatology, the patient with dementia associates a series of general health problems that hinder the evolution of the underlying disease. Through the correct, multidimensional approach of the patient with dementia, by managing all psychiatric symptoms such as depression, general health problems, including oral pathology and the risk of edentation, can be avoided or alleviated.

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EXPRESSION OF EXTRACELLULAR MARKERS AS CRITERIA FOR TREATMENT OF PERIPHERAL ARTERIAL OCCLUSIVE DISEASE

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EXPRESSION OF EXTRACELLULAR MARKERS AS CRITERIA FOR TREATMENT OF PERIPHERAL ARTERIAL OCCLUSIVE DISEASE (Abstract): During the last decade, mesenchymal stromal cells (MSC) have been used in medical therapeutics, especially for reperfusion in peripheral arterial disease, ischemic cardiomiopathy and myocardial infarction. Studies demonstrated that membrane proteins are important in MSC endothelial differentiation. **Aim:** To determine the expression of extracellular markers of MSC, in order to ascertain if some expressed proteins (MSC specific cluster of differentiation proteins, essential for MSC characterization and proteins which have a role in adhesion and migration) have capability to induce angiogenesis **Material and methods**: To detect the expression of these proteins and confirm the presence of MSC and other essential proteins with important role in adhesion and migration, cells from five donors were fully assessed by flow cytometry (surface expression), differentiation assays (MSC differentiation into osteocytes, adipocytes, and chondrocytes), and qRT-PCR (gene relative expression). **Results**: Our results were in agreement with other reports, with small exceptions, related especially to donor dependent conditions. The surface expression of beta 1 subunits correlated with a high gene expression on RNA level. **Conclusions**: MSC had all specific characteristics. Surface marker expression was in agreement with pervious findings. These cells can be used for endothelial progenitor cells further differentiation. Beta 1 expression suggests a high potential of the MSC to form capillary lumen, thus for further artery formation and efficient angiogenesis. **Key words**: PERIPHERAL ARTERIAL DISEASE, ADULT STEM CELL, MEMBRANE PROTEINS.

In the last decade mesenchymal stromal cells (MSC) were used as medical therapeutics. MSC can be isolated from bone marrow, peripheral blood, fat, skin, vasculature, and muscle, where they most likely are responsible for normal tissue regeneration, as well as for a response to injury (1). MSC are negative for primitive hematopoietic cell markers. Individual clones of cell lines derived from MSC have different potentials for differentiation, indicating different stages of determination and levels of plasticity. MSCs are a varied population of plastic-adherent cells that show a fibroblast-like morphology, they are forming colonies when grown at clonal densities (referred to as colony forming units fibroblast or CFU-F) (2), and can differentiate into bone, cartilage, and fat cells (3). Human MSCs have been defined by the positive expression of the cell surface antigens CD73, CD90, CD105 and a lack of expression of hemato-

poietic antigens including CD11b or CD14, CD34, CD45, CD79 or CD19, and HLA-DR (4). A comprehensive list of surface antigen expression on MSC was recently published, but variability still exists within MSC populations due to differences in the species, tissue source, and the *in vitro* culture conditions used. To deal with this problem it was released an accord position paper stating the nominal criteria required for essential MSC characterization (4).

Molecular cardiology is a new direction of modern research world with promising results in the field of angiogenesis, not only in the periphery but also heart. In this disease the opening of new horizons for cell therapy, especially therapy with adult stem cells and vascular growth factors that have direct action on progenitor cells to endothelium, which is supposed to circulate freely within the bloodstream and have the ability to become endothelial cells to form capillaries, arterioles and finally arteries, or by autollogus MSC transplantation, to improve angiogenesis, by rising the number of endothelial progenitor cells at the lesion site.

Integrins are receptors that mediate intercellular and intracellular contacts as well as the extracellular matrix (ECM). They also play a role in cell signaling and regulate cellular shape, motility, and the cell cycle. The receptors inform a cell about the environment and the cell responds in consequences. These molecules have an inside-outside signaling possibility. They transduce information from the ECM to the cell as well as revealing the status of the cell outside, allowing rapid and flexible responses to changes in the environment (5). There are many types of integrin, hosted on cells surface. Integrins are of vital importance to all animals and have been found in all animals investigated, from sponges to mammals, being extensively studied in humans. Integrins are working with other proteins such as cadherins (transmembranare molecules which mediate cellular adhesion by calcium dependent mechanism), immunoglobulin superfamily cell adhesion molecules, selectins and syndecans, have the role to mediate cell-cell and cell-matrix interaction and communication. The integrin role is not fully known, a recent study showed that beta 1 integrin have an important role in establishing the endothelial cell polarity and arteriolar lumen formation (6). A final aspect of the vascular occlusion phenotype is that the ECs occluding the lumen exhibited an over accumulation of vacuoles within their cytoplasm. This accumulation will merge and in the end will form endothelial lumen. MSC can home to damaged tissue, process regulated in part by integrins.

MATERIAL AND METHODS

Human MSC were obtained from BM, from five donors, it was harvested from the iliac crest and collected. The obtained sample is further used to isolate MSC, using their property of adherence to culture plastic flask under standard culture condition. Mesodermal differentiation assays were performed using standardized differentiation media, and the proposed protocol was respected. The staining was performed with: Oil-red-O4 for adipocytes, alkaline phosphatase for osteocytes, and with Toluidin Blue for chondrocytes. Differentiation was visualized using light microscopy.

Surface expression was detected by flow cytometry. The cells were labeled to identify the specific markers (4). MSC were phenotyped to ensure the absence of the leukocyte markers CD45, endothelial marker CD34, and the presence of CD105

and CD73. IgG2a and IgG2b isotype control antibodies were used. To identify specific integrins fluorescently labeled conjugated or unconjugated rat anti-mouse antibodies were used: CD11a, CD11b, CD29, ITGAL, ITGAM, ITGAX, ITGAD, ITGB2, ITGB3, ITGB1, ITGA4, and ITGA5.

Quantitative Real-Time Polimerase Chain Reaction (qRT-PCR) was performed on Applied Biosystem StepOne. Real Time PCR System, with TaqMan primers, using the same estimated amount of DNA for each experiment, it was obtained from 1 μg RNA transcription. Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) was used as a positive control, being one of the most commonly housekeeping genes used for gene expression. It serves as a positive control for northern and other expression studies. StepOne® program was used and after assigning target samples on plate setup, the method was set to run. The amplification plot (target amplification generates fluorescent signals which are collected by the instrument) and the data analyze is done via StepOne® software and results were export to Graph Prism for analysis.

RESULTS

We identified the differentiation potential of MSC into osteocytes, adipocytes, and chondrocytes (fig. 1). Oil Red-O stain was used to identify neutral triglycerides. The microscope analysis reveals the red fat vesicles in adipocytes differentiated cells (fig. 1 B). Kossa Stain was used to identify the presence of calcium deposits (fig. 1 C). Chondrocytes differentiation can be assessed after Toluidin Blue staining, (stains in lila colour) (fig. 1 D).

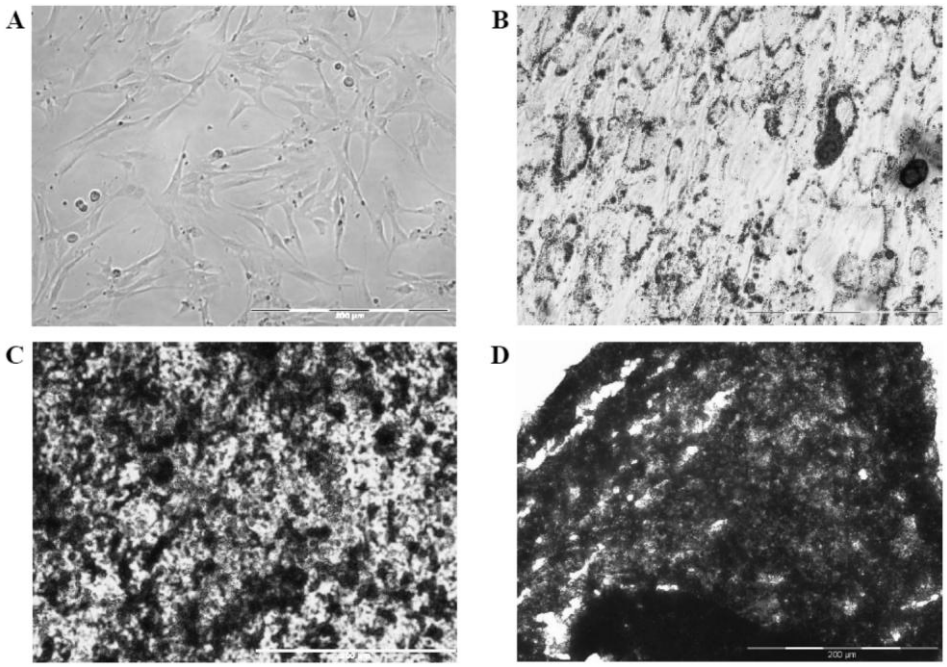

Fig. 1. Differentiation of MSC (n=5). A. MSC culture before differentiation, B. Adipocyte differentiation - Oil Red O staining, C. Osteocyte differentiation - von Kossa staining. D. Chondrocyte differentiation - Toluidin Blue staining (100 x magnification).

To complete the MSC characterization specific markers had to be identified on their surface. The isolated MSC respected the minimal characteristics of surface markers. They were positive for: CD105, CD90, CD73, and negative for: CD45, CD34, CD11b (as it is seen in fig. 2). The right shift at CD105, Cd73 and CD90, is representative for positive cells. The overlapping of CD11b, CD45, CD34, represents their absence in comparison with IgG isotype control, which as usual is considered negative. As a result we know that the isolated cells were MSC. They respect all tree conditions of tissue growth for MSC (adherence to plastic, tri-lineage differentiation and specific surface markers).

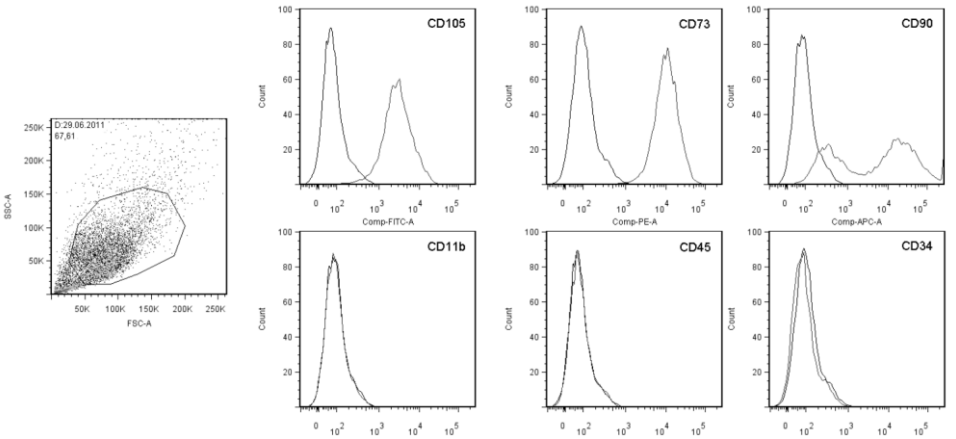

Fig. 2. MSC characteristic markers expression (n=5)

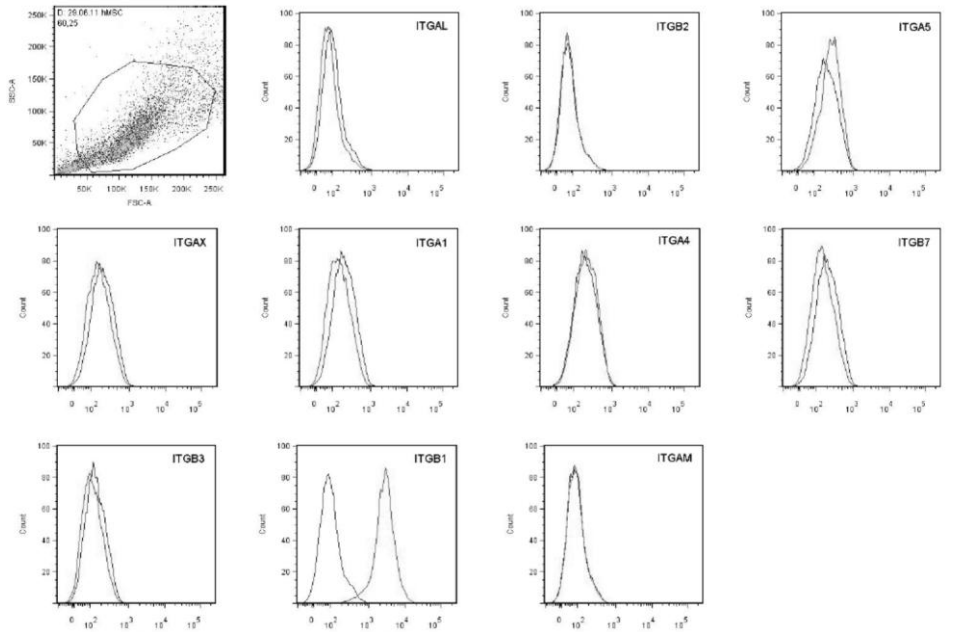

Fig. 3. Integrin expression on MSC surface (n=5)

The flow cytometric analysis was performed to identify integrin subunits expression on isolated cells. The results show that **l**ymphocyte function-associated antigen 1 (LFA-1, also known as alpha L/beta 2 integrin) is not expressed, compared with IgG isotype control (fig. 3). All tested alpha subunits (alpha 5, alpha X, alpha 1, alpha 4, alpha M, alpha L) are absent, some beta tested subunits (beta 2, beta 3 and beta 7) are negative, in contrast with beta 1 subunit.

To confirm the results from pervious experiment we decided to evaluate integrin expression at RNA level. For this purpose we performed qRT-PCR analysis, in order to assess gene expression Using GAPDH as the housekeeping gene. The analysis confirmed the flow cytometry for absence of all alpha integrin subunits, exception alpha X, discreet expression in two donors. Beta subunits have a relative good expression in beta 3, beta 5 integrins, with a small donor dependent variation. In contrast beta 1 integrin subunit has a high donor dependent variation, seen in a high standard deviation (fig. 4).

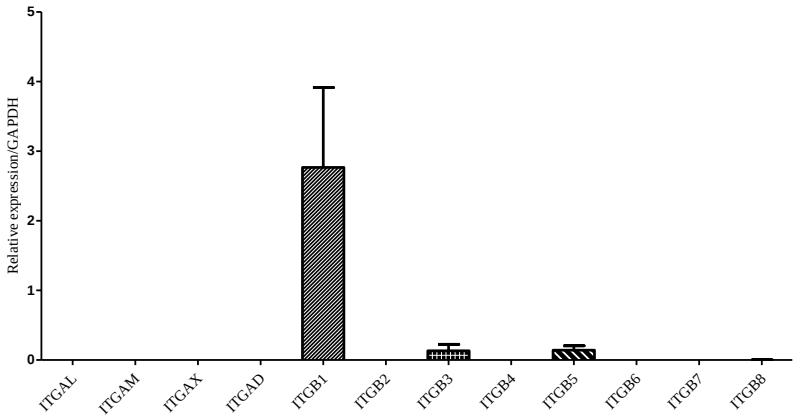

Fig. 4. Relative expression of integrins in five different donors (We can see donor dependent expression clearly in beta 1 integrin subunit and in consequence a high standard deviation) (n=5).

DISCUSSION

Stem cell therapy is a type of interventional medicine, which has the aim to introduce new cells into damaged tissue in order to treat a disease or injury (7). The ability of stem cells to give rise to subsequent generations, with variable degrees of differentiation capacities, offers significant potential for generation of tissues that can potentially replace damaged areas in the body, with minimal risk of rejection and side effects (8).

A number of stem cell therapies exist, but most are either at experimental stages or costly, with the notable exception of bone marrow transplantation for leukemia treatment. Medical researchers anticipate that adult and embryonic stem cells will soon be able to treat many types of disorders using stem cells. Nevertheless, before stem cell therapeutics can be applied in the clinical setting, more research is necessary to understand their behavior upon transplantation, as well as the mechanisms of interaction with the microenvironment (9). As described in literature over the last years, the MSC have a significant therapeutically effect over the other medicine cell types. One of the most

important advantages of MSC is their relevant immune modulatory effect post intravenous administration (10). It has been shown that these cells have the possibility to be transplanted without causing immune rejection. Their potential has been proposed to confer main functions in securing the support of hematopoiesis, and has been involved in functions in tissue repair such as osteogenic remodeling or cartilage generation (11). It is known that after donor isolation, the MSC can be expanded in culture, cryopreserved, and then used for different acute phases and chronic clinical conditions in various patients (12). Currently, it is known that MSC follow a cascade of signaling molecules, due to their mobilization into bloodstream, but there are several other factors involved in, which are still unknown (13).

The current work aimed to analyze five MSC donors. The isolated MSC were differentiated into three specific lineages as it was described before. These findings were according to other researches findings, where MSC were able to differentiate into three mesodermal layers: adipocytes, osteocytes, and chondrocytes (3), (14). MSC were able to differentiate in our case into tree mesodermal layers: adipocytes, osteocytes and chondrocytes. The results from flow cytometry confirm the expression of MSC markers like: CD105, CD90, CD73 as well as the absence of Cd11b, CD34 and CD45 markers (4). Integrin expression was assessed using flow cytometry and gene expression quantification. All analyzed alpha integrin subunits were absent (alpha 1, alpha 4, alpha 5, alpha X, alpha M, alpha D, alpha L) as well as some beta subunits (beta 2, beta 7, beta 4, beta 6). A relative high expression for beta 3 and beta 5 integrin subunits was observed. Only beta 3 was analyzed flow cytometry and qPCR, and is negative in flow cytometry test, while gene expression is low. This can be explained by a possible cellular modification at integrin level when we prepared cells for flow cytometry (the surface molecules are cut off in the time of trypsinisation), or the protein is not expressed on surface. Beta 5 integrin subunit has a relative gene expression, but the surface expression couldn't be detected in flow cytometry. Beta 5 integrin subunit has a relative gene expression, but the surface expression couldn't be analyzed by flow cytometry, because we didn't had the specific surface antigens.

Our results are in agreement with Semon (15), that observed that over 80% of MSC express beta 1 integrin subunits. In contrast we did not find any expression of beta 2 and other alpha subunits. Furthermore, beta 1 is the integrin which has variation among different MSC donors.

CONCLUSIONS

The experiment performed isolated MSC that have all three specific characteristics: adherence to plastic flasks, trilineage differentiation, and surface marker characterization. The integrin expressions on MSC are donor dependent, more pronounced in the case of beta 1 integrin subunit, in this case we had high donor variability. The surface marker expression was in agreement with pervious findings, and this want to demonstrate that we will work with MSC, for EPC further differentiation. The high beta 1 integrin expression suggests a high potential of the MSC to form capillary lumen, for further artery formation and an efficient angiogenesis. The results weren't fully in agreement with previous findings and the literature discrepancies were not totally understood.

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INVESTIGAŢII FARMACOEPIDEMIOLOGICE ASUPRA INTERACŢIUNILOR MEDICAMENTOASE ALE ANTICOAGULANTELOR ORALE LA PACIENŢI CU TULBURĂRI ALE HEMOSTAZEI

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3. Disciplina Medicină de Urgenţe

PHARMACOEPIDEMIOLOGIC INVESTIGATIONS REGARDING THE INTERACTIONS BETWEEN ORAL ANTICOAGULANTS AND OTHER DRUGS IN PATIENTS WITH HAEMOSTATIC DISTURBANCES (Abstract): A**im:** To evaluate drugs interactions with oral anticoagulants and their clinical relevance, in patients from two emergency departments in hospital. **Material and methods:** Retrospective study, during one year, on patients (between 18 and 85 year old) with haemostatic disturbances admitted at two emergency departments in „Sf. Spiridon" Hospital, Iaşi and in Piatra Neamţ Public Hospital, consisted in medical records analysis**:** the diagnostic, therapy recommended. Data were scored and statistically analyzed by t test (Windows Excel). The experimental protocol was in agreement with recommendations of the "Gr. T. Popa" University Committee for Research and Ethical Issues. **Results and conclusions:** Analysis and statistical processing of data shows that during the period of the study, 69 patients, which received oral anticoagulant therapy associated with different other medication belonged to the 60 and 80 years age group. The findings suggests the pathology is represented especially by atrial fibrillation and cerebral ischemic diseases. The correlation between the therapy consisting in OAC and amiodaronum or statines drugs association and International Normalized Ratio (INR), revealed a high level of anticoagulation and an increase of hemorragic complications. **Key words:** ORAL ANTICOAGULANTS, DRUG INTERACTIONS, PHARMACOEPIDEMIOLOGY, HAEMOSTATIC.

Medicaţia anticoagulantă este folosită pentru prevenirea trombemboliilor sistemice la pacienţii cu fibrilaţie atrială cronică, cu afecţiuni cardiace valvulare sau cu proteze valvulare, în tromembolismul pulmonar primar sau secundar. Sunt, de asemenea, utilizate în prevenţia trombemboliilor la pacienţii cu infarct miocardic acut şi angină pectorală, la cei cu valve biologice sau după intervenţii chirurgicale sau ortopedice (1,2,3).

International Normalized Ratio (INR) este indicatorul cel mai utilizat în evaluarea eficacităţii terapiei anticoagulente orale. Riscul complicaţiilor hemoragice sau tromboembolice creşte dacă INR este în afara intervalului terapeutic recomandat. Efectul ACO este influenţat de diverşi factori genetici, medicali, alimentari, comorbiditate, etc (4,5).

Studiul urmăreşte realizarea unei analize retrospective privind interacţiunile medicamentoase ale terapiei cu anticoagulante orale, la pacienţii cu tulburări ale hemostazei la Unitatea de Primire Urgenţe (UPU) din Iaşi şi Piatra-Neamţ.

MATERIAL ŞI METODE

S-a realizat un studiu descriptiv transversal, pe un lot de 368 de pacienţi desfăşurat în cursul anului 2009 constând în culegerea datelor specifice privind interacţiunile medicamentelor la pacienţii trataţi cu anticoagulante orale şi nivelul acestora anticoagulare la cei asistaţi în UPU de nivel judeţean, pentru diverse afecţiuni care necesită administrarea de anticoagulante orale pe termen lung; evaluarea nivelului anticoagulării la pacienţi care au primit alte tratamente, cu referire directă la amiodaronă şi la statine; standardizarea şi predictibilitatea aplicării terapiei anticoagulante concomitent cu alte terapii în practica medicală umană; stabilirea unor corelaţii între nivelul terapeutic al anticoagulantelor orale şi diverse medicamente utilizate simultan.

Criteriile de includere lotul de studiu au fost reprezentate de: persoane cu domiciliu stabil în localitate; vârsta între 18-85 ani; prezentarea la UPU pentru efectuarea analizelor lunare sau de urgenţă. Criteriile de excludere au fost vârsta sub 18 sau peste 85 ani; date incomplete; persoane necooperante sau cu domiciliu stabil în alte localităţi. Studiul s-a efectuat cu acordul Comisiei de Etică a UMF ''Gr.T. Popa'', Iaşi.

Informaţiile preliminare s-au obţinut analizând datele din registrele de consultaţii şi fişa de evaluare a stării de sănătate a pacientului elaborată in cadrul UPU, reţinând vârsta, statusul marital, nivelul educaţional laborator şi rezultatele de laborator Timpul Quick, INR, aPTT şi cele clinice: TA, parametrii antropometrici, tratamentul.

Pentru prelucrarea şi evaluarea informaţiilor acumulate s-a stabilit un mod de codare şi înregistrare a datelor; stocarea acestora în calculator cu sistem de operare Windows 7; interpretarea statistică cu ajutorul programului SPSS 17.0(8).

REZULTATE ŞI DISCUŢII

1.Unele caracteristici demografice ale loturilor incluse în studiu. În anul 2009 la UP„Sf. Spiridon" Iaşi au fost asistaţi 237 pacienţi cu tulburări ale hemostazei, iar la UPU a Spitalului Judeţean de Urgenţe Piatra Neamţ, 131, la ambele unităţi predominând grupurile de vârstă 61 – 80 de ani, urmate de 51 – 60, şi peste 81 ani (Tab. I).

Analiza repartiţiei pe sex a evidenţiat că la UPU „Sf. Spiridon" Iaşi, 128 (53,8%) au fost pacienţi de sex feminin, iar la Piatra Neamţ 80 (61,1%) de sex masculin.

TABELUL I

Structura pe grupuri de vârstă a pacienţilor asistaţi la UPU Iaşi şi Piatra Neamţ, în anul 2009

Grupul de vârstă (ani)	Unitatea Primire Urgenţe			
	Spitalul „Sf. Spiridon" Iaşi		Spitalul Judeţean Piatra Neamţ	
	Nr.	%	Nr.	%
20 - 30	3	1,3	1	0,8
31 - 40	1	0,4	3	2,3
41 - 50	6	2,5	8	6,1
51 - 60	30	12,6	16	12,2
61 - 70	45	18,9	31	23,7
71 - 80	91	38,2	56	42,7
peste 81	61	25,6	16	12,2
TOTAL	238	100	131	100

2. Distribuţia pacienţilor incluşi în studiu, la cele două UPU, în relaţie cu diagnosticul principal şi cel secundar.

La UPU „ Sf. Spiridon ” Iaşi, diagnosticul principal a inclus 7 afecţiuni din care pe primul loc s-a situat Fibrilaţia atrială (FA – 57,1%), urmat de Sindromul coronarian acut (SCA – 15%) şi de Accident la ACO (14,3%).

Rezultatele determinărilor TQ, INR şi aPTT diferenţiat pentru fiecare din cele două UPU (tab. II, III, IV).

Rezultatele determinărilor TQ, INR şi aPTT ale pacienţilor admişi în studiu în Spitalul Judeţean de Urgenţe din Piatra Neamţ sunt sistematizate în tabelele V, VI, VII.

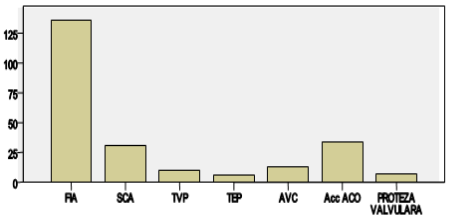

Fig. 1 Diagnosticul principal al pacienţilor de la Spitalul de Urgenţe „Sf. Spiridon” Iaşi

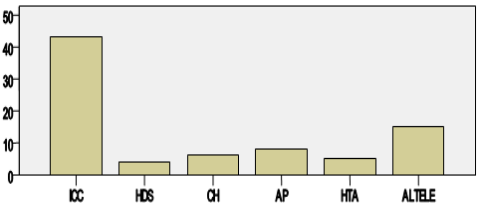

Fig. 2 Diagnosticul secundar al pacienţilor de la Spitalul de Urgenţe „Sf. Spiridon” Iaşi

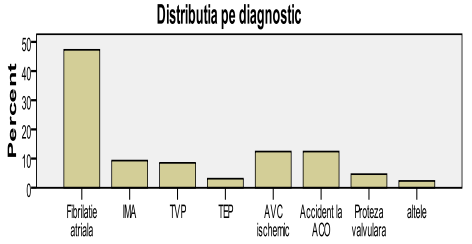

Fig. 3 Diagnosticul principal al pacienţilor asistaţi la UPU Piatra Neamţ

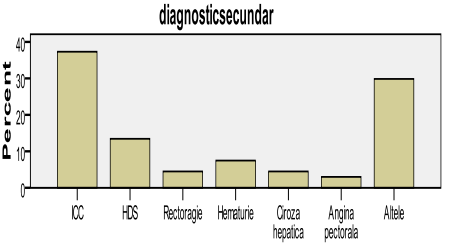

Fig. 4. Diagnosticul secundar al pacienţilor de la UPU Piatra Neamţ

TABELUL II

Distribuţia valorilor TQ în Spitalul Clinic de Urgenţe „Sf. Spiridon” Iaşi

Grupe TQ	Frecventă			
	Nr.	%	% valid	% cumulativ
80 - 100	24	10,1	10,1	10,1
60 - 80	19	8,0	8,0	18,1
40 - 60	38	16,0	16,0	34,2
20 - 40	93	39,1	39,2	73,4
10 - 20	51	21,4	21,5	94,9
< 10	11	4,6	4,6	99,6
45	1	,4	,4	100,0
Total	237	99,6	100,0	

TABELUL III

Distribuția valorilor INR la pacienții incluşi în studiu din Spitalul de Urgențe „Sf. Spiridon” Iaşi

Grupuri INR	Frecvență			
	Nr.	%	% valid	% cumulativ
< 2	83	34,9	35,0	35,0
2 - 2,5	52	21,8	21,9	57,0
2,5 - 3	17	7,1	7,2	64,1
3 - 3,5	10	4,2	4,2	68,4
3,5 - 4	14	5,9	5,9	74,3
4 - 5	19	8,0	8,0	82,3
5 - 6	9	3,8	3,8	86,1
> 6	33	13,9	13,9	100,0
Total	237	99,6	100,0	

TABELUL IV

Distribuția valorilor aPTT la pacienții incluşi în studiu la Spitalul de Urgențe „Sf. Spiridon” Iaşi

Grupuri aPTT	Frecvență			
	Nr.	%	% valid	% cumulativ
20 - 40	17	7,1	34,7	34,7
40 - 60	14	5,9	28,6	63,3
60 - 80	7	2,9	14,3	77,6
80 - 100	2	,8	4,1	81,6
> 100	9	3,8	18,4	100,0
Total	49	20,6	100,0	

TABELUL V

Distribuția valorilor TQ la pacienții din Spitalul Județean de Urgențe din Piatra Neamț

Grupuri TQ	Frecvență			
	Nr.	%	% valid	% cumulativ
80 - 100	11	8,4	8,4	8,4
60 - 80	10	7,6	7,6	16,0
40 - 60	20	15,3	15,3	31,3
20 - 40	59	45,0	45,0	76,3
10 - 20	26	19,8	19,8	96,2
< 10	5	3,8	3,8	100,0
Total	131	100,0	100,0	

TABELUL VI

Distribuția valorilor INR la pacienii din Spitalul Județean de Urgențe Neamț

Grupuri INR	Frecvență			
	Nr.	%	% valid	% cumulativ
1 - 2	42	32,1	32,1	32,1
2 - 2,5	34	26,0	26,0	58,0
2,5 - 3	11	8,4	8,4	66,4
3 - 3,5	5	3,8	3,8	70,2
3,5 - 4	8	6,1	6,1	76,3
4 - 5	11	8,4	8,4	84,7
5 - 6	7	5,3	5,3	90,1
> 6	13	9,9	9,9	100,0
Total	131	100,0	100,0	

TABELUL VII

Distribuția valorilor aPTT la pacienți din Spitalul Județean de Urgențe Neamț

Grupuri aPTT	Frecvență			
	Nr.	%	% valid	% cumulativ
20 - 40	6	4,6	20,7	20,7
40 - 60	12	9,2	41,4	62,1
60 - 80	4	3,1	13,8	75,9
80 - 100	4	3,1	13,8	89,7
> 100	3	2,3	10,3	100,0
Total	29	22,1	100,0	

TABELUL VIII

Tratamentul recomandat pacienților de la UPU „Sf. Spiridon” Iaşi

Preparate terapeutice	Frecvență			
	Nr.	%	% valid	% cumulativ
Trombostop	175	73,5	73,8	73,8
Sintrom	17	7,1	7,2	81,0
Trombostop + Amiodarona	34	14,3	14,3	95,4
Trombostop + statine	11	4,6	4,6	100,0
Total	237	99,6	100,0	

TABELUL IX

Terapia pacienților în Spitalul Județean de Urgențe din Piatra Neamț

Preparate terapeutice	Frecvență			
	Nr.	%	% valid	% cumulativ
Trombostop	94	71,8	71,8	71,8
Sintrom	13	9,9	9,9	81,7
Trombostop + amiodarona	17	13,0	13,0	94,7
trombostop + statine	7	5,3	5,3	100,0
Total	131	100,0	100,0	

Tratamentul pacienților incluşi în studiu a fost stabilit în relație cu rezultatele observațiilor clinice şi ale evaluărilor statistico – matematice (tab. IX).

Prin utilizarea concomitentă a ACO şi a amiodaronei există riscul creşterii efectului anticoagulant datorită interacțiunii celor două tipuri de medicamente. Efectul apare ca urmare a inhibării de către amiodaronă a metabolizării ACO. Astfel amiodarona este incriminată în sensul inhibării enantiomerilor R şi S ai warfarinei prin intermediul citocromului P-450 *in vivo*, dar şi *in vitro* (9,10). Timpul până la apariția incidentelor şi a accidentelor datorate hipocoagulabilității sangvine este variabil şi efectul poate continua câteva săptămâni după identificarea interacțiunii, chiar după întreruperea administrării amiodaronei, datorită timpului de înjumătățire plasmatică prelungit şi variabil al acesteia. Factorii predispozanți pentru apariția acestor efecte ar putea fi şi inducerea de către amiodaronă a tireotoxicozei, care poate creşte clereanceul unor factori ai coagulării dependenți de vitamina K, scăzând, astfel, nivelul coagulabilității sangvine (11,12). Se recomandă controlul mai frecvent al timpului de protrombină, monitorizarea INR-ului şi ajustarea dozei de anticoagulant oral (3,9).

În unele studii (12,13) se recomandă scăderea cu 1/3 până la 1/2 a dozelor de warfarină pentru menținerea unui nivel satisfăcător al activității protrombinei. Existența interacțiunii dintre ACO şi amiodaronă necesită ajustarea dozei cu 32,9% - 70% în cazul warfarinei şi cu 20% - 60% în cel al acenocumarolului.

CONCLUZII

În urma studiului realizat pe un lot de 43 de pacienți, s-a constatat necesitatea ajustării dozei de warfarină cu până la 44% după şapte săptămâni de administrare concomitentă cu amiodaronă. Datorită necesității menținerii amiodaronei în doze de 400, 300, 200, 100mg/dl în terapie concomitent cu warfarina, s-a recomandat scăderea dozei acesteia din urmă cu 40%, 35%, 30% şi respectiv 20%. S-a observat că menținerea dozei de amiodaronă asigură o bună interacțiune între aceasta şi ACO. În cazul utilizării concomitente a ACO şi a amiodaronei s-a constatat modificarea drastică a TQ, INR şi aPTT cu riscul apariției accidentelor hemoragice, urmare a inhibării de către amiodaronă a metabolizării ACO.

În studiile noastre, asocierea statinelor

cu ACO a determinat modificarea coagulabilității, efectul manifestându-se, în special, asupra INR. Acest aspect indică controlul frecvent, la intervale scurte de timp, a TQ, INR şi aPTT, atât în monoterapia cu ACO, cât şi în cazul asocierii statinelor cu ACO, respectiv, în cazul asocierii ACO cu statine şi amiodaronă.

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SPAŢIUL PARAFARINGIAN – ISTORIC ŞI IMPLICAŢII CHIRURGICALE

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PARAPHARYNGEAL SPACE – HISTORY AND SURGICAL STUDY (Abstract): One area of anatomy, which is a true passage of secrecy, is the anatomy of the potential anatomical spaces. The study of the fascial layers of the head and neck and the potential spaces they make-up is both fascinating and confusing. Much of the confusion is a function of the multiple synonymous terms and classifications that various authors use, and not the basic anatomical description. Their anatomy and communications, to the base of skull and downward to diaphragm, acts like a route through which infection from mouth and throat can reach the superior mediastinum. Conclusions: Understanding the anatomy of the parapharyngeal space is important in making a correct diagnosis and surgical plan for removing tumors in this region. **Key words**: FASCIAL SPACE, PARAPHARYNGEAL SPACE, CLASSIFICATION, SURGICAL APPROACHES.

Spaţiul parafaringian (SPF) reprezintă o zonă anatomică complexă atât prin structură şi organizare, cât şi prin patologie şi de la prima descriere făcută de Burns în 1811, a fost motivul unei mari controverse. Cunoaşterea anatomiei spaţiilor fasciale ale capului şi gâtului constituie suportul înţelegerii etiologiei, diseminării, simptomatologiei şi complicaţiilor variatelor procese patologice ale regiunilor feţei, gâtului şi toracelui (1,4).

Termenul de spaţiu potenţial este utilizat pentru a defini un spațiu ce devine evident prin distensie sau disecție exploratorie, fără distrugerea sau degradarea integrității structurale şi funcţionale a ţesuturilor care participă la delimitarea acestuia (4, 6, 9, 16).

În 1605, pentru prima dată, Francis Bacon a atras atenția asupra „pasajelor" dintre structurile anatomice propriu-zise.

În 1811, BURNS a descris aceste spaţii fasciale pentru prima dată. O importantă clasificare a spațiilor cervicale a fost elaborată de Grodinsky și Holyoke în 1938, când a fost demonstrată și continuitatea acestora cu spațiile mediastinale, până la nivel toracic inferior (1, 4, 5, 9, 12) (fig.1).

Spaţiul parafaringian este constituit, de fapt, atât din spaţiul retrofaringian cât şi din cel laterofaringian.

Trebuie subliniată importanța cunoașterii structurilor anatomice naturale de limitare a SPF, și anume rafeul pterigomandibular (fig.2), tunelul stilomandibular şi mușchiul stiloglos (2, 3, 13, 15)(fig. 3).

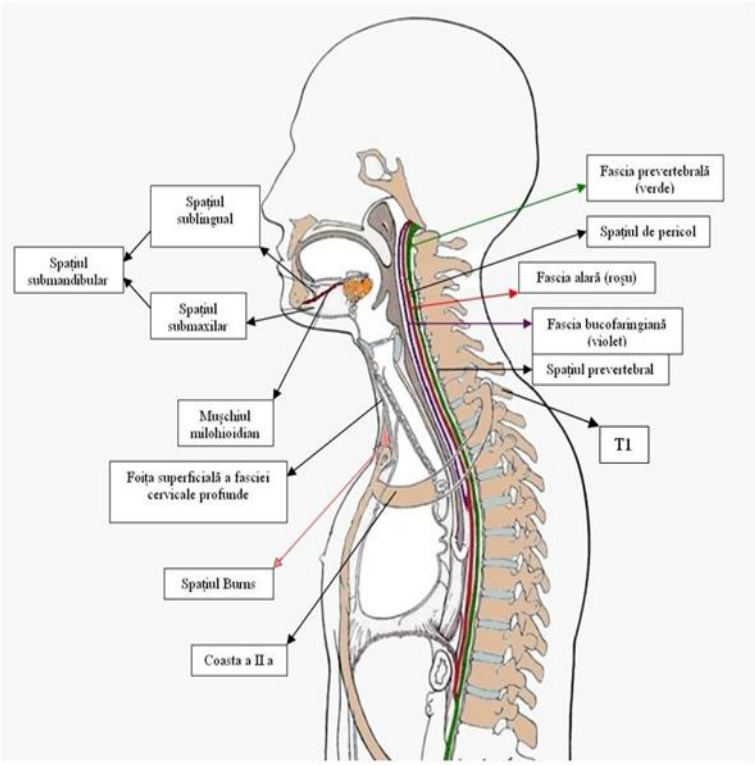

Fig. 1. Spaţii fasciale posterioare – secţiune sagitală

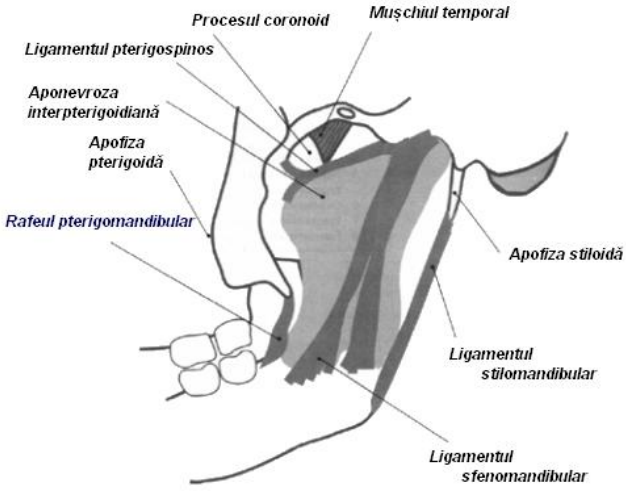

Fig. 2. Rafeul pterigomandibular

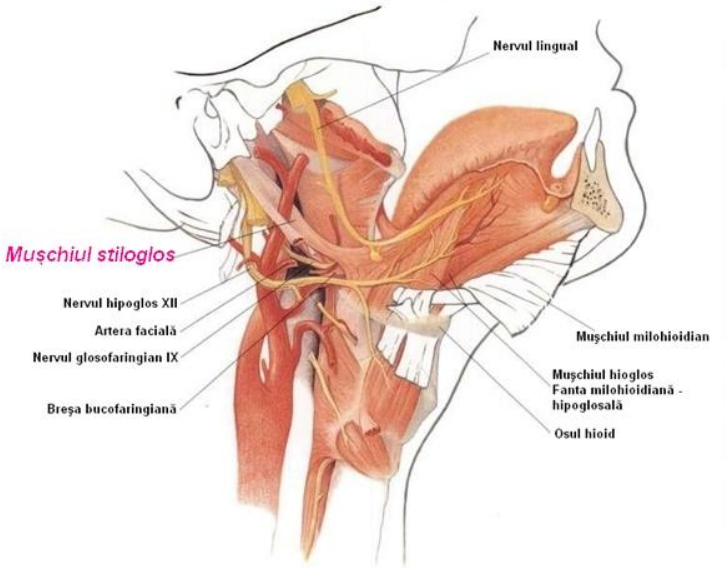

Fig. 3. Muşchiul stiloglos

Scopul acestui articol este de a face o conexiune între compartimentarea acestor spații fasciale și diferitele tipuri de abord chirurgical care pot fi utilizate pentru excizia formațiunilor tumorale parafaringiene.

MATERIAL, METODĂ ŞI TEHNICI DE CERCETARE

Au fost incluși în studiu un număr de 192 de cazuri noi de pacienți cu formațiuni tumorale parafaringiene, internați în Clinica ORL a Spitalului „Sf. Spiridon" Iași, pe o perioadă de 20 ani (1990 -2009).

Toate aceste cazuri au fost evaluate clinic și ca status general, pacienții au fost investigați imagistic și supuși unor intervenții chirurgicale.

ANALIZA STATISTICĂ

Toate datele medicale au fost prelucrate statistic utilizându-se programe specifice, *SPSS 13.0* și Microsoft Office, utilizându-se un grad de încredere de 95%, și obținându-se rezultate cu prag semnificativ $p < 0{,}001$.

REZULTATE

Dezvoltarea deosebită a tehnicilor imagistice au permis specialiștilor radiologi stabilirea unor repere noi de compartimentare a spațiului parafaringian, mai utile chirurgilor otorinolaringologi decât cele clasice, anatomice (8, 10).

Astfel, SPF este împărțit într-un spațiu anterior şi unul posterior de un plan dus de la apofiza stiloidă până la mușchiul tensor al vălului palatin (fig. 4).

Utilizând secțiunile coronale CT sau IRM, fiecare dintre aceste două spații poate fi subdivizat în plan vertical, în 3 etaje (fig. 5):

- Superior (S)
- Mijlociu (M)
- Inferior (I),

Etajele superior (S) şi mijlociu (M), sunt separate printr-un plan situat la nivelul marginii inferioare a mușchiului pterigoidian lateral, iar cel mijlociu (M) și inferior (I) sunt delimitate de un plan situat la nivelul unei linii ce unește marginea inferioară a lamelor laterale ale apofizelor pterigoide.

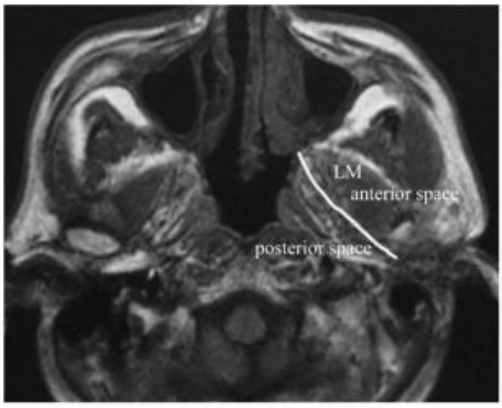

Fig. 4. Compartimentarea anteroposterioară a SPF

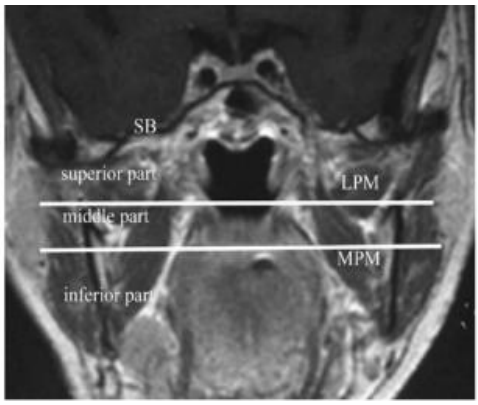

Fig. 5. Compartimentarea în plan vertical a SPF

Astfel se realizează o topogramă cu 6 compartimente, care se va suprapune cu imaginea TC sau IRM a formaţiunii tumorale (tab. I).

TABELUL I

Topograma spaţiului parafaringian

Cadrane ale spaţiului parafaringian	**Posterior P**	**Anterior A**
Superior S	Postero-superior P-S	Antero-superior A-S
Mijlociu M	Mijlociu-posterior M-P	Mijlociu-anterior M-A
Inferior I	Postero-inferior P-I	Antero-inferior A-I

În cadrul acestei topograme, pentru stabilirea localizării formaţiunii tumorale, se va lua în considerare limita superioară a acesteia.

TABELUL II

Tipurile de abord chirurgical preconizat

Cadrane afectate ale spaţiului parafaringian	**Tipul de abord chirurgical preconizat a fi utilizat**
Superior S	-Abordul fosei infratemporale
Mijlociu M	-Abord transoral -Abord transparotidiancu /fără mandibulotomie
Inferior I	-Abord submandibular transcervical
Afectarea simultană a mai multor cadrane ale spaţiului parafaringian	Abord combinat -tr.parotidian+tr.cervical -tr.oral+tr.cervic -tr.oral+tr.parotidian -abord FIT+tr.parotidian

Tipurile de abord utilizate în cazul afectării unuia sau mai multor cadrane ale SPF sunt prezentate în tabelul II.

În cadrul studiului efectuat, tipurile de intervenţii chirurgicală care au fost utilizate sunt cuprinse în tabelul III, fiind reprezentate grafic şi procentele acestor intervenţii în cadrul numărului total de pacienţi la care s-a instituit tratament chirurgical (fig. 6).

TABELUL III

Tipuri de intervenţii chirurgicale	Număr de cazuri	Frecvenţa tipului de intervenţie chirurgicală
Abord transcervical submandibular = Cervicotomie înaltă	144	75%
Abord transoral	18	9,4%
Abord transcervical – transmandibular Mandibulotomie laterală	6	3,1%
Abordul fosei infratemporale	1	0,5%
Abord mixt Cervicotomie + abord transoral	23	12,0%
Total cazuri	192	100%

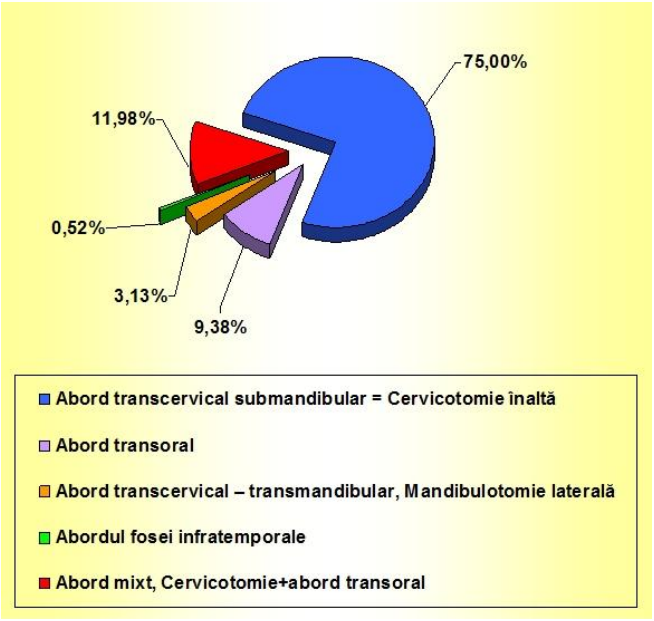

Fig. 6. Tipuri de abord chirurgical utilizat

CONCLUZII

Cunoaşterea anatomiei spaţiilor fasciale cervicale şi a încadrării spaţiului parafaringian între acestea este esenţială în abordul chirurgical al tumorilor parafaringiene.

Cu toate progresele imagisticii realizate până în prezent, abordul tumorilor parafaringiene ridică probleme importante de diagnostic şi mai ales de tratament.

Îmbunătăţirea semnificativă a tehnicilor imagistice au permis un diagnostic mai exact şi în acest mod, creşterea numărului formaţiunilor considerate operabile.

Alegerea căii de abord chirurgical a spaţiului parafaringian trebuie să ţină cont de împărţirea radiologică a acestuia în cele şase compartimente**.**

În cadrul acestei topograme, pentru stabilirea localizării formaţiunii tumorale, se va lua în considerare limita superioară a acesteia.

Managementul eficient al acestor cazuri necesită o colaborare interdisciplinară, a unei echipe formate din chirurgul otorinolaringolog, specialistul radio-imagist, anatomo-patolog, oncolog, neurochirurg, de chirurgie vasculară, plastică şi nu în ultimul rând, psihologul terapeut.

Având în vedere complexitatea zonei pe care se intervine şi probabilitatea survenirii unor situaţii neprevăzute, intempestive şi uneori critice, pacientul trebuie informat cât mai amănunţit asupra tuturor situaţiilor clinice ce pot decurge în timpul şi după actul operator.

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TUMORI RARE ALE REGIUNII CERVICALE - PREZENTARE DE CAZ

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RARE TUMORS OF THE NECK- REPORT OF TWO CASES (Abstract): Of the tumors of the neck some are very uncommon, difficult to diagnose both clinically and paraclinically. Two of the most rare tumors are osteolipoma and soft tissue sarcoma. We present 2 such cases admitted to and operated at the ENT Department of the Iasi „St. Spiridon" Emergency Hospital in the last 5 years, with emphasis on the atypical clinical and imaging features of these tumors. Surgery and histopathological examination were the methods used to make the diagnosis. **Key words**: OSTEOLIPOMA, SOFT TISSUE SARCOMA, RARE TUMORS

Tumorile latero-cervicale reprezintă o entitate destul de des întâlnita în patologia O.R.L. fie ca localizare primară, fie ca diseminare secundară a unui proces neoplazic de cele mai multe ori. Deşi diagnosticul etiologic uneori se poate pune doar pe baza examenului clinic (ex. tumori vasculare), de cele mai multe ori se stabileşte analizând şi datele biologice (hemoleucograma, serologie pentru infecții specifice bacteriene sau virale etc.) şi imagistice (ecografie, CT, RMN) (1).

Cu toată dezvoltarea tehnicii şi specificitatea testelor de laborator pot să apară surprize intraoperatorii şi histopatologice, în special in cazul varietăților cu incidență scăzută.

Raritatea acestor tipuri de tumori face ca un studiu riguros al lor să fie dificil de efectuat, astfel încât principiile terapeutice sunt rezultatul experienței personale sau a unor studii retrospective limitate şi nu se bazează pe studii clinice extinse efectuate pe loturi mari populaționale (2, 3, 4).

PREZENTAREA CAZURILOR

Au fost selectate şi analizate două cazuri internate şi operate în Clinica O.R.L. a Spitalului Clinic de Urgență „Sf. Spiridon" din Iaşi. În aceste cazuri diagnosticul de certitudine preoperator a fost imposibil de stabilit datorita particularităților şi caracterului atipic al formațiunilor tumorale cervicale cât şi a absenței corelațiilor semnificative, clinice sau a modificărilor sugestive ale tabloului biologic.

CAZUL 1

Pacientul M. L. în vârsta de 50 de ani, se internează pentru deformarea regiunii bazicervicale drepte de către o formațiune tumorală de aproximativ 7/6 cm, aparent

bine delimitată, suprafața neregulată, aderenta de planurile profunde muşchiul sternocleidomastoidian (SCM), mobilă pe planurile superficiale (fig. 1).

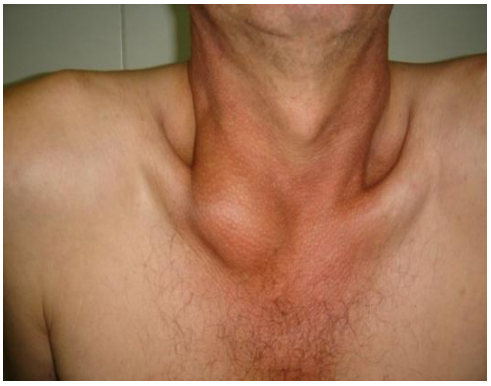

Fig. 1. Formațiune tumorală bazicervicală

Palparea evidențiază consistența elastică a formațiunii care însă devine dură cu caracter osos spre planurile profunde musculare. Pacientul relatează debutul afecțiunii insidios, în urma cu 4-5 ani prin apariția unei formațiuni de mici dimensiuni (1 cm), moale, mobilă, localizată în treimea mijlocie şi anterior de muşchiul SCM, care în timp, coboară, devine dură şi se fixează în regiunea bazicervicală dreaptă, crescând progresiv în dimensiuni. Tabloul biologic este uşor modificat, nesemnificativ (discretă anemie). Panendoscopia nu identifică existența unor zone suspecte care să sugereze o eventuală localizare cervicală, neoplazică secundară.

Ecografia cervicala descrie o formațiune hiperecogenă de 7/4/6 cm (cranio-caudal/orizontal/antero-posterior), fără semnal Doppler, cu efect de masa asupra muşchiului SCM, fără limita de demarcație față de acesta şi fără rapoarte cu pachetul vascular al gâtului.

Se intervine chirurgical şi intraoperator se observa o formațiune cu caracter lipomatos dar consistență dură, osoasă, ce formează un bloc cu fibrele posterioare şi externe din treimea inferioara a muşchiului SCM, excizia largă, completă necesitând şi rezecția parțiala a acestora (fig. 2, 3).

Fig. 2. Aspect intraoperator cu evidențierea zonei dure, aderente de muşchiul SCM

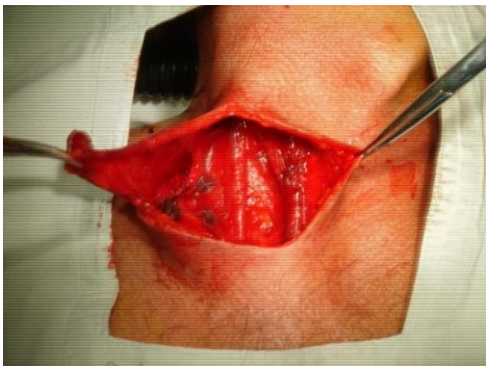

Fig. 3. Aspect postexcizional

Evoluția favorabila permite externarea pacientului la 2 zile postoperator.

Examenul histopatologic descrie o „formațiune tumorală benignă, constituită dintr-un nodul sclerohialin cu metaplazie osoasă ce înglobează fibre musculare striate degenerate sau atrofice şi izolați lobuli de țesut adipos" şi stabileşte diagnosticul de „fibrolipom cu metaplazie osoasă".

CAZUL 2

Pacientul I. F. în vârsta de 50 de ani se internează pentru deformarea regiunii latero-cervicale drepte, în treimea inferioară de către o formațiune tumorala de aproximativ 10/10 centimetri, cu consistență elastică, suprafața regulată, margini bine delimitate, fixată de planurile profunde, ulcerată la tegument pe o suprafață de 7/7 centimetri, uşor sângerânda spontan şi la atingere, cu evoluție în ultimii 3 ani (fig. 4).

Fig. 4. Aspect preoperator

Tabloul biologic şi explorările paraclinice evidențiază un sindrom de citoliză hepatică toxică, cu trombocitopenie şi discretă alterare a coagulării.

Examenul clinic O.R.L. şi panendoscopia nu au evidențiat modificări patologice, formațiunea latero-cervicală fiind considerată astfel o localizare primară.

Ultrasonografia latero-cervicală precizează existența formațiunii tumorale hiperecogene, de 9/8/7 centimetri (cranio-caudal/antero-posterior/orizontal) situată posterior de muşchiul SCM pe care îl invadează şi îl împinge anterior cu efect de masa asupra pachetului vascular al gatului, cu semnal Doppler la interior.

Intraoperator se observă extensia formațiunii retroclaviculare şi se excizează în bloc cu lanțul ganglionar spinal (grupul V), parțial cu fibre din muşchiul SCM, disecându-se cu dificultate de clavicula şi vena subclavie (fig. 5, 6).

Evoluția favorabilă permite externarea la 5 zile postoperator.

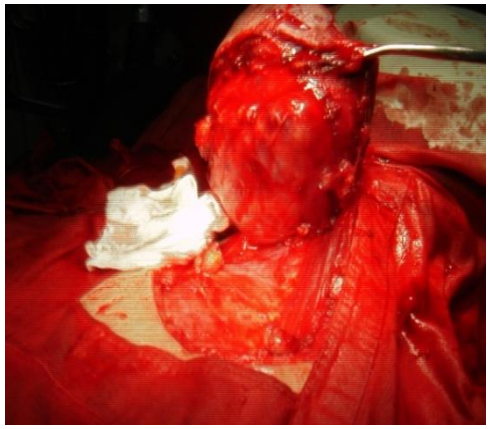

Fig. 5. Aspect intraoperator cu evidențierea muşchilor SCM şi trapez

Fig. 6. Piesa de excizie

Examenul histopatologic evidențiază existența unor „fragmente tumorale cu aspecte de sarcom cu grad înalt de malignitate şi plaje întinse de necroza cu hemoragii". Pentru a se stabili *pattern*-ul tumoral diferențiind un eventual NPNST de un alt sarcom se recomanda reacții imunohistochimice care însă, nu se pot efectua.

DISCUȚII

Deşi aproximativ 13% din tumorile lipomatoase apar la nivelul capului si gatului, majoritatea sunt superficiale şi alcătuite din țesut adipos separat de trabecule fibroase. În cazul în care proporția de țesut fibros crește, vorbim despre fibrolipom.

Uneori se întâmpla ca acest țesut adipos să „infiltreze" structurile musculare din jur (lipom intramuscular) şi, extrem de rar, pot apărea metaplazii osoase sau cartilaginoase (osteolipom, lipom condroid) (1, 2).

Sarcomul este o tumora mezenchimală şi reprezintă sub 1% din totalul neoplaziilor parților moi ale gâtului. (4)

Dificultățile de diagnostic apar datorită faptului că factorii care pledează pentru benignitate coexistă cu caracterele maligne (consistența dură, fixarea pe planurile profunde, evoluția lentă, lipsa unei „tumori primare" şi a modificărilor tabloului biologic).

Nici explorările imagistice, extrem de utile de obicei, în aceste situații nu aduc clarificări asupra tipului tumoral ci doar informații despre invazia de vecinătate.

În acest context, rolul exciziei-biopsie (în limite oncologice) şi a examenului histopatologic rămâne determinant în stabilirea unui diagnostic de certitudine (5).

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ALEXITHYMIA AND THE BIG FIVE PERSONALITY TRAITS AS PREDICTORS OF BURNOUT AMONG MEDICAL STUDENTS

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ALEXITHYMIA AND THE BIG FIVE PERSONALITY TRAITS AS PREDICTORS OF BURNOUT AMONG MEDICAL STUDENTS (Abstract): Professional exhaustion or burnout in health professionals has become a serious problem, as previous studies documented its effects on personal and professional life, such as a lower quality of care, reduced level of patient satisfaction, and problems with patient safety. **Aim:** Our study aimed to assess the influence of several psychological, demographic and academic characteristics on Romanian medical students' levels of burnout. **Material and methods:** Participants (315 students in general medicine) were administered three psychological instruments: Maslach Burnout Inventory, measuring the general level of burnout and its three dimensions: emotional exhaustion, depersonalization and personal accomplishment; Big Five Inventory, evaluating the Big Five Factors (dimensions) of personality: Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness; and Toronto Alexithymia Scale, assessing one's difficulties with emotional processing and emotional awareness on three dimensions: difficulty of describing feelings, difficulty of identifying feelings, and externally-oriented thinking. **Results** show that students with higher levels of Neuroticism also report higher levels of Emotional Exhaustion. Also, more extravert, conscientious, open, and less neurotic students experience a greater sense of Personal Accomplishment, while students low on Agreeableness score high on Depersonalization. Associations between the three facets of burnout and demographic and academic variables were also found. **Conclusions:** such psychological profiles of burnout vulnerability among medical students could inform future academic guidance programs designed to prevent burnout at younger stages of medical training. **Keywords**: BURNOUT, PERSONALITY TRAITS, ALEXITHYMIA, MEDICAL STUDENTS.

In order to improve the quality of the medical act, it is important to consider doctors' risk of professional exhaustion, which can hinder their work performance and quality of care towards their patients. The professional activity of doctors has been analyzed from different perspectives: professional, moral, social, ethical but also economic. In recent years, studies have shown that the level of professional exhaustion has effects on personal and professional life (increasing the rate of errors, dropping out of work, substance abuse, increasing suicide attempts). Generally, burnout is also correlated with less free time, risk of experiencing work-home con-

flicts or high level of depression (1).

Burnout in doctors has become a serious problem, many researchers highlighting the relationship between burnout and lower quality of care, reduced level of patient satisfaction, and problems with patient safety. The phenomenon of professional exhaustion in healthcare professionals has also become an economic problem analyzed through investments, costs and profits in health services. A more satisfied physician will provide better medical services and a more compassionate care for patients, which, in return, will reduce the total costs of care (2, 3).

Previous studies on Romanian physicians or other healthcare professionals showed a moderate level of burnout among different specializations but also identified relationships between burnout or job satisfaction and different variables such as socio-demographic variables, alexithymia or personality traits like neuroticism (4-8).

To develop the ability to cope with difficult situations it is necessary to have a good theoretical knowledge acquired during the university curriculum, but also to apply it in practice, both in the framework of the university training stages and during one's professional activity as a doctor. Some studies focusing on medical students from Romanian identified that freshman students are the most depressed and graduating students are the most stressed students, comparing to medical students from other years of study. Also, perceived stress and the level of alexithymia were found to influence the level of depression among medical students, with great impact on academic results (9, 10). The present study aims to assess the influence of several psychological, demographic and academic characteristics on Romanian medical students' levels of burnout.

MATERIAL AND METHODS

Participants

The study developed between June and October 2017. A number of 400 questionnaires were distributed among medical students from General medicine. Students had to complete a document with socio-demographic data and to answer the items of three psychological instruments. A number of 368 questionnaires were returned to the researchers. The exclusion criteria were: incomplete questionnaires and documents returned after 1st of October. After analyzing the returned questionnaires, 315 were finally included in the study.

Instruments

Several demographic and academic data were registered: age, sex, year of study (from one to six year of study), environment (urban/rural), department, admission mark, and information related to the accommodation in the University City (staying with parents or living by themselves in a rented apartment). One item questioned whether at least one of their parents is working abroad.

Three psychological instruments were administered: *Maslach Burnout Inventory* (MBI), *Big Five Inventory* (BFI) and *Toronto Alexithymia Scale* (TAS-20).

MBI (11) has 22 items and it was applied to measure the level of burnout referring to its three general scales: emotional exhaustion (EA), depersonalization (D) and personal accomplishment (PA). Emotional exhaustion evaluates feelings of being emotionally overextended and exhausted by work. Depersonalization refers to the cynic responses, negative attitudes and behaviors at work, in relationship with colleagues or beneficiaries. The Personal achievement scale evaluates one's feelings of competence and successful achievement

at work. A person is professionally exhausted if she/he has a high level of emotional exhaustion and depersonalization and a low level of professional accomplishment.

The second instrument, BFI (12), was used to evaluate the Big Five Factors (dimensions) of personality: Extraversion (E), Agreeableness (A), Conscientiousness (C), Neuroticism (N), and Openness (O). The questionnaire consists of 44 items divided into these five broad domains, each of them rated on a 7-point Likert scale. Neuroticism is characterized by individuals who are frequently worried, tense and fearful. Extraversion measures the tendency to be self-confident, active and sociable. Openness assesses being sensitive, artistic, and imaginative. Agreeableness is captured by aspects as being cooperative, friendly, and generous, while conscientiousness embodies efficiency, competence, and self-discipline.

The third instrument, TAS-20 (13), has three subscales measured using 20 items and a general score. The instrument assesses the difficulties with emotional processing and emotional awareness, respectively, on three domains: difficulty of describing feelings, difficulty of identifying feelings, and externally-oriented thinking. Students rated their answers on a Likert - scale, from 1 (strongly disagree) to 5 (strongly disagree).

Ethical approval

The study was approved by ethical committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi. Informed consent was delivered together with the documents. Students were informed about the purpose of the study, the confidentiality of collected data and the fact that they could withdraw from the research with no consequences.

Statistical analysis of data

Collected data were analyzed using IBM *SPSS Statistics, version 23*. Means and standard deviations revealed the levels of burnout dimensions among medical students. Pearson and partial correlations were used to investigate the relationships between variables, and hierarchical regression analyses were used to identify the significant predictors of burnout.

RESULTS

Demographic and educational data

All participants to the research (246 females and 69 males) were enrolled in general medicine studies in "Grigore T. Popa" University of Medicine and Pharmacy from Iasi. Their gender distribution corresponds to the gender proportion among the population of students in medicine in the university. Most of the participants are coming from urban areas in the north-eastern part of the country. Almost 40% declared that they are living in the University City or in suburbia. The average age is M = 22 ±3 (ranging from 18 to 27 years). 156 students were registered in preclinical (1st and 2nd) and 159 in clinical years of study (3rd to 6th).

The admission process in the university is based on a final mark (minimum 5 from 10), resulted from a written exam with multiple answers, covering items from different disciplines (biology, chemistry and physics). The mean admission mark for the researched sample is 9.01 (±.9). 59 (18%) of them reported having at least one parent working abroad and more than half (66%) reported living in a rented apartment or in campus.

Participants' demographic and educational characteristics are presented in first table.

TABLE I
Demographic and educational characteristics of the sample

Characteristic	Mean (±SD) N (%)
Age	22 (±3)
Gender – female	246 (74)
Year of study Pre-clinic	156 (47)
Clinic	159 (53)
Department - Iasi	125 (39)
Environment - urban	232 (71)
Admission mark	9.01 (±.9)
Having at least one parent working abroad	59 (18)
Living in a rented apartment or in campus	217 (66)

Psychological results

First, the relations between the psychological factors were examined through Pearson and partial correlations while controlling participants' demographic and educational characteristics (tab.II). Regarding the three dimensions of students' burnout, both types of statistical indexes show significant associations to most of the five factors of the BFI and to two of the alexithymia subscales.

Specifically, the pattern of associations suggests that students' emotional exhaustion is positively associated to their neuroticism, difficulty describing feelings and difficulty identifying feelings, and negatively related to their extraversion, agreeableness, conscientiousness and openness. In other words, the higher the score on neuroticism, the higher students` emotional exhaustion. Also, the more students have difficulties identifying and describing feelings, the higher they score on emotional exhaustion. On the other hand, students low on extraversion, agreeableness, conscientiousness and openness have high scores on emotional exhaustion.

Conversely, Personal Accomplishment emerged as having an opposite pattern of correlations, being positively related to these personality factors and negatively to neuroticism and participants' difficulty in identifying feelings. Thus, students who score high on extraversion, agreeableness, conscientiousness and openness also score high on Personal Accomplishment also. Likewise, students who have higher scores on neuroticism and difficulties in identifying feelings also score low on Personal Accomplishment.

Second, hierarchical regression analyses were used in order to examine for the personality and the alexithymia factors as predictors of burnout, while controlling for the effect of participants' demographic and educational characteristics. Each of the three dimensions of burnout was included as criterion in a different set of regression analyses. In each the demographic and educational characteristics were entered as predictors in Step 1, to assess and to control their effects on the criterion. The five personality factors and the three dimensions of alexithymia were entered in Step 2.

TABLE II

Descriptive statistics, reliability, Pearson and partial correlations between the MBI, BFI, and TAS-20 factors

		M and SD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
MBI	(1) EA	2.23 1.23	.85	-.12*	.52**	-.27**	-.27**	-.17**	.42**	-.20*	.23**	.42**	.03
	(2) PA	3.91 1.12	-.16**	.84	-.05	.24**	.21**	.28**	-.21**	.29**	-.11*	-.20**	.06
	(3) D	1.45 1.14	.50**	-.05	.73	-.25**	-.30**	-.19**	-.17*	-.12*	.18**	.27**	.03
BFI	(4) E	3.63 .69	-.23**	.23**	-.21**	.81	.27**	.26**	-.22**	.28**	-.32**	-.24**	-.03
	(5) A	3.62 .60	-.26**	.24**	-.28**	.23**	.70	.28**	-.46**	.25**	-.18**	-.37**	.01
	(6) C	3.48 .65	-.15*	.27**	-.14*	.21**	.26**	.78	-.17**	.23**	-.10	-.23**	.07
	(7) N	2.93 .72	.44**	-.27**	.20**	-.20**	-.50**	-.18**	.76	-.14**	.22**	.44**	.05
	(8) O	3.68 .58	-.20**	.28**	-.11	.24**	.25**	.22**	-.17**	.77	-.07	-.17**	.10
TAS-20	(9) Difficulty Describing Feelings	2.86 .73	.25**	-.08	.23**	-.34**	-.17**	-.07	.21**	-.06	.70	.49**	.22**
	(10) Difficulty Identifying Feelings	2.44 .91	.39**	-.19**	.33**	-.24**	-.43**	-.26**	.46**	-.17**	.46**	.83	.15**
	(11) Externally-Oriented Thinking	3.30 .52	-.01	.05	.05	-.04	.04	.03	.06	.08	.23**	.12*	.71

Notes: The upper triangle of the matrix shows Pearson correlation coefficients. The lower triangle of the matrix shows partial correlation coefficients controlling for age, gender, year of study, department, environment, admission mark, parents' migration and accommodation. The matrix's diagonal presents reliability coefficients (Cronbach's α). * p<.05; ** p<.001.

The first regression analysis tested the effect of these factors on Emotional Exhaustion (tab. III). The first model, including only the demographic and educational characteristics, significantly predicted this facet of burnout (p<.01). Two of these characteristics emerged as significant predictors (both ps <.05), specifically year of study, with students in their clinical years reporting less emotional exhaustion, and admission mark, with students who had higher admission marks reporting more intense emotional exhaustion. The second model also significantly predicted this facet of burnout. Two of the variables in this model emerged as significant and positive predictors, namely Neuroticism and Difficulty Identifying Feelings (both ps <.01). Specifically, increases in neuroticism and difficulty

identifying feelings are related with increases in emotional exhaustion. Students reporting high levels of neuroticism and experiencing difficulties identifying feelings tend to also report high levels of emotional exhaustion.

TABLE III

Results of hierarchical regression analysis showing amount of variance in Emotional Exhaustion accounted for by the five personality factors and the three dimensions of alexithymia (Step 2) while controlling for participants' demographic and educational characteristics

		Step 1	Step 2	
		B	*β*	ΔR^2
Socio-demographic data	Gender	.16	.001	
	Age	-.01	-.01	
	Study year	-.58**	-.16*	
	Department	-.24	-.03	
	Environment	-.04	-.01	
	Admission mark	.23*	.12*	
	Migration	-.21	-.03	
	Accommodation	.15	.06	
				.07**
BFI	Extraversion		-.09	
	Agreeableness		.04	
	Conscientiousness		-.01	
	Neuroticism		.31**	
	Openness		-.09	
TAS-20	Difficulty Describing Feelings		.07	
	Difficulty Identifying Feelings		.20**	
	Externally-Oriented Thinking		-.06	
				.25**

Note: *p <.05; ** p<.01

The second regression analysis included Personal Accomplishment as criterion (tab. IV). The first model, including only the demographic and educational characteristics, significantly predicted this facet of burnout (p<.01). In this set, gender (with females having higher self-assessments on this facet of burnout) and age (with older students having higher means) yielded the significance level as predictors. The second model also significantly predicted Personal Accomplishment. Three personality factors in this model emerged as significant and positive predictors, namely Extraversion, Conscientiousness and Openness, while Neuroticism emerged as a negative predictor of Personal Accomplishment (all ps<.05). Specifically, increases in Extraversion, Conscientiousness and Openness and decreases in Neuroticism are related to increases in Personal Accomplishment. Students who are more extroverted, conscientious, and open, while also scoring low on neuroticism, report feeling accomplished on personal issues.

TABLE IV

Results of hierarchical regression analysis showing amount of variance in Personal Accomplishment accounted for by the five personality factors and the three dimensions of alexithymia (Step 2) while controlling for participants' demographic and educational characteristics

		Step 1	Step 2	
		B	*β*	ΔR^2
Socio-demographic data	Gender	.32*	.11*	
	Age	.06*	.12*	
	Study year	.11	.04	
	Department	.12	-.02	
	Environment	.13	.04	
	Admission mark	-.05	.003	
	Migration	-.12	-.07	
	Accommodation	.01	.007	
				.06*
BFI	Extraversion		.12*	
	Agreeableness		.04	
	Conscientiousness		.18**	
	Neuroticism		-.17*	
	Openness		.16**	
TAS-20	Difficulty Describing Feelings		.01	
	Difficulty Identifying Feelings		.04	
	Externally-Oriented Thinking		.06	
				.17**

Note: *p <.05; ** p<.01

The third regression analysis included Depersonalization as criterion (tab. V). The first model, including only the demographic and educational characteristics, significantly predicted this facet of burnout (p<.01). Three characteristics in this set gender emerged as significant predictors (all ps<.05), specifically gender, environment and migration of parents. Results show that females, students from urban environments and those who have at least one parent abroad have lower self-assessments on this facet of burnout, although this last variable did not yield the significance level in the second step of the analysis, in which the psychological traits were also introduced.

The second model also significantly predicted Depersonalization. Two factors in this model emerged as significant predictors, specifically Agreeableness, which is negatively related to Depersonalization, and Difficulty Identifying Feelings, which emerged as positive predictor of Depersonalization (both ps<.01). More explicitly, increases in Difficulty Identifying Feelings and decreases in Agreeableness are related with increases in Depersonalization. Students who have problems with identifying feelings and are less agreeable tend to report high levels of depersonalization.

TABLE V

Results of hierarchical regression analysis showing amount of variance in Depersonalization accounted for by the five personality factors and the three dimensions of alexithymia (Step 2) while controlling for participants' demographic and educational characteristics

		Step 1	Step 2	
		B	*β*	ΔR^2
Socio-demographic data	Gender	-.47**	-.19**	
	Age	-.005	.02	
	Study year	-.07	-.01	
	Department	-.05	-.02	
	Environment	-.35*	-.14*	
	Admission mark	.17	.11	
	Migration	-.39*	-.09	
	Accommodation	.14	.05	
				.09**
BFI	Extraversion		-.09	
	Agreeableness		-.15*	
	Conscientiousness		-.02	
	Neuroticism		-.001	
	Openness		-.008	
TAS-20	Difficulty Describing Feelings		.08	
	Difficulty Identifying Feelings		.20**	
	Externally-Oriented Thinking		-.005	
				.13**

Note: *p <.05; ** p<.01

DISCUSSION

Burnout affects medical students from the first years (14) to the final years of study (15) and beyond (16), with prevalence rates ranging from 7.0% to 75.2% (17). Furthermore, emotional exhaustion has been shown to be strongly associated with risk of psychiatric morbidity (18). Rates of emotional exhaustion and depersonalization also appear to rise significantly during the graduate's intern year in the public hospital system (18).

Besides involving health professionals to provide psychological support to burnout sufferers, institutional involvement has also become a way to prevent or cure burnout in physicians, which emphases the need to have a deep understanding of the mechanisms and factors that predispose medical personnel to burnout. The aim of our study was to examine the factors of burnout in medical students in a Romanian University. Specifically, we tested whether personality (five factors: Extraversion, Conscientiousness, Openness, Agreeableness, Neuroticism) and alexithymia (Difficulty Describing Feelings, Difficulty Identifying Feelings, Externally-Oriented Thinking) predict burnout (EE, PA, D). We found significant predictors for each of the three burnout dimensions.

Concerning personality factors, the results show that students with higher levels of Neuroticism also report higher levels of EE. Also, more extravert, conscientious,

open, and less neurotic students experience a greater sense of Personal Accomplishment. Last, but not least, students low on Agreeableness score high on Depersonalization.

A study on Japanese nursing students (19) found some correlations supporting the idea of an association between personality factors and burnout. In their research, Neuroticism was positively, and Agreeableness, Conscientiousness, and Extraversion was negatively correlated with Emotional Exhaustion. Extraversion, Conscientiousness, and Agreeableness was negatively, and Neuroticism was positively correlated with Depersonalization. Extraversion, Openness, and Agreeableness was positively, and Neuroticism negatively correlated with PA.

The findings of the present study are consistent with a study by McManus *et al*. (20) who also showed, among others, that Emotional Exhaustion is related to Neuroticism, Personal Accomplishment is related to Extraversion, and Depersonalization is related to Agreeableness, although their study focused on doctors aged 29 and 30. Also, a research of Magnano *et al*. showed that emotional stability negatively predicted exhaustion in health professionals (21).

Our results are also partially supporting those of Cañadas-De la Fuente (22), who focused on nurses. Specifically, these authors found Neuroticism, Agreeableness, and Extraversion to predict Emotional Exhaustion, while we found only Neuroticism to be linked to Emotional Exhaustion. Also, our results showed Agreeableness to be predictive of Depersonalization, while their study also added Neuroticism and Conscientiousness as predictors of this dimension of burnout. Additionally, the authors found all five dimensions to be predictors of PA, while we found support for four of them (Extraversion, Conscientiousness, Openness, and Neuroticism).

Furthermore, in a study by Bakker *et al*. on volunteer counselors, emotional stability predicted EE (23), a relationship that also emerged in our study; emotional stability, Extraversion, and intellect/autonomy predicted Depersonalization (in our study, only agreeableness negatively predicted Depersonalization); Extraversion and emotional stability predicted PA (as in our study).

However, not all studies found a link between the five personality factors and burnout. For example, (18) showed that Extraversion, Agreeableness, and Consciousness did not significantly predict any burnout factor.

Nevertheless, personality seems to relate consistently to all dimensions of burnout. Neuroticism and Extraversion appear to be consistent predictors of burnout (22-25). Some traits of people high in Neuroticism (the predisposition to underestimate self-performance and to respond with strong emotions in stressful situations) seem to lead to symptoms of burnout. The predilection of extraverts to join intense personal interactions may help them prevent burnout. Conscientiousness is associated with a person's perseverance and self-discipline to finish a task and, as a result, that person could also experience higher levels of PA (23). Individuals with high scores on Openness tend to view challenges as opportunities and are inclined to learn and, thus, feel more personally accomplished. Agreeable individuals have lower levels of EE and Depersonalization probably because they are more likely to be caring, nurturing, responsible, and sympathetic (22).

Concerning alexithymia, our results show an association with burnout. Specifically, students reporting higher levels of Difficulties Identifying Feelings also have high levels of Emotional Exhaustion and Depersonalization. The results of other studies point to an association between Alexithymia and burnout in the medical

field. Thus, Bratis *et al.* (26) found a positive correlation with Emotional Exhaustion and Depersonalization and a negative one with personal achievement. Also, Katsifaraki *et al.* (27) found a significant association between Externally Oriented Thinking (a facet of alexithymia) and PA and Depersonalization. Additionally, Popa-Velea *et al.* (28) found alexithymia to have a significant role in the development of burnout syndrome, with a prevalence of 6.02% in a sample of Romanian medical students. Researchers should focus on the impact of alexithymia and its possible connections with various psychological problems and its relationship with stress and depression during medical studies (29).

This study has some limitations that should be taken into consideration when interpreting the results. A cross-sectional survey design was utilized, and longitudinal data will be necessary to establish causality among the variables considered. Also, it included a relatively small number of participants and the context was restricted to a university, thus the generalizability of the statistically significant associations that emerged is limited. However, this study offers some valuable insights into burnout among medical students.

CONCLUSIONS

Overall, this study adds to the identification of personality factors and alexithymia associated with burnout among medical students in Romania and confirms the role they play in the experience of burnout. Our findings highlight the need for academic guidance programs to prevent burnout at younger stages of medical training. We could also take into consideration personality profiling with the aim of identifying resources to prevent burnout among medical students with vulnerable personality traits.

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EVALUĂRI PRIVIND UNELE INVESTIGAŢII DE LABORATOR, IMAGISTICE ŞI ANATOMO-PATOLOGICE PE UN LOT DE 104 PACIENTE DIAGNOSTICATE CU TUMORI MAMARE ŞI ÎNSCRISE ÎN LISTA MEDICULUI DE FAMILIE, DIN MUNICIPIUL IAŞI

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ASSESSMENT OF SOME LABORATORY, IMAGING AND ANATOMICAL-PATHOLOGICAL INVESTIGATIONS ON A LOT OF 104 PATIENTS DIAGNOSED WITH BREAST CANCER AND APPEAR IN THE LIST OF FAMILY DOCTOR FROM THE CITY OF IASI (Abstract) : **The aim** of study was to assess some investigations in female patients with benign and malignant breast diseases. **Material and methods** : The study was performed on a sample of 104 patients registered with breast tumors, at family physicians' offices from Iasi, Romania. **Results :** Impaired glucose tolerance was associated with 34.2% of patient with breast tumors, and hypercholesterolemia with 25.9% of them. Anatomo-pathological assessments included aspiration puncture and biopsy, extemporaneous biopsy, and paraffin-embedded exam. Invasive carcinoma was the most frequent histo-pathological type (42.1%). **Conclusions** Further investigations on tumor markers are needed in all patients with in situ neoplasia and breast carcinomas. **Key words** : BENIGN BREAST DISEASE, BREAST CANCER, BIOCHEMICAL AND HISTO-PATHOLOGICAL EXAMINATIONS, RISK FACTORS

La nivel mondial, cancerul mamar este principala cauză de deces la femeile de peste 40 de ani, cu o rată a incidenţei de două ori mai mare decât cea a cancerului de col uterin şi colorectal. În 2008, maladia a cauzat aprox. 800.000 decese mondial – 8% din toate decesele prin cancer şi aprox. 1% din decese prin toate cauzele (1,2). Se apreciază că între 6 şi 10% din totalul femeilor vor dezvolta boala cancer de sân şi 1 din 4 femei suferinde de cancer are, drept localizare a bolii, glanda mamară.

În ultimii ani s-a remarcat o scădere a ratei mortalităţii prin cancer mamar, ajungându-se la aproximativ 25%ooo. Această tendinţă se poate datora diagnosticării cancerului mamar în stadii din ce în ce mai precoce - prin metode de screening aplicate populaţional, în special prin mamografie (3,4,5).

MATERIAL ŞI METODĂ

Studiul a fost realizat pe un lot de 104 paciente diagnosticate cu formaţiuni mamare şi înscrise la cabinete individuale de medicină de familie, din Iaşi. Au fost luate în consideraţie investigaţiile de laborator (glicemie, colesterol total), imagistice (ecografia mamară, mamografia) şi anatomopatologice (examenul citologic al frotiului obţinut prin puncţie mamară, biopsia extemporanee). Datele au fost culese retrospectiv din fişa individuală a fiecărei paciente şi ulterior prelucrate statistic.

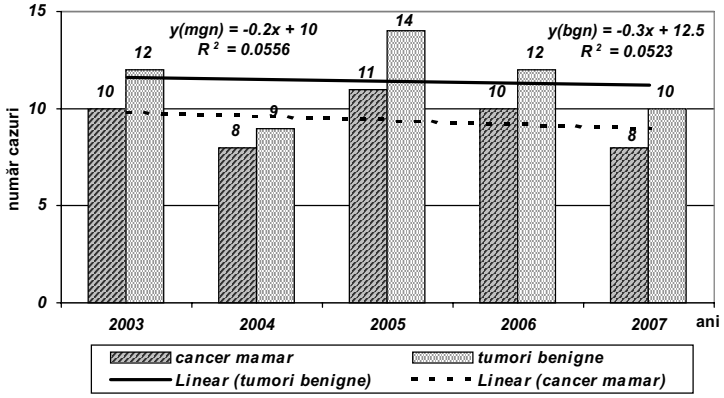

Fig. 1. Repartiţia anuală a numărului de cazuri de tumori mamare

REZULTATE ŞI DISCUŢII

1. Distribuţia anuală a cazurilor de tumori mamare, atât maligne (44,2% din total), cât şi benigne (55,8%), arată o tendinţă descrescătoare, fapt care poate fi explicat prin creşterea adresabilităţii la medicul de familie, dar şi îmbunătăţirea mijloacelor de diagnostic (fig. 1).

Derularea programelor de screening în tumorile mamare, în ultima perioadă, a conştientizat populaţia feminină de riscul apariţiei unei tumori mamare, crescând astfel şi adresabilitatea femeilor la medicul de familie, în vederea aplicării unei metode de screening (5, 6, 7).

2. Repartiţia pe grupuri de vârstă. Cele mai multe cazuri de tumori benigne (25,0%) au fost înregistrate la grupul 36-45 ani, iar de tumori maligne (25,9%, $p > 0,05$), la femeile între 45-65 ani, grup cu numeroşi factori de risc, cumulaţi pe parcursul existenţei (fig. 2).

3. Repartiţia pe categorii profesionale a arătat că, în lotul de paciente luat în studiu, cele mai multe cazuri au fost întâlnite la femeile încă în activitate profesională şi la profesii care ar cumula factori de risc precum sedentarismul (funcţionare), stresul ocupaţional (cadre medicale şi didactice), pauciparitatea (femei angajate – 39 cazuri *versus* casnice – 3 cazuri) (fig. 3).

4. Examene biochimice. Evoluţia de lungă durată a diabetului zaharat, asociată cu modificări la nivelul glandei mamare, poate fi luată în consideraţie ca factor etiopatogenic în tumorile sânului. În lotul studiat, 34,2% dintre femei au prezentat *scăderea toleranţei la glucoză*, iar 11,5% diabet zaharat tip II. S-a constatat că în 27 cazuri (25,9%), *hipercolesterolemia* a fost asociată cu cancerul de sân (fig. 4). Această asociere poate fi explicată prin factori de risc comuni (consumul crescut de grăsimi animale, sedentarismul, stresul). Trebuie luat în consideraţie faptul că, biochimic, colesterolul este precursorul hormonilor sexuali, iar o creştere a acestuia conduce la dezechilibre ale balanţei estrogeni-progesteron şi, deci, la hiperestrogenemie, factor de risc important în etiopatogenia tumorală mamară (3,4,9). Corelaţia între categoria profesională (angajate / neangajate) şi hipercolesterolemie nu a fost găsită semnificativă statistic ($p > 0,05$, coeficient de corelaţie 0,36; dispersia între cele două teste – 12,3%); un studiu pe loturi mai mari ar putea conduce la rezultate valide fiziopatologic.

5. Unele explorări paraclinice. Pacientele luate în studiu au beneficiat de ecografie mamară (23,1% paciente diagnosticate cu cancer mamar) şi mamografie (44,2%). La

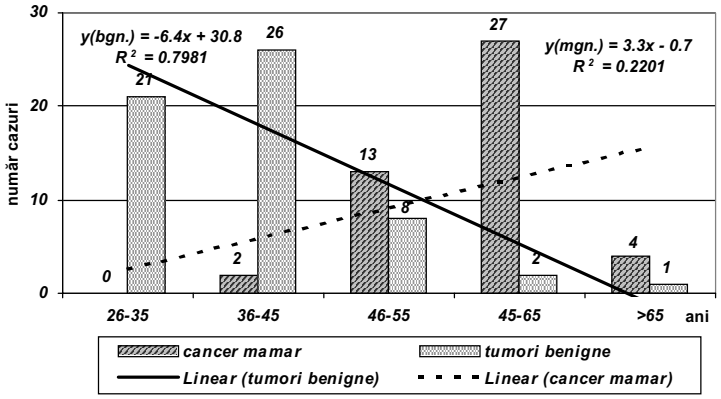

Fig. 2. Repartiţia pe grupuri de vârstă

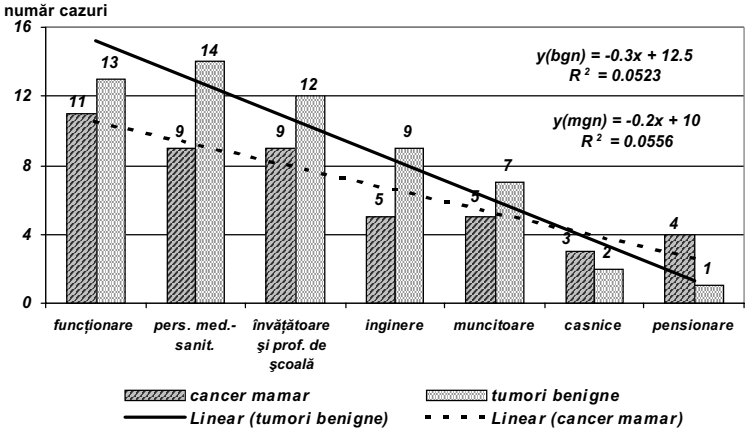

Fig. 3. Repartiţia pe categorii ocupaţionale

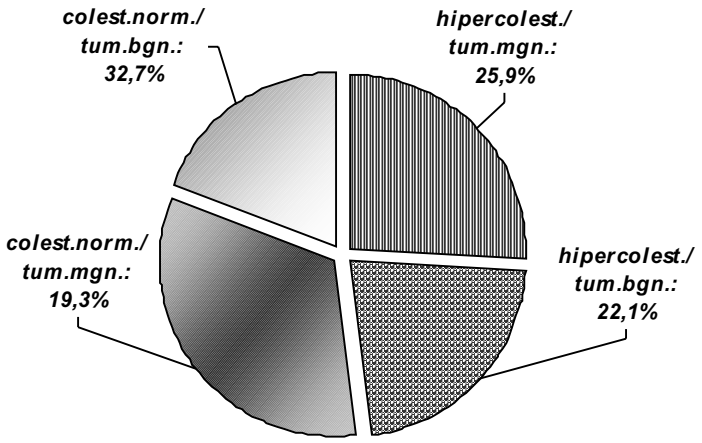

Fig. 4. Relaţia între nivelul colesterolemiei şi tipul tumoral

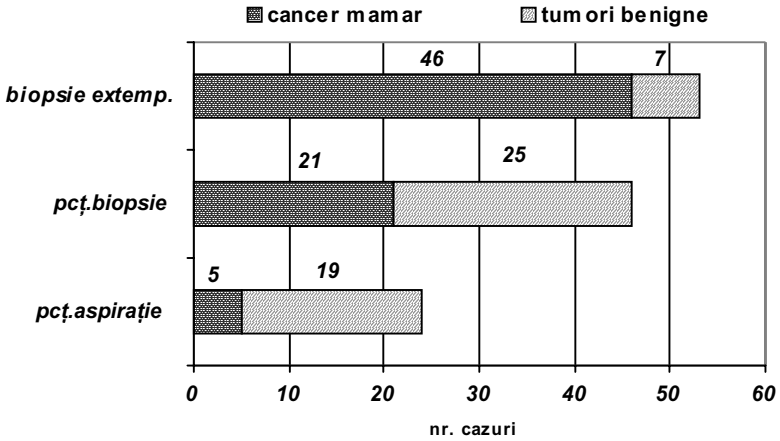

Fig. 5. Rezultatele puncţiilor şi biopsiei

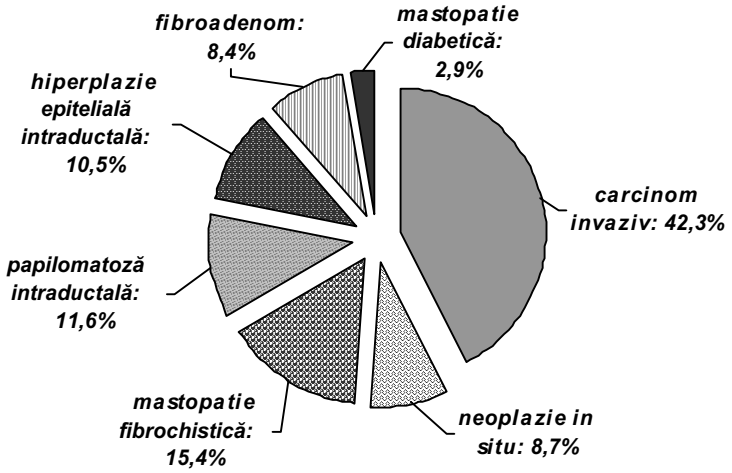

Fig. 6. Rezultatele examenului anatomopatologic

biopsia extemporanee, au fost decelate 46 (44,2%) cazuri de tumori mamare maligne şi doar 7 (6,7%) benigne. *Puncţia biopsie* a fost utilizată în special pentru tumorile benigne (24,1%), la fel ca şi puncţia aspiraţie (18,2%) (fig. 5).

Examenul anatomopatologic (extemporaneu şi / sau după includerea la parafină) a evidenţiat (din buletinele anatomo-patologice au fost selectate unele elemente mai importante de diagnostic) că tipul histopatologic de carcinom invaziv a fost prezent în 44 cazuri (42,1%) (fig. 6), rezultate similare cu cele publicate în studii de anatomie patologică a tumorilor de sân (3, 4, 8, 9, 10).

CONCLUZII

Studiul descriptiv efectuat a cuprins 104 paciente aflate în listele medicilor de familie din cabinete din Iaşi, diagnosticate cu tumori mamare. Distribuţia anuală a cazurilor de tumori mamare, atât maligne, cât şi benigne, arată faptul că, în ultimul deceniu, a crescut numărul de cazuri diagnosticate cu cancer de sân. Repartiţia pe grupuri de vârstă a cazurilor de tumori maligne arată faptul că

cele mai multe au fost diagnosticate la grupul de vârstă 45-65 ani, grup cu numeroşi factori de risc pentru cancerul de sân.

Ca investigaţii paraclinice au fost menţionate : evaluarea glicemiei, a colesterolului total seric, evaluări imagistice prin ecografie mamară şi mamografie, evaluări anatomo-patologice prin puncţie aspiraţie, biopsie extemporanee şi examenul la parafină. Carcinomul invaziv a fost cea mai frecventă forma histopatologică întâlnită în lotul studiat.

De asemenea, evaluarea prin identificarea markerilor tumorali este necesară la toate pacientele cu neoplazie *in situ* şi cancer mamar.

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THE INFLUENCE OF PREMATURE UNILATERAL LOSS OF MANDIBULAR DECIDUOUS MOLARS ON CONDYLAR AND RAMUS VERTICAL SYMMETRY

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THE INFLUENCE OF PREMATURE UNILATERAL LOSS OF MANDIBULAR DECIDUOUS MOLARS ON CONDYLAR AND RAMUS VERTICAL SYMMETRY (Abstract): **Aim**: This study evaluates the influence of premature unilateral loss of mandibular deciduous molars on condylar and ramus vertical symmetry, on digital orthopantograms of a sample of Romanian patients. **Material and methods:** The retrospective study was performed on 200 patients (128 boys and 72 girls), between 6-9 years old, during 2010-2015, in the Clinic of Pediatric Dentistry Ambulatory of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, Romania. The digitally measurements were made with Planmeca Romexis Viewer 3.0.1®, according method to a known method. **Results:** Statistically significant differences were found between the control group (without loss of deciduous molars) and the study group (with premature unilateral loss of mandibular deciduous molars) for mean values of the condylar asymmetry index (p=0.000), which were over 5-7%, indicating the condylar asymmetry. **Conclusions:** The premature unilateral loss of the mandibular deciduous molars is a risk factor for the condylar symmetry. **Keywords**: PREMATURE UNILATERAL LOSS, MANDIBULAR DECIDUOUS MOLARS, DIGITAL ORTHOPANTOGRAMS, CONDYLAR ASYMMETRY.

The premature loss of mandibular deciduous teeth is a consequence of untreated carious lesions and lead to three-dimensional disorders of the dental arch and occlusion. Thus, there may appear local modifications (delays or accelerations of permanent teeth eruptions), intra-arch modifications (migration of permanent teeth with loss of space, arch shrinking and crowding) and inter-arch modifications (super-eruptions of the opposite teeth, crossbites, premature contacts and occlusal interferences) (1, 2, 3 and 4).

During the first decade of life, the mandibular condyles become less vascularized and the major morphological changes are completed. Intra-articular modifications in mixed dentition period are unclear. Some studies have shown that dental occlusion is adjusted very rapidly in growing children and there may be no influence on condylar morphology or temporo-mandibular joint (5, 6). However, a recent study reported that the unilateral loss of the mandibular permanent first molar before the age of 12 would have an influence on the mandibular

condylar symmetry, but without clinical importance (7).

Recently, studies of condylar asymmetry associated with malocclusions have increased (8, 9, 10), but no study has been conducted to evaluate the association with premature unilateral loss of mandibular deciduous molar. The objective of present study is to investigate whether the premature unilateral loss of mandibular deciduous molars has an influence on the symmetry of the condyles and the ramus vertical of the mandible. The purpose of our study is to establish the effects of premature unilateral loss of mandibular deciduous molars on the height of the condyles and the vertical ramus of mandible, using the method described by Habets *et al.* (11), on digital orthopantograms (OPGs) of a sample of Romanian patients with early mixed dentition.

MATERIAL AND METHODS

The retrospective study was carried out on the digital OPGs of 200 patients aged between 6-9 years, who asked for paedodontic and orthodontic treatment during 2010-2015 period, in the Clinic of Pediatric Dentistry Ambulatory of the "Sf. Spiridon" County Clinical Emergency Hospital in Iasi, Romania. The informed consent was acquired from all patients or their parents.

The patients included in this study had early mixed dentition and no carious lesions on permanent incisors and first molars and no previous orthodontic treatment. Patients with syndromes, cranio-facial or neuro-muscular malformations, facial asymmetries and dental fillings were excluded.

The patients were divided into two groups: 64 (32%) patients without deciduous teeth absent in the lateral area and no signs or symptoms of temporo-mandibular dysfunction (TMD), as control group; 136 (68%) patients with at least one premature loss of a mandibular deciduous molar, as study group. The study group was divided into subgroups, depending on the premature unilateral loss of mandibular deciduous molars: subgroup 1 consisting of 24 (12%) patients with the premature loss of mandibular deciduous second molar on the right side; subgroup 2 consisting of 18 (9%) patients with the premature loss of mandibular deciduous first molar on the right side; subgroup 3 consisting of 14 (7%) patients with the premature loss of mandibular deciduous first and second molars on the right side; subgroup 4 consisting of 22 (11%) patients with the premature loss of mandibular deciduous second molar on the left side; subgroup 5 consisting of 44 (22%) patients with the premature loss of mandibular deciduous first molar on the left side; subgroup 6 consisting of 14 (7%) patients with the premature loss of mandibular deciduous first and second molars on the left side.

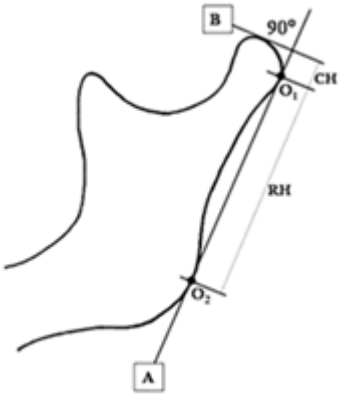
*Measurements made on digital OPGs***.** At the moment of exposure, the head of patient were positioned on to the chin support with relaxed lips and with the Frankfort plane parallel with the horizontal plane, according to Azevedo *et al*. (12). All selected OPGs were made within a single radiological center, using the radiological device PlanmecaProMax®2D, to be saved afterwards in electronic format. Digital OPGs which presented the bilateral radiological anatomical features of the jaws, including the head of the condylar mandibular and no artifacts were included.

The measurements were made digitally by a single examiner, using the Planmeca

Romexis Viewer 3.0.1® software, after the proper calibration. All measurements were made in millimeters (mm), according method described by Habets *et al.* (11) (fig. 1). For the calculation of the condylar asymmetry index (CAI), the ramus vertical asymmetry index (RAI) and the condyle and ramus vertical asymmetry index (CRAI), the formulas suggested by the same authors ware used:

$$CAI = \frac{CHr - CHl}{CHr + CHl} \times 100; \quad RAI = \frac{RHr - RHl}{RHr + RHl} \times 100; \quad CRAI = \frac{CRHr - CRHl}{CRHr + CRHl} \times 100;$$

where CHr/l – condylar height right/left;
RHr/l– ramus height right/left;
CRHr/l(CHr/l+RHr/l) –condylar and ramus height right/left.



O_1– the most lateral point on the mandibular condyle
O_2 – the most lateral point on the mandibular ramus vertical
line A – tangent to the most lateral points of the mandibular condyle (O_1) and ramus vertical (O_2)
line B – perpendicular to line A, tangential to the highest point of the mandibular condyle
CH – condylar height, that correspond to the distance measured between the line B to the point O_1
RH – ramus height, that correspond to the distance measured between the points O_1 and O_2

Fig. 1. The measurement method described by Habets *et al*. (11)

*Statistical analysis***.** The software used for the data analysis was *SPSS 20.0* (Chicago, Illinois, USA), for Windows. We calculated the frequency and descriptive parameters – mean value, standard error, standard deviation (SD), minimum and maximum value, for each group. The *t*-paired test was used to find statistically significant differences between the right and left sides for the measurements of the CH, RHand CRH in the control group and the subgroups. The *t*-student test was used to verify whether there are significant differences of the calculated indices (CAI, RAI and CRAI) for the groups and subgroups, since their values comply with the normal law. The normal law was checked with the Kolmogorov-Smirnov test.

In order to identify the possible errors during measurements, two weeks after the first measurements, 30 OPGs were selected for which the measurements were repeated by the same author. The error values as per Dahlberg's method (13) were within acceptable limits and the difference between the first and second measurements was insignificant. The difference between the two measurements was tested for signifi-

cance with *t*-paired test and no statistically significant difference was identified between the two measurements ($p>0.05$).

RESULTS

Out of the 200 analyzed OPGs, 128 (64%) belong to boys and 72 (36%) to girls, with the mean age of 7.53±0.98 years (tab. I).

No statistically significant difference was founded between the right and left side of mean values measurements of the heights of mandibular condyle and ramus vertical in the control group and subgroups with premature unilateral loss of mandibular deciduous molars on the right/left side ($p>0.05$), but the mean values are more decreased on the side with premature lost molars than on the normal side (tab. II).

TABLE I

Characteristics of patients' groups

Groups	Sex	N	Age	
			Mean	SD
Control group	male	38	7.74	1.14
	female	26	7.85	1.06
	total	64	7.78	1.09
Study group	male	90	7.38	0.85
	female	46	7.48	1.02
	total	136	7.41	0.91
Total		200	7.53	0.98

TABLE II

Statistical comparison of right and left side's measurements by groups

Groups	Measurements	Right		Left		p value
		Mean (mm)	SD	Mean (mm)	SD	
Control group	CH	3.87	1.00	3.86	0.90	0.765
	RH	33.93	3.00	33.65	2.80	0.084
	CRH	37.81	3.20	37.51	3.00	0.055
Subgroup 1, 2 and 3	CH	3.65	0.90	3.67	0.70	0.649
	RH	32.57	3.10	32.80	3.20	0.065
	CRH	36.22	3.30	36.47	3.30	0.067
Subgroup 4, 5 and 6	CH	3.70	0.90	3.68	0.98	0.785
	RH	33.02	2.60	32.70	2.32	0.076
	CRH	36.72	3.10	36.38	2.90	0.052

Statistically significant differences we found between the control group and the study group for mean values of the condylar asymmetry index (CAI)(p=0.000) (tab. III).

Also, statistically significant differences we found between the control group and 1 and 4 with premature loss of mandibular deciduous second molar on the right/left side subgroups, as well as 3 and 6 with premature loss of mandibular deciduous first and second molar on the right/left side subgroups, for mean values of the condylar asymmetry index (CAI)(p=0.000) (tab. IV).

TABLE III

Statistical comparison of calculated indices by groups

Indices	Groups	N	Mean	SD	*p* value
CAI	Control group	64	3.60	3.29	0.000*
	Study group	136	5.66	3.65	
RAI	Control group	64	1.53	1.30	0.371
	Study group	136	1.70	1.22	
CRAI	Control croup	64	1.32	1.09	0.401
	Study group	136	1.46	1.13	
*statistically significant differences when p<0.05					

TABLE IV

Statistical comparison of calculated indices by subgroups

Indices	Groups	N	Mean	SD	*p* value
CAI	Control group	64	3.60	3.68	
	1 and 4 subgroups	46	6.75	3.34	0.000*
	2 and 5 subgroups	62	4.07	3.15	0.442
	3 and 6 subgroups	28	7.40	1.41	0.000*
RAI	Control group	64	1.53	1.22	
	1 and 4 subgroups	46	1.69	1.42	0.452
	2 and 5 subgroups	62	1.63	1.17	0.702
	3 and 6 subgroups	28	1.86	1.42	0.259
CRAI	Control group	64	1.32	1.14	
	1 and 4 subgroups	46	1.55	1.06	0.295
	2 and 5 subgroups	62	1.30	1.25	0.920
	3 and 6 subgroups	28	1.55	0.67	0.334
*statistically significant differences when p<0.05					

DISCUSSION

Although the computed tomography (CT) and cone beam computed tomography (CBCT) have progressively become a top method used in many comparative studies (14, 15, 16), the OPG still represents a screening method for children aged 6-12 years (17). OPGs have been used in many studies which analyzed mandibular (18, 19) and condylar asymmetries (8, 9, 10), as a risk factor for TMD. Kambylafkas *et al.* (20) found the OPG was not very sensitive in the diagnosis of height total mandibular asymmetries, suggesting can be used to evaluate the posterior mandibular vertical asymmetry. Reproducibility of vertical and angular measurements on OPG is acceptable, if during exposure the patient's head is correctly positioned in the horizontal plane (21). The impossibility of positioning the head's mid-sagittal plane on the median rotation line of the device will lead to a distorted image on the horizontal plane (8, 20). Fuentes *et al.* (21) reported that Habets' technique did not show any statistically significant differences in the x-rays at 5° and 10° horizontal angles compared to the 0º angle.

The specialized software used in this study had the advantage of accurately iden-

tifying bone structures, radiographic landmarks, and measurements, due to the zoom and image contrast editing options. The correctness of the digital measurements was verified by the *t*-paired test.

We used the method of Habets *et al*. (11) to measure the height of condyles and the ramus vertical (CH, RH, CRH) on right/left side and to calculate asymmetry indices (CAI, RAI, CRAI), because was shown the reliability (22), although there is no consensus with respect to the utility of the different techniques used to measure mandibular asymmetries (18). Comparison of the mean values of these right/left measurements was not statistically significant in the studied groups, but more decreased average values were observed in the side of the lost mandibular deciduous molars. For control group in importance the variability of the mandibular constitutional, the masticator type, the mandibular dynamics typology and the differentiated preferences of children regarding food consistency. The loss of the unilateral occlusal support of mandibular deciduous molars leads to a displacement upwards-downwards of the condyle in the glenoid fossa, lead progressively to a different growth of the right condyle compared to the left one (23).

By the comparison the values of the calculated indices, we found significant differences in the mean values of the condylar asymmetry index (CAI) between the groups, which shows that the premature unilateral loss of mandibular deciduous molars determines an asymmetry of mandibular condyles. Also, we found significant differences for the condylar asymmetry index between the control group and subgroups with the premature loss of mandibular deciduous second molar on the right/left side and with premature loss of mandibular deciduous first and second molars on the right/left side. These results are the most important of our research and are not found in previous studies. Condylar asymmetry determined by comparing the vertical condylar height between the left and right condyle can be considered a risk factor for the development of a TMD (24, 25).

According to Habets *et al*. (11) a relation of asymmetry index (CAI, RAI, CRAI) of 3% appear to a change of up to 1cm of the position of the head during the OPG expose (25). Thus, the asymmetry index with an exceeded value of 3% should be considered as being vertical asymmetry (23). In the control group, the mean values of the condyle asymmetry index were over 3%, probably due to occlusal disorders. These results are comparable with those obtained in other studies performed on patients with normal occlusion (8, 9). In the study group the mean values of the condyle asymmetry index (over 5-7%) were different from the results obtained in other studies performed on patients with malocclusions (8, 15).

The mean values of the ramus vertical asymmetry index (RAI) and the condyle and ramus vertical asymmetry index (CRAI) were not statistically significant, indicating that symmetry was not affected by the unilateral absence of mandibular deciduous molars.

Similar future studies may extend to premature unilateral loss of maxillary deciduous molars or use of CT and CBCT measurements. Clinical implications of premature unilateral loss of deciduous molars may be reversible, if intervening on time with interceptive treatment for dental arch and occlusion rehabilitation.

CONCLUSIONS

Our research has shown that there may be other new risk factors for the condylar

symmetry compared to previous studies, clinical and therapeutic significance for children and practitioners. The premature unilateral loss of the mandibular deciduous molars can influence the symmetry of condyles. The mean values of the condyle asymmetry index were statistically significantly different between the control group and the study group with premature unilateral loss of deciduous mandibular molars, and the percentages were over 3% to be interpreted as condylar asymmetry.

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NEWS

IT'S ALL IN THE EYES: ASSOCIATION BETWEEN RETINAL NERVE FIBER LAYER THICKNESS AND RISK OF COGNITIVE DECLINE

The global burden of people with neurodegenerative pathology is increasing and this is why there is a great desire to discover the perfect screening tool for cognitive decline. A group of researchers used the retina as "mirror image" of the central nervous system and conducted a study on 32 038 UK residents aged 40 to 69 who underwent cognitive testing and optical coherence tomography imaging. They discovered that participants with a thinner retinal nerve fiber layer (RNFL) were more likely to fail at least 1 or more of the 4 cognitive tests compared to those who had a RNFL of at least 60.2μm. Furthermore, a repeated assessment of 1251 participants after 3 years showed that those with a thinner baseline RNFL did worse on at least 1 follow up cognitive test. This is a very exciting study because it opens new directions for research in the prevention and treatment of dementia and it is the largest one of this kind so far. (Ko F, Muthy ZA, Gallacher J, *et al*. Association of Retinal Nerve Fiber Layer Thinning with Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. *JAMA Neurol* 2018; 75(10): 1198-1205).

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IMMUNOCHEMICAL ASSESSMENT OF P16 AND HPV L1 CAPSID PROTEIN IN CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS

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IMMUNOCHEMICAL ASSESSMENT OF p16 AND HPV L1 CAPSID PROTEIN IN CERVICAL INTRAEPITHELIAL LESIONS (Abstract): The behavior of the cervical squamous intraepithelial lesions cannot be predicted, many of them, particularly of the low grade type, may disappear without treatment. Invasive cervical carcinoma occurs in approximately 10% of the intraepithelial precursor lesions, being strongly associated with HPV infection. The aim of this study was to make a comparative assessment between immunohistochemical and immunocytochemical expression of p16 and L1 HPV capsid protein respectively, in low grade and high grade cervical squamous intraepithelial lesions. **Material and method**: The study involved 20 patients with cytological diagnosis of LSIL/CIN1 (low grade squamous intraepithelial lesion/cervical intraepithelial neoplasia) and HSIL (CIN2 and CIN3) (high grade squamous intraepithelial lesion), which underwent a subsequent cervical biopsy. The conventional smears were evaluated for the immunoexpression of L1HPV protein and the corresponding biopsies for the immunoexpression of p16. **Results**: The HPV L1 capsid protein was expressed in 46% of LSIL and 24% of HSIL. P16 was positive in 68% of LSIL, 84% of CIN2 and 100% of CIN3. The correlative analysis of p16 status and protein L1HPV expression can be very useful in the assessment of progression risk of cervical squamous intraepithelial lesions. **Key words**: HPV, L1 CAPSID PROTEIN, p16, LSIL, HSIL

It is now accepted that invasive cervical squamous carcinomas and the correspondent precursor lesions are determined by specific types of Human papillomavirus (HPV), especially high oncogenic risk types, which infect the anogenital tract. At present, the proportion of cervical carcinoma attributed to HPV infection is estimated to 99%. Many observations have indicated the importance of immune response in HPV infection. Antibodies against capsid proteins of different types of HPV have been previously studied, using fusion bacterial proteins or chemically synthesized peptides (1,2). As an essential condition of these studies, HPV infecting type has to be detected. Until recently, the detection of HPV type in a certain tissue was performed by hybridization arrays with nucleic acids. Polymerase chain reaction (PCR) was developed as a more specific and sensitive method of HPV DNA amplification, used either for general detection or for the HPV typing, particularly in genital infections. The issue of quantification is the main limit of the procedure, the distinction between latent subclinical infection and evident clinical lesions being difficult. As an alternative to

hybridization and PCR, the immunologic detection of viral capsid antigens can be used for the diagnostic of productive HPV infections.

The tumor suppressor protein p16 is a cyclin-dependent kinase inhibitor that regulates transition from the G1 to the S phase of the cell cycle (3,4).

The high immunoexpression of the p16 was previously reported to be characteristic for dysplastic and neoplastic epithelium of the cervix (5,6,7). P16 overexpression slows down the cell cycle by inactivating the cyclin--dependent kinases that phosphorylate retinoblastoma protein (pRb) (8,9). The viral oncogenes E6 and E7 of HPV, whose expression is associated with malignant transformation of the cervical epithelial cells (10,11), have the ability to bind and inactivate pRb which, in turn, influence the p16 expression in cervical intraepithelial squamous lesions (SIL) (12). Recent studies have proposed that p16 is a useful marker for HR (high risk)-HPV type related cervical neoplasia (9,13) and for predicting SIL progression (14,15).

The behavior of the cervical squamous intraepithelial lesions cannot be predicted, many of them, particularly of the low grade type, may disappear without treatment. Invasive cervical carcinoma occurs in approximately 10% of the intraepithelial precursor lesions, being strongly associated with HPV infection (16,17,18). The aim of this study was to make a comparative assessment between immunohistochemical and immunocytochemical expression of p16 and L1 HPV capsid protein respectively, in low grade and high grade cervical squamous intraepithelial lesions in order to determine, by clinico-morphological correlations, their practical applicability in patients diagnosis and follow-up.

MATERIALS AND METHODS

Twenty patients were included in our study, 10 cases with cytological diagnosis of LSIL (low grade squamous intraepithelial lesion) and 10 cases with HSIL (high grade squamous intraepithelial lesion), which underwent a subsequent cervical biopsy. The cervico-vaginal smears were fixed and stained using Papanicolaou method. The conventional smears were evaluated for the immunoexpression of L1HPV capsid protein and the corresponding biopsies for the immunoexpression of p16.

After Pap cytodiagnosis, the cervico-vaginal smears were used to detect HPV L1 capsid protein by immunocytochemistry, using the monoclonal antibodies (Cytoactiv HPV L1 High Risk Set REF SCA0850, Cytoimmun Diagnostics GmbH) in a standardized protocol. Epithelial cells with positive nuclear staining were scored as positive, considering one stained nucleus enough for scoring.

The cervical biopsies were investigated by routine histopathological exam and by immunohistochemistry, using p16-D25 antibodies. Collected tissues were fixed for 24 hours in buffered formalin and processed for paraffin embedding. Serial sections of 4-5 μm were dewaxed and stained with Hematoxylin-Eosin. After performing the standard histopathologic exam, supplementary sections were used for immunohistochemical exam. Heat-induced epitope retrieval technique was performed using Target Retrieval Solution pH 6 (code S1700, DAKO, Denmark). After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with one of the primary antibodies, anti-p16 mouse monoclonal antibody (clone D25, code sc-81613, Santa Cruz, USA), dilution range 1 : 100. The immune reaction was amplified using the appropriate secondary antibody and the Streptavidin–Biotin– Peroxidase HRP complex (code K5001, DAKO, Denmark). Sections were then developed using 3,3'-diaminobenzidine tetrahydrocloride chromogen (DAB) (code K5001, DAKO, Denmark), under microscope control. The sections were finally counterstained with Mayer's Hematoxylin. Negative control was also performed.

TABLE I
p16 Immunohistochemical score

Score	p16
0	none
1	Weak (<25% immunopositivity)
2	Moderate (25-75% immunopositivity)
3	Intense (75-100% immunopositivity)

The quality control represented by external and internal negative and positive controls was necessary to monitor the accuracy of tissue processing, staining procedures and reagents effectiveness. The primary antibody specificity sought to be assesed by their negative controls.

Protein p16 was scored based upon the estimated proportion of immunopositive cells (table I).

RESULTS

In our study, the histopathological diagnoses were consistent with the cytodiagnoses, as follows : 10 cases with LSIL (CIN1) and 10 cases with HSIL (4 cases CIN2 and 6 cases CIN3).

HPV infection was morphologically confirmed by the presence of cytopatic HPV effect (koilocytes) in the smears and biopsies.

The p16 staining pattern was predominantly nuclear with occasional cytoplasmic positivity. Most cases presented heterogeneity of staining with positive cells admixed with negative cells.

From all cervical biopsies, p16 was positive in 68% of LSIL, 84% of CIN2 and 100% of CIN3. The proportion of biopsies with intense immunoexpression of p16 increased with the severity of cytological abnormality. In HSIL cases, the staining distribution was as follows : 65% full thickness (figure 3), 35% basal and intermediate. The staining intensity for HSIL cases was strong in 80% (figures 1, 3), moderate in 16%, and weak in 4% accordingly. Regarding LSIL category, the staining distribution was basal in 75% of cases and occasional in the remaining 25%. There was no LSIL case with full thickness of p16 positivity. The staining intensity of LSIL cases was strong in 20% (figures 2, 4, 5), moderate in 13%, and weak in 67%.

From all cervical smears, the HPV L1 capsid protein was expressed in 46% of LSIL and 24% of HSIL. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated.

Positive reaction was characterized by the strong staining of the whole nucleus, surrounded by a cytoplasm with no background. In most cases, positive reaction for HR-HPV L1 was positive in typical koilocytes or in dyskeratocytes, presenting nuclear characteristics for HSIL (CIN 2 or CIN 3). In LSIL cases, the positivity of the nuclei was presented only in typical koilocytes (figure 6).

All HPV-positive cases (with morphologic evidence of HPV infection) were also p16-positive, but no significant relationship between HPV infection immunopositivity (active infection) and the intensity and distribution of p16 was found.

DISCUSSIONS

L1 capsid protein is expressed in the active phase of HPV infection and is necessary in viral cellular cycle completion. Consequently, viral protein detection, by immunohistochemical reaction is an evidence of active HPV infection in examined tissue (19). L1 viral capsid protein is considered a major target of the cellular immune

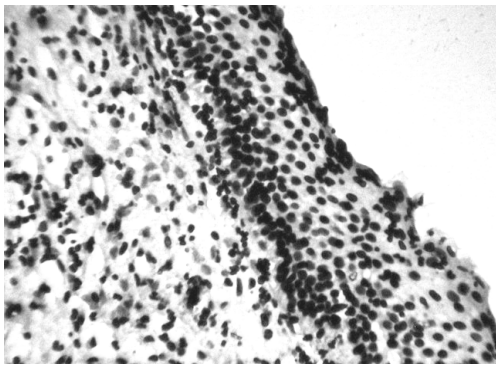

Fig. 1. HSIL (CIN2), strong p16 immunostaining (x10)

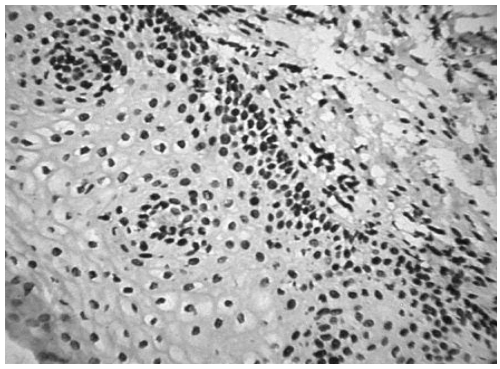

Fig. 2. LSIL, strong p16 immunostaining (x10)

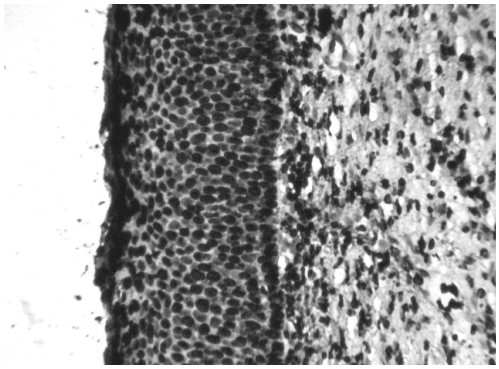

Fig. 3. HSIL (CIN3), strong full thickness p16 immunostaining (x10)

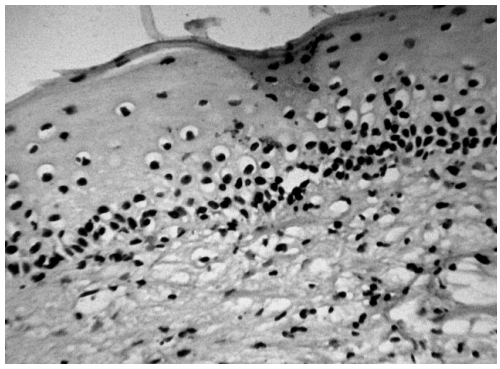

Fig. 4. LSIL, strong p16 immunostaining (x10)

response (20). LSIL and moderate SIL without immunohistochemically detected L1 are correlated, in more than 80% of cases, with dysplasia progression. Griesser et al. certify these aspects, highlighting that minor and moderate lesions without L1 capsid protein expression are significantly more exposed to a progression in comparison to L1 positive cases (21). Most probably, the lack of HPV antigen is determined by a weak protein synthesis, under immunohistochemical test minimum level. As L1 represents the major target of the immune cellular response (22), its deficient translation may result in an inefficient depuration of the infected cells, promoting viral DNA integration in host cellular genome and the transformation of immature epithelial cells. The observation that the decrease of the HPV16 capsid positivity in cervical cancer patients' serum is an indicator of a poor prognosis sustains the importance of a specific humoral response. Immunohistochemical detection of L1 capsid, on conventional Papanicolau smears, may consequently indicate the locally induced defence status on HPV infection and may offer prognosis information, mainly in LSIL lesions.

From all cervical smears, our data revealed that the HPV L1 capsid protein was expressed in 46% of LSIL and 24% of HSIL. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. Because of the low rate of

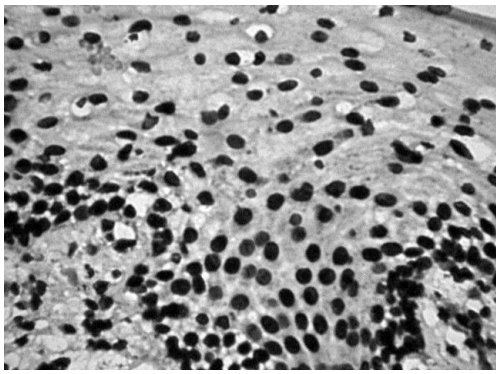

Fig. 5. LSIL, strong p16 immunostaining (x20)

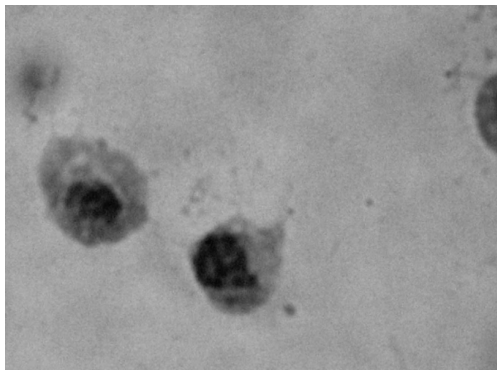

Fig. 6. LSIL, HPV L1 positive (x100)

HR-HPV L1 positivity found in LSIL cases in our study, we can admit that HPV is not helpful in grading cervical SIL, which is in accordance with the literature data (12).

In our study, patients' mean age was 34 years. This fact is consistent with the literature data, where it has been demonstrated that the prevalence of HPV infection varies with age and geographical region, reaching highest rates below 35 years of age (23).

The presence of L1 HPV positively stained nuclei of squamous epithelial cells could be correlated with the clinical course. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. Mild and moderate dysplastic cervical lesions without immunohistochemical positive reaction of HPV L1 capsid protein are more likely to progress as compared to positive cases (21). Researches consider that lack of detectable HPV antigen in the Pap smears is due to low protein synthesis in squamous epithelial cells below the limit of the immunocytochemical test. The loss of L1 capsid protein immunoexpression can be the result of the integration of the viral DNA into the human genome. Although most of cervical carcinomas show integration of viral DNA, it is detectable only in a small proportion of LSIL and HSIL (24). As was already reported in the literature (20), perhaps there are additional control mechanisms which lead to the L1-negative immunoexpression in the mentioned cases.

The development of viral capsid antigen L1 depends upon transcriptional factors which only can be expressed during maturation process from basal epithelial cell to superficial epithelial cell (19). In HSIL, the natural structure as well as maturation of the epithelium are disturbed, thus the dysplastic basal squamous cells represent the predominant cell type with reduced L1 capsid protein expression.

P16 is a highly sensitive marker for cervical epithelial dysplasia. Strong and full thickness staining of p16 in the cervix epithelium is highly supportive of HSIL, while weak and basal/rare staining favors LSIL (4).

In our study, from all cervical biopsies, p16 was positive in 68% of LSIL, 84% of CIN2 and 100% of CIN3. The proportion of biopsies with intense immunoexpression of p16 increased with the severity of cytological abnormality. Our results are consistent with the data reported in the literature (25, 26, 27, 28).

As previously reported (14), we also found that all cases morphological HPV positive expressed the p16 protein. No significant relationship between HPV infection immu-

nopositivity (active infection) and the intensity and distribution of p16 was found. This is also supported by Lin et al. (29), who admitted that p16 expression in cervical squamous and glandular epithelium is the marker of dysplasia or malignancy irrespective of the HPV infection status.

Other studies reported the existence of a significant association between cervical squamous intraepithelial lesion grade and p16 intensity and distribution of the immunostaining (30, 31). Regarding the localization of p16 staining, there are authors who report predominant cytoplasmic staining (7) as well as predominant nuclear staining (32, 33), or a combination of both patterns (34).

According to our results, we cannot formulate any definitive statements regarding the applications of p16 in routine practice, excepting its recognized value in certifying the diagnosis of doubtful cases.

CONCLUSIONS

The immunoexpression of L1 HPV has clinical application in the progression assessment of the cervical precancerous lesions without a correlation to the grade of the cervical SIL. The analysis of p16 status, in parallel with protein expression L1 HPV, can be very useful in the assessment of progression risk of cervical squamous intraepithelial lesions.

ACKNOWLEDGEMENTS

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MORPHOLOGIC PROFILE OF TWO LEUZEA SPECIES HARVESTED FROM WILD FLORA OF EASTERN ROMANIA

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MORPHOLOGIC PROFILE OF TWO LEUZEA SPECIES HARVESTED FROM WILD FLORA OF EASTERN ROMANIA (Abstract): *Leuzea carthamoides DC and Leuzea salina* are two plant species found in Chinese and Russian traditional medicine for their adaptogen and stress relieving properties. There are still many unknown bioactive compounds that may influence the mechanism of action of these species. Nevertheless, all chemical investigations are directly related to the proper identification of the raw material. **Aim**: In Romania, it is known that two different species of *Leuzea* grow in the wild flora, but there is no data that confirms the difference between the species. Moreover, there are specialists that consider that *L. carthamoides* DC. is a synonym for *Rhaponticum carthamoides* (Willd) Iljin, which rises a series of questions regarding the botanical belonging. Our aim was to observe and assess possible taxonomic differences between the two *Leuzea species*. **Material and methods**: The investigations consisted in the morphologic assessment (microscopy, scanning electronic microscopy) of each important structure found in the roots, stems and leaves of both species on plants harvested from Iasi and Neamt counties in 2017. **Results**: The microscopic observations indicated both similar and different structures that are related to the environmental conditions. Some of the observed features can confirm the species and the growth conditions. **Conclusions**: Although, the results confirmed the taxonomic variability between the investigated samples, further research is still undergoing to assess the chemical and the biological profile based upon the maker compounds important for taxonomy. **Keywords:** *LEUZEA*, ROOTS, LEAVES, MICROSCOPY, VARIABILITY, TAXONOMY.

Leuzea species belong to *Leuzea* genus although, there are researchers that insist that *Leuzea* and *Rhaponticum* genus are synonyms. Moreover, the existent scientific literature presents mainly *Rhaponticum carthamoides* (Willd) Iljin and thus, there is still great need for more data to confirm the taxonomic characteristics of *Leuzea* species. *Leuzea carthamoides* has a long history in traditional Siberian medicine being used to tone the body and combat following a long illness (1-3). It is still presently used as a remedy for male sexual dysfunctions. The bioactive compounds of this species are ecdysteroids, flavonoids and polyphenolic acids (4-9). From this

species, hydroalcoholic extracts from roots administered at a dose of 500 mg / kg body weight in rats increase the locomotor activity, antagonize the narcotic effect of chloralhydrate, increase brain excitability and increase learning memory (4, 7, 10-11). *Leuzea carthamoides* roots decoction corrects depressive status in humans and increases health in patients with somatic gastrointestinal pain. Typically, roots and rhizomes are used to prepare extracts used for the adaptogen and tonic qualities, being incorporated in their entirety or as separate fractions in nutraceutical supplements to promote muscle development, to treat impotence and to eliminate physical and mental fatigue. For this reason, since 1958 *Leuzea* has been in the composition of well-known preparations in mixtures with *Schisandra chinensis, Panax ginseng, Aralia mandshurica* and *Eleutherococcus senticosus*, species with adaptogen properties (9-10).

Regarding *Leuzea salina* there is not much mentioned in scientific literature and according to our observations the plant has a short and thick rhizome with numerous long, thick, black-and-brown roots. Also, the erect stem is thick, cylindrical, simple, finely striated, 60-75 cm tall, while the basal leaves form long rosettes, but the rest of the leaves are petiolate, ovate or elliptic. The flowers of magenta color are similar to *Leuzea carthamoides* (2-3, 11).

The purpose of our research is the comparative investigation of two species of Leuzea, namely a species recognized as a medicinal species, *Leuzea carthamoides* DC, and a species about which there is almost no data in specialized literature: *Leuzea salina*. Our intention was to study the two *Leuzea* species in terms of morphological and histological characteristics in order to identify possible taxonomic similarities and differences.

MATERIAL AND METHODS

The plant material was harvested from the wild flora in the summer of 2017; *Leuzea carthamoides* was collected from Neamt county, whereas *Leuzea salina* raw material was obtained from Iasi County. The sampling of individuals was done for each species within the Biology Faculty, "Al. I. Cuza" University Iasi. The vegetal material was represented by the underground and aerial vegetative organs taken from plants in the antecedent stage. Vegetative organs were fixed and conserved in 70º alcohol. For both samples various types of sections were made using botanical microtome and shock marrow, as follows: upper, middle and inferior transversal axial sections through root, rhizome and stem, also transversal and superficial sections through the lamina (at the median and lateral ribs) were obtained.

Clarification and coloring of the sections

The sections thus obtained were collected and the cellular content was removed by introducing the vegetal material sections into a few drops of sodium hypochlorite, where different time for contact was used depending on the vegetative organ from which they originated (underground organs - 20 minutes, stems - 15 minutes, leaf-10 minutes). Superficial sections of the leaf and leaf stalks have not been mapped. The clarified sections were processed in conformity with double coloring technique (iodine green and red ruthenium), but the superficial sections of the leaves and stems were colored only once with one of the mentioned reagents. As a result of the double coloration: the lignified cell walls are colored in

blue, and the non-lignified in violet; the suberus is colored green, and the parenchyma and the phloem in red and pink.

Thus, the plant material was fixed in glutaraldehyde, dried at the critical point, metallized and then observed and photographed with a photonic microscope (Teskan Electronic Scanning Microscope).

Reagents and equipment

The used apparatus was first Novex Holland Microscope with Sony Cyber-shot DSC-W730 and then Teskan Electronic Scanning Microscope (SEM).

Green iodine is obtained from one gram of iodine green is dissolved in 100 ml of hexane, whereas red ruthenium is obtained by dissolving 0.02 g of ruthenium red in 100 ml of distilled water; add a thymol crystal for preservation.

RESULTS AND DISCUSSION

The results indicated both similarities and differences for the investigated samples. Briefly, at the severed level, *Leuzea carthamoides* root has a typically secondary structure. On the outside of the bark there was a very thin periderm with 1-2 layers of cork cells. The primary bark is parenchymal (relatively thick, 10-12 layers), consisting of slightly elongated tangential cells. The most internal layer is an endodermis with cells having lignified and suberized walls. The central cylinder showed parenchymal cells, many vascular bundles (phloem and xylem solitary or grouped vessels). The secondary fibers have fairly numerous pits and were often arranged in radar strings which intertwine the phloem resulting in incomplete rays. The phloem vessels of variable diameter are composed of cells with moderately thick walls irregularly arranged, in incomplete radar areas as shown in the image below (fig. 1).

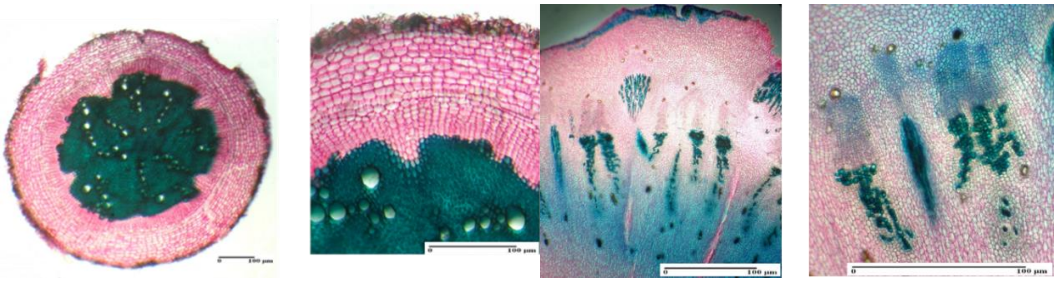

Fig. 1. *Leuzea carthamoides* roots and rhizomes – sectional view with detail

Our findings indicate that at the sectional level Leuzea salina presents mainly a primary structure, as opposed to what we have seen in *L. carthamoides*. The cortical parenchyma is very thick, with small cells, including numerous air cavities. The inner layer is made of small cells elongated tangentially, alternating with those of the pericardium. The central cylinder is relatively thick with parenchymal cells, larger air cavities surrounded by interfascicular cellular parenchyma are observed between the phloem and xylem fascicles. A detailed view is presented in the following image (fig 2).

At the electronic scanning microscope (SEM) we noticed that the cortical cells are heavily elongated and all the thin wall (fig. 3).

The SEM images for *Leuzea salina* roots are shown in the figure 4. The details seem different from the other investigated species.

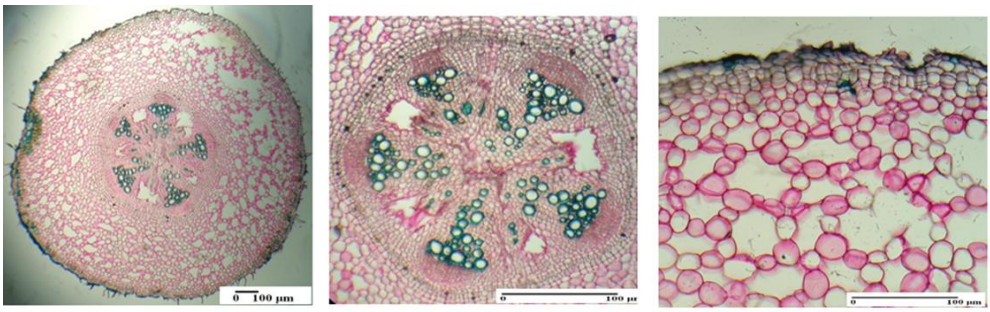

Fig. 2. *Leuzea salina* roots and rhizomes – sectional view with detail

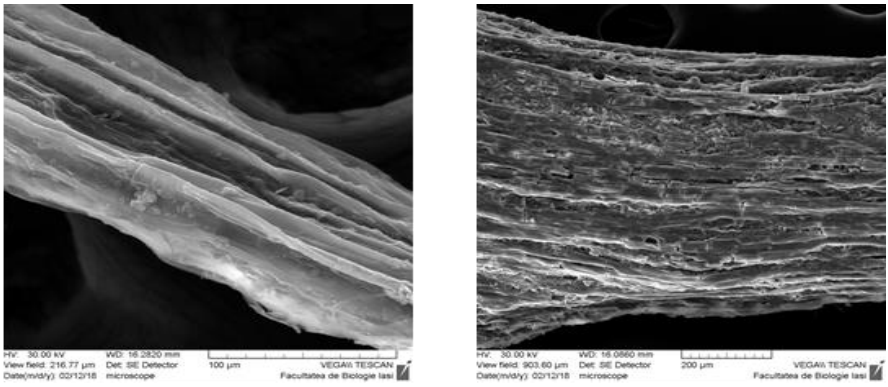

Fig. 3. The root of *Leuzea carthamoides* seen on the SEM:
lateral root to the top (left); cortical cells (right)

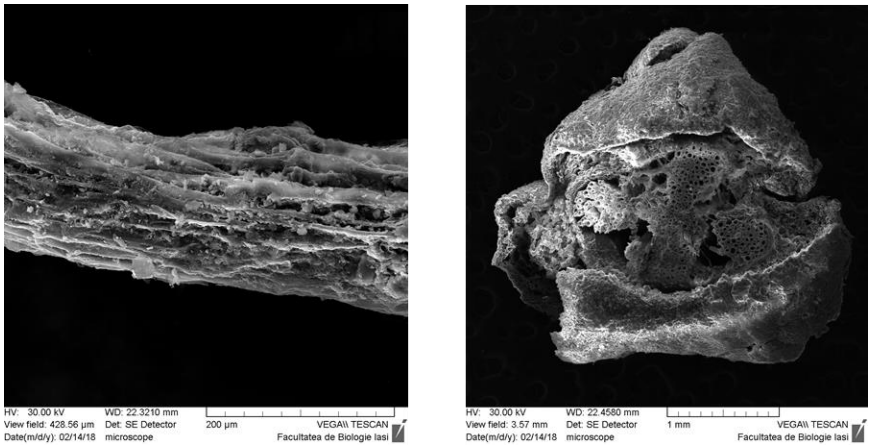

Fig. 4. *Leuzea salina* seen at the scanning electron microscope:
rhizoderma (left); the root seen in cross-section (right)

Considering the rest of the analyzed plant parts, the stem of *Leuzea carthamoides* is extremely short, with early primordial structure, where the conductor bundles did not differentiate, being surrounded by several leaves of different size and age, making several concentric circles. Nevertheless, the characteristics of *Leuzea salina* stem are different, the contour of the cross section is circularly with a thin bark, air cavities and the hypodermis is composed of collenchyma within the ribs section. The epidermis exhibits isodiametric cells at the ribs and slightly elongated tangentially to the valleys. All these elements are clearly visible in the photographs included below (fig 5).

A particular feature that is visible only for *L. salina* in SEM, is the presence of salt glands of particular structure, among the epidermal cells.

Fig. 5. Stem cross-section general view and detail:
A - *Leuzea carthamoides*; B- *Leuzea salina*

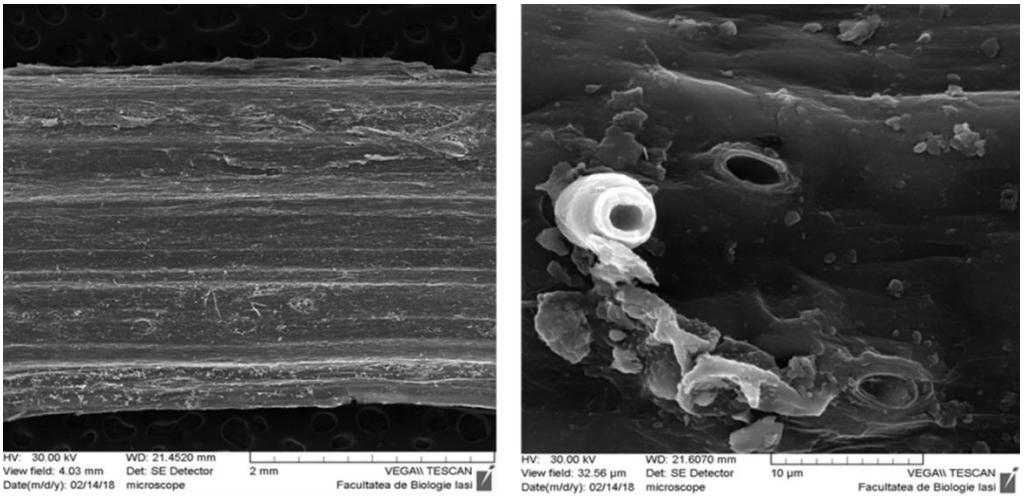

Fig. **6.** Salt glands from the epidermis of the stem in *Leuzea salina* (SEM)

Such elements, which were not observed in *L. carthamoides*, are largely discussed in the old scientific literature as a characteristic for halophytic plants. Nevertheless, there is a lack of proper information in regards to these glands and their actual role for the plants (12-13). This is why we need to further investigate the type of the mineral elements that are secreted.

The mature leaf of *Leuzea carthamoides* has a semicircular cross-sectional shape

with a wide and superficial adaxial groove delimited by two latero-adaxial ridges in which homogeneous parenchyma is observed and a small vascular bundle located in front of a thin cordon of hypodermic collenchyma. The epidermis exhibits isodiametric cells with the external wall slightly thicker than the others and with a thin cuticle. From place to place, stomata are observed. Moreover, the fundamental parenchyma has cells whose size decreases towards the inside of the organ. The hypodermic layer presents a thin layer of collenchyma cells, and in the two latero-adaxial ridges the collenchyma cells have visibly thickened walls (fig. 7).

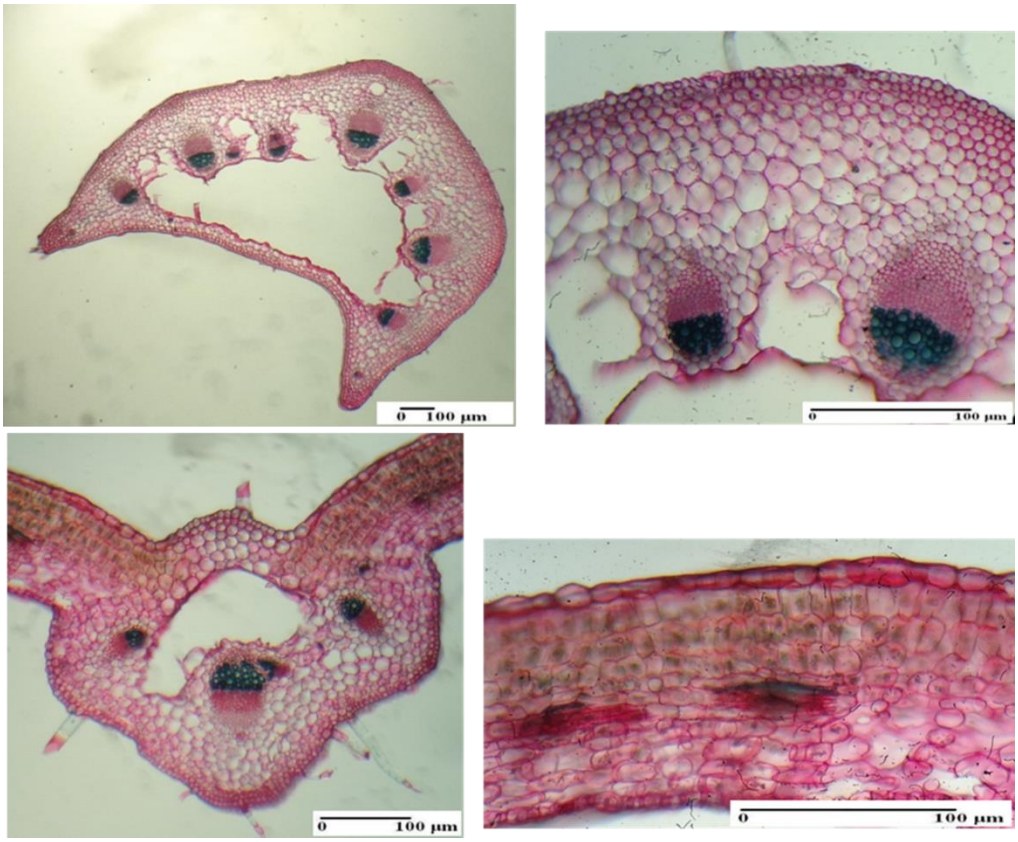

Fig. 7. Transverse section through *Leuzea carthamoides* leaves: petiole (upper images), lamina (lower images)

In the transverse section through the limb of *Leuzea salina*, the medial rib is thick and protruding at the lower face. In the thickness of the median rib, the chloroplast-free parenchyma predominates in which there are usually between 10-12 vascular bunches of different size, arranged on a spring form, all of which have a primary structure. The epidermis presents slightly elongated cells tangentially to the external wall thicker than the others and thinly cutinized. From place to place we see pluricellular, relatively short salt glands, nonexistent in *Leuzea car-*

thamoides. The mesophyll is almost totally palisaded, with relatively short and wide cells, so the limb has a bifacial structure (equiaxial) (fig. 8)

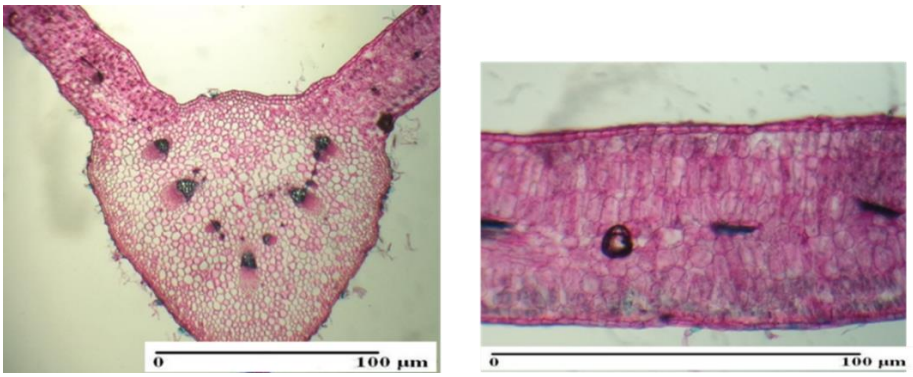

Fig. 8. Cross-section through the leaf of *Leuzea salina*: median rib (left); mesophyll (right)

Irrespective of size, all vascular bundles have pits, tracheid, sieve-tubes elements and intermediary cells. The xylem is formed of separate parenchymal cell vessels with thick and lignified walls. A peripherical cord of collenchyma elements and some mechanical cells with moderately thick and lignified walls were noted along with the larger vascular bundles.

In the scanning electron microscope, the epidermal cells on the lower and upper face of the leaf appear irregular with irregularly curved lateral walls, and among them there are stomata, which are elliptical, and the walls defining the ostiole are very thick (fig. 9).

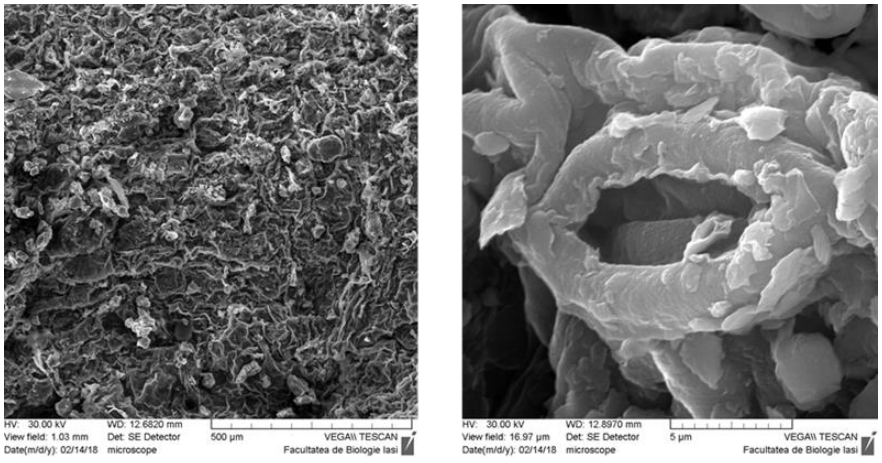

Fig. 9. Epidermis (left) and stomata (right) from *Leuzea salina* in SEM view

CONCLUSIONS

In the present paper we presented the results of the histoanatomical studies conducted for the rhizomes, roots and the aerial part of two *Leuzea* species. Our conclusion is that the cross sections of each ana-

lyzed sections sustain that the species are related and justify the inclusion in the same genus. Nevertheless, the noted differences especially in terms of tissue type and certain elements found only in *Leuzea salina*, suggest that the investigated species are different. Such features important for taxonomic classification at the generic level represent the novelty of our research. However, the chemical composition and the pharmacological action of the extracts that follow closely the similarities and differences between the two species remains to be further investigated. Also, *Leuzea salina*, unknown for its bioactive potential may also become interesting as a supplier of ecdysteroid compounds.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ASPECTE TOPOGRAFICE ŞI ANATOMOPATOLOGICE ALE MENINGIOAMELOR DE FOSĂ POSTERIOARĂ

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POSTERIOR FOSA MENINGIOMAS TOPOGRAPHIC AND ANATOMOPATHOLOGIC ASPECTS (Abstract): Meningiomas are the most frequent met intracranial tumors. Many authors tried to order them according to location, origin and histopatologic type. **Material and method**: Our study is focused on posterior fosa meningiomas, initially classified according to their origin based on IRM and surgical findings in order to identify correlations between demographic data, topographic tumor origin and anatomopathologic characteristics for each subgroup. 35 posterior fosa meningioma patients that have been subject to neurosurgery in Iaşi "Prof.Dr. N. Oblu" Hospital Neurosurgery Clinic between January 1st 2005 and December 1st 2009 were assessed. **Results**: In our study group the majority of patients were females, with an average age in their early 60's. Benign meningiomas (Ist degree) represented the unique subtype in tumors located in cerebelum convexity and foramen magnum. Other locations have different chracteristic subtypes (fibrous vs. angiomatous meningioma). **Key words**: MENINGIOMA, HISTOPATHOLOGY, POSTERIOR FOSA

Meningioamele reprezintă una din cele mai frecvente tumori intracraniene. În 1938, Cushing şi Eisenhardt au realizat o clasificare a meningioamelor funcţie de locul de origine, clasificare ce se foloseşte şi în prezent în practica neurochirurgicală (1). După această dată au urmat şi alte clasificări ale meningioamelor intracraniene funcţie de originea lor durală. Aceste clasificări nu oferă informaţii privind malignitatea sau tipul lor histologic, dar neurochirurgul foloseşte aceste date pentru stabilirea abordului chirurgical (2).

Roberti et al. (3) au clasificat meningioamele de fosă posterioară în şase grupe: tipul I: tumori localizate pe convexitatea cerebeloasă şi tentoriul lateral (tentorium, sinusurile transverse sigmoide); tipul II: tumori localizate în unghiul cerebelopontin (aripa pietroasă, conductul auditiv intern); tipul III: tumori localizate la nivelul foramen jugular (unghiul cerebelo-medular, vena jugulară internă); tipul IV: tumori petroclivale (2/3 superioare ale clivusului, sinusul cavernos, cavum Meckel, aripa pietroasă); tipul V: tumori de foramen magnum (1/3 infe-

rioară a clivusului, aria C1-C2); tipul VI: tumori neclasificate (tumori gigante, extinse în mai multe arii).

Bradac et al. (4) au împărţit meningioamele de fosă posterioară în patru grupe: 1. meningioame tentoriale - tumorile localizate pe suprafaţa tentorium cerebelli; 2. meningioame clivale – tumorile cu originea la nivelul treimii superioare a clivusului în direcţia fosei craniene mijlocii sau în direcţia trunchiului cerebral; 3. meningioame de unghi pontocerebelos – tumorile cu originea la nivelul porţiunii medii a osului pietros; 4. meningioamele de foramen magnum – tumorile localizate la nivelul sau în apropierea marginii anterioare a foramenului.

Studiul de faţă analizează meningioamele de fosă posterioară, clasificate iniţial funcţie de locul lor de origine pe baza CT, IRM şi a observaţiei chirurgicale pentru a identifica posibilele corelaţii dintre datele demografice ale pacienţilor cu meningioame de fosă posterioară, locul de origine a acestor tumori şi caracteristicile anatomopatologice ale fiecărui subgrup.

MATERIAL ŞI METODĂ

Au fost luaţi în studiu 35 de pacienţi cu meningioame de fosă posterioară care au fost supuşi unei intervenţii neurochirurgicale pentru ablaţia tumorii în perioada 1 ianuarie 2005 – 1 decembrie 2009 în Clinica de Neurochirurgie a Spitalului Clinic de Urgenţă „Prof.Dr. N. Oblu" Iaşi. Analiza a inclus: vârsta şi sexul pacienţilor, localizarea şi aspectele anatomopatologice ale tumorii rezecate.

În funcţie de relaţiile anatomice cu structurile fosei posterioare, cele 35 de meningioame au fost clasificate în 5 tipuri: 1. tumori de convexitate cerebeloasă (tentorium cerebelli, sinusurile sigmoide transverse); 2. tumori de unghi pontocerebelos; 3. tumori petroclivale (2/3 superioare ale clivusului, foramen jugular); 4. tumori de foramen magnum (1/3 inferioară de clivus, aria C1-C2) şi 5. tumori neclasificate (ataşament dural larg în arii multiple ale fosei posterioare) (tab. I).

Piesele de exereză chirurgicală au fost prelucrate cu tehnica histopatologică clasică (fixare în formaldehidă 10%, includere la parafină şi colorare cu hematoxilină-eozină şi van Gieson). Pentru determinarea gradului de proliferare al meningioamelor de fosă posterioară, secţiunile au fost studiate cu anticorpii Ki-67 folosind tehnica imunohistochemică EnVision.

Pe baza schemei de clasificare a Organizaţiei Mondiale a Sănătăţii, 2007 (5) şi luând în considerare rezultatele obţinute prin investigaţia imunohistochimică, toate cazurile au fost clasificate în trei grupe histopatologice: benigne (I), atipice (II) şi maligne (III) şi s-a notat fiecare subtip histopatologic (tab. II).

REZULTATE

În seria noastră, meningioamele de fosă posterioară au prezentat sex ratio femei/bărbaţi de 3,33:1. Din cele 35 de cazuri, 27 de cazuri (77,14%) au fost femei cu o vîrstă ce a variat între 36 şi 73 ani (vârsta medie fiind de 52,8 ani). Restul de 8 cazuri (22,86%) au fost pacienţi de sex masculin cu o vârstă variind între 35 şi 57 ani (vârsta medie fiind de 47,1 ani).

Vârsta medie pentru toate meningioa-

TABELUL I

Meningioamele de fosă posterioară : clasificare funcţie de locul de origine, vîrsta şi exul pacienţilor

LOCALIZAREA TUMORII	Neclasificate		Convexitatea cerebeloasă		Unghi pontocerebelos		Petroclival		Foramen magnum	
Sexul pacientului	F	M	F	M	F	M	F	M	F	M
Vîrsta medie a pacientului (ani)	52	43,33	64,25	49	50,93	49,33	55	50	52,66	-

TABELUL II

Meningioame de fosă posterioară: locul de origine, sexul pacienţilor şi gradul de malignitate

Gradul de malignitate a tumorii	Neclasificate		Convexitate cerebeloasă		Unghi ponto-cerebelos		Petro-clival		Foramen magnum		Nr. cazuri (%)
	F	M	F	M	F	M	F	M	F	M	
Gradul I	1	2	4	1	12	3	3		3	-	**29 (82,85%)**
Gradul II	1				2			1			**4 (11,42%)**
Gradul III	1	1									**2 (5,71%)**

TABELUL III

Meningioamele de fosă posterioară: clasificare funcţie de locul de origine şi subtipurile histologice

Subtipul histologic	Ne-clasificate	Convexitate cerebeloasă	Unghi ponto-cerebelos	Petro-clival	Foramen magnum	Nr. cazuri (%)
Fibros	1	5	5			11 (37,79%)
Psamomatos	2		2	1	2	7 (24,13%)
Meningotelial			3	1		4 (13,79%)
Secretor			2	1	1	4 (13,79%)
Angiomatos			3			3 (10,34%)
Atipic	1		2	1		4 (11,42%)
Anaplastic	2					2 (5,71%)
Total	6 (17,14%)	5 (14,28%)	17 (48,57%)	4 (11,42%)	3 (8,57%)	35 (100%)

mele de fosă posterioară a fost de 51,6 ani. Meningioamele gigante ce au afectat o mare parte din fosa posterioară s-au întâlnit la pacienţii de sex masculin mai tineri decât vârsta medie (43,33 ani). Vârsta pacienţilor de sex feminin a fost mai mare decât vârsta medie a grupului în cazul meningioamelor de convexitate cerebeloasă (64,25%).

Meningioamele de grad I (fig. 1 şi 2) au fost întâlnite în majoritatea cazurilor (82,85%), în timp ce tumorile de grad II (fig. 3 şi 4) au fost diagnosticate în 11,42% din cazuri, iar tumorile anaplazice doar în 5,71% din toate cazurile analizate. Meningioamele atipice

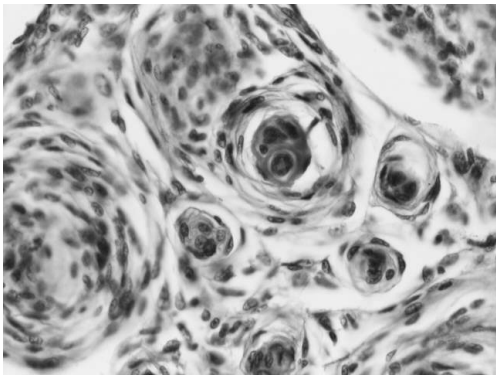

Fig. 1. Meningiom meningotelial grad I (H&E, x200)

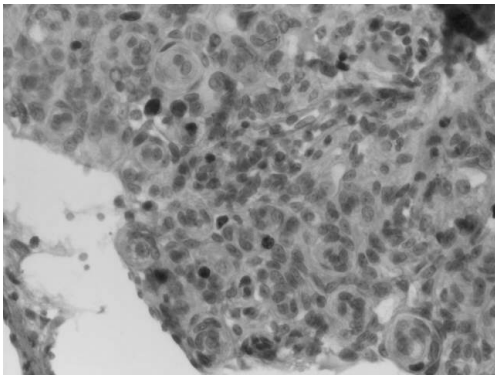

Fig. 2. Meningiom meningotelial grad I – 2% din celulele tumorale sunt pozitive pentru anticorpul Ki-67 (reacţia de imunocolorare cu anticorpul Ki-67, x200)

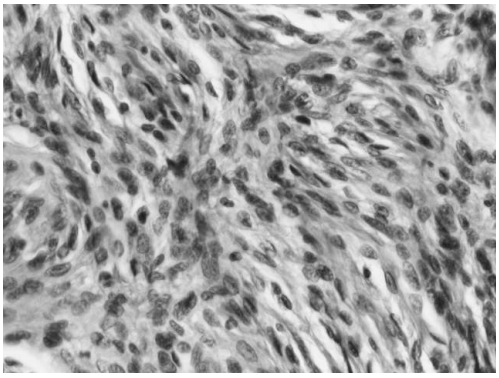

Fig. 3. Meningiom atipic grad II (H&E, x200)

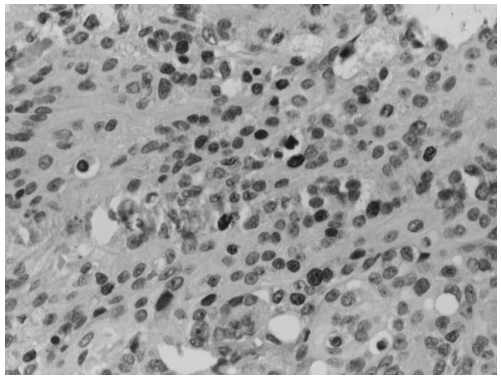

Fig. 4. Meningiom atipic grad II (8% din celule tumorale sunt pozitive pentru anticorpul Ki-67 (reacţia de imunocolorare cu anticorpul Ki-67, x200)

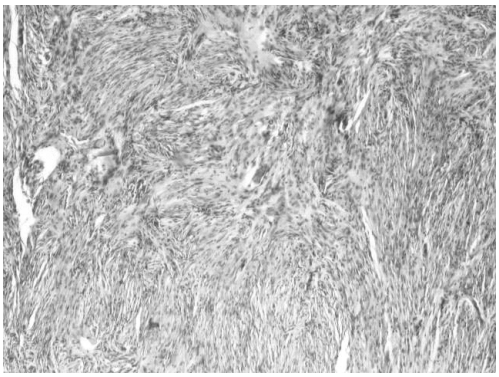

Fig. 5. Meningiom fibros (H&E, x100)

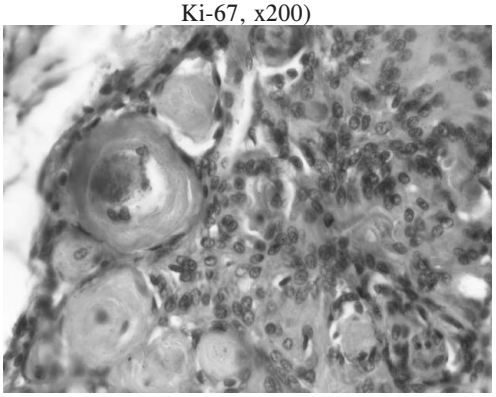

Fig. 6. Meningiom psamomatos (H&E, x200)

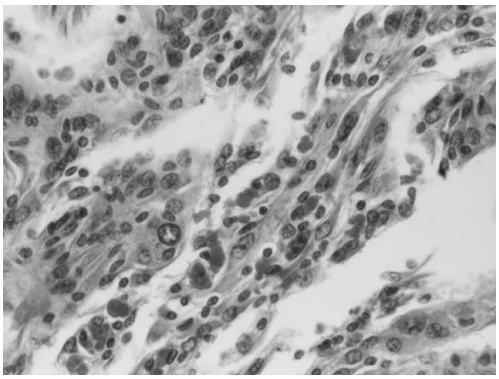

Fig. 7. Meningiom secretor (H&E, x200)

(grad II) au fost diagnosticate în unghiul ponto-cerebelos şi în aria petroclivală. Meningioamele maligne (grad III) nu au putut fi localizate într-un compartiment specific al fosei posterioare deoarece au fost operate în momentul în care au atins dimensiuni foarte mari.

29 de pacienţi au prezentat ataşament dural distinct la unul din cele patru compartimente menţionate ale fosei posterioare (convexitatea cerebeloasă – 14,28%, unghiul ponto-cerebelos – 48,57%, petro-clival – 11,42% şi foramen magnum – 8,57%), iar 6 pacienţi (17,14%) au avut ataşament dural larg în multiple arii ale fosei posterioare.

Cel mai frecvent, la nivelul fosei posterioare s-au dezvoltat subtipurile histologice benigne fibros (37,79%) (fig. 5) şi psamomatos (24,13%) (fig. 6). Puţine cazuri au fost de tip angiomatos (10,34%). Meningioamele atipice şi anaplazice au reprezentat 17,13% din toate meningioamele de fosă posterioară. Luând în considerare subtipul

histologic şi locaţia tumorilor, am constatat că la nivelul convexităţii cerebeloase s-au dezvoltat numai meningioame fibroase. Unica locaţie a subtipului angiomatos a fost unghiul ponto-cerebelos. Subtipul psamomatos a predominat în locaţia foramen magnum. Unghiul ponto-cerebelos a prezentat toate subtipurile histologice de meningioame, inclusiv subtipul secretor (fig. 7).

DISCUŢII

Meningioamele de fosă posterioară prezintă o provocare formidabilă pentru neurochirurg şi multe tumori, mai ales cele localizate în regiunea petro-clivală au fost considerate „inoperabile" până în anii 1970. Însă, o dată cu dezvoltarea noilor tehnologii (microscop operator, computertomografie, rezonanţa magnetică nucleară) şi cu dezvoltarea unor noi abordări neurochirurgicale asupra bazei craniului, a devenit posibilă intervenţia neurochirurgicală „în siguranţă" asupra majorităţii meningioamelor de fosă posterioară.

Locaţia tumorii este cel mai important aspect ce poate influenţa terapia deoarece defineşte modalitatea intervenţiei chirurgicale. Aceste tumori apar mai frecvent la nivelul tentoriumului, emisferelor cerebeloase şi unghiului ponto-cerebelos, dar ele pot fi găsite de asemenea de-a lungul clivusului şi pe marginea anterioară a foramen magnum (6).

Frecvenţa meningioamelor funcţie de variatele locaţii intracraniene a variat de la studiu la studiu. Unele din ele au arătat că meningioamele de fosă posterioară reprezintă 6-15% din toate meningioamele intracraniene, meningioamele dezvoltate la nivelul convexităţii cerebeloase se întâlnesc în aproximativ 5% din cazuri, meningioamele de unghi ponto-cerebelos apar în 2-4% din cazuri, iar la nivelul clivusului apar mai puţin de 1% din toate meningioamele intracraniene (7).

Meningioamele unghiului ponto-cerebelos reprezintă cele mai frecvente meningioame de fosă posterioară. Meningioamele de foramen magnum, pe de altă parte, sunt foarte rare. Meningioamele de clivus sunt, de asemenea, mai puţin frecvente (8). Cohen-Gadol şi Al-Mefty (9) au raportat procentaje asemănătoare: 50% din meningioamele de fosă posterioară sunt localizate în unghiul ponto--cerebelos, 40% apar în jurul tentoriumului sau al convexităţii cerebeloase, 9% sunt la nivelul clivusului, iar 6% apar în vecinătatea foramen magnum.

Roberti et al. au găsit alte procente privitoare la frecvenţa meningioamelor de fosă posterioară raportat la localizare. În studiul lor, 68,32% din tumori erau meningioame petroclivale, 13,04% erau meningioame de forame magnum, 8,69% erau meningioame de convexitate cerebeloasă, 5,59% erau meningioame de unghi ponto-cerebelos, iar 4,34% erau meningioame de foramen jugular (3).

În studiul nostru, cea mai frecventă localizare a meningioamelor de fosă posterioară a fost unghiul ponto-cerebelos (48,57%). Numai 8,57% din meningioamele de fosă posterioară s-au dezvoltat la nivelul foramen magnum.

Meningioamele intracraniene au afectat predominant pacienţii aflaţi în decadele a 5-a – a 7-a de viaţă. În literatura de specialitate, sunt prezentate variate valori pentru sex ratio femei/bărbaţi (de la 1,4 : 1 la 2,7 : 1) (3,10), dar meningioamele de fosă posterioară sunt în toate sursele citate mai frecvente la femei. García-Navarrete şi Sola au prezentat 26 de pacienţi cu meningioame de fosă posterioară în cadrul seriei existând sex ratio femei : bărbaţi de 12 : 2 (11). Roberti et al. au raportat 161 de cazuri de meningioame de fosă posterioară şi a găsit un raport femei : bărbaţi de 3,9 : 1 (3), valori aproape similare cu raportul găsit în studiul nostru (3,3 : 1).

Vasta majoritate (92%) a meningioamelor intracraniene au o histologie benignă, în timp ce 8% arată aspecte atipice sau maligne. În ceea ce priveşte meningioamele benigne, cel mai frecvent subtip histologic este de obicei cel meningotelial (63%), urmat de cel tranziţional (19%), fibros (13%) şi psamomatos (2%). Mai puţin de 10% din

meningioame sunt maligne (12).

În studiul de faţă, cele mai frecvente subtipuri histologice benigne dintre meningioamele de fosă posterioară au fost subtipul fibros (37,79%) şi cel psamomatos (24,13%). Meningioamele atipice şi cele maligne au reprezentat 17,13% din toate meningioamele de fosă posterioară luate în studiu, dar seria noastră cuprinde relativ puţine cazuri pentru a avea o concluzie pertinentă.

Meningioamele petroclivale reprezintă aproximativ 3-10% din toate meningioamele de fosă posterioară (13,14,15). Ele prezintă o uşoară predominanţă feminină, fiind înregistrate rapoarte variabile femei : bărbaţi (de la 1,4 : 1 până la 2,8 : 1). Vârsta medie a pacienţilor la prezentare se situează în decada a 5-a de viaţă.

În studiul nostru, meningioamele petroclivale au prezentat o incidenţă mai mare ca în alte studii (11,42% din toate meningioamele de fosă posterioară). Vârsta medie la prezentare pentru pacienţii-femei a fost mai mare (55 ani) decât cea raportată în alte studii, iar pentru pacienţii –bărbaţi a fost de 50 de ani. Meningioamele petroclivale au inclus diferite subtipuri histologice (psamomatos, meningotelial şi secretor) şi chiar un caz anaplastic la un pacient bărbat.

Dintre meningioamele de fosă posterioară, cele localizate la nivelul foramen magnum merită o atenţie deosebită deoarece ele prezintă cel mai rău prognostic atunci când ne referim la rezultatele chirurgicale şi la morbiditatea postoperatorie.

Aceste tumori sunt rare, reprezentând doar 0,3% până la 3,2% din toate meningioamele şi între 4,2% şi 20% din toate meningioamele de fosă posterioară (9,13, 15). Datorită rarităţii cazurilor prezentate, există puţine informaţii privitoare la histologia acestor tumori. Tsao et al. au prezentat histopatologia unui meningiom de foramen magnum arătând că era subtipul meningotelial (16), iar Avninder et al. au prezentat un caz de meningiom bogat în limfoplasmocite în această localizare (17).

În studiul de faţă, meningioamele de foramen magnum au afectat doar femeile aflate în decada a 4-a de viaţă până în decada a 6-a de viaţă (vârsta medie fiind de 52,66 ani). Toate meningioamele de foramen magnum au fost benigne (gradul I), iar subtipul psamomatos a fost cel mai frecvent întâlnit.

Meningioamele de convexitate cerebeloasă reprezintă 6-18% din toate meningioamele de fosă posterioară (9). În studiul de faţă, 14,26% din cele 35 de cazuri de meningioame de fosă posterioară s-au localizat la nivelul convexităţii cerebeloase. Raportul femei : bărbaţi a fost de 4 : 1. Vârsta medie a pacienţilor-femei cu meningioame de convexitate cerebeloasă a fost mai mare (64,25 ani) decât vârsta medie a întregii serii. În această localizare, toate tumorile identificate au prezentat numai histologie benignă (subtipul fibros, 100%).

Deşi mai puţin de 20% din toate meningioamele intracraniene apar în fosa posterioară, aproape jumătate din ele sunt localizate în unghiul ponto-cerebelos. În studiul nostru, meningioamele de unghi ponto-cerebelos reprezintă 48.57% din toate cele 35 de cazuri ale seriri. Am întâlnit o evidentă predominenţă a pacienţilor-femei (raportul femei/bărbaţi = 5 : 1). Vârsta medie, indiferent de sexul pacienţilor, a fost relativ apropiată de vîrsta medie a seriei. 11,74% din meningioamele de unghi pontocerebelos au avut gradul II de malignitate iar restul de 88,23% au evidenţiat o histologie benignă. În această locaţie s-au întâlnit variate subtipuri histologice (fibros, meningotelial, secretor). Numai în această locaţie s-a identificat subtipul angiomatos.

CONCLUZII

În seria noastră, meningioamele de fosă posterioară s-au dovedit a avea un spectru variat în ceea ce priveşte datele demografice şi histologice atunci când le comparăm funcţie de locul de origine a tumorii. Am

identificat o asociere semnificativă între vârstă şi sexul pacienţilor, histologia şi locul de origine a acestor tumori.

S-a constatat o predominenţă a pacienţilor-femei cu o vârstă medie situată în decada a 6-a de viaţă. Meningioamele benigne (grad I) au reprezentat categoria unică în cazul localizării la nivelul convexităţii cerebeloase şi la nivelul foramen magnum, existând subtipuri diferite (de ex. cel fibros versus cel angiomatos) ce au caracterizat fiecare locaţie.

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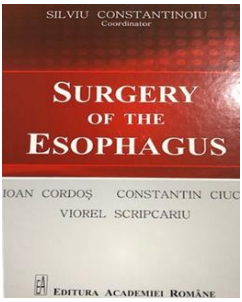
BOOK REVIEW



SURGERY OF THE ESOPHAGUS

Silviu Constantinoiu, Ioan Cordoș, Constantin Ciuce, Viorel Scripcariu

Editura Academiei Române 2019

This year, the Romanian Academy Publishing House published a treatise on esophageal pathology and surgery, under the coordination of Professor S. Constantinoiu and the editorial staff comprised of I. Cordoş, C. Ciuce, V. Scripcariu, a remarkable editorial event for the Romanian medical literature. The conception, organization and publication of such a treatise covering all aspects of the esophageal surgical pathology demonstrates the strength of the contemporary Romanian surgical community, which the publisher managed to mobilize, and the continuation of an internationally recognized tradition through the graces of Professor Dan Gavriliu, who published about esophageal surgery in 1957.

This work reflects the achievements of Romanian surgery and a synthesis of the progresses that esophageal surgery has seen over recent years.

Throughout its 839 pages, the treatise, well-structured in 17 chapters, brings together a wide range of national and international collaborations (47 authors) and provides specialists with the latest data on esophageal surgical pathology.

The first chapter, signed by Professor S. Constantinoiu, also passionate about the history of medicine, describes in detail the history of esophageal surgery. Along with the general history of esophageal surgery, the chapter presents the first efforts of the Romanian school of surgery regarding the esophagus - Amza Jianu, Iancu Jianu, Dan Gavriliu, as well as other Romanian surgeons from the past or present and their surgical centers in Bucharest, Iasi, Cluj, Timisoara, Oradea, Constanta, Craiova and Sibiu.

The following 4 chapters address the surgical anatomy and the physiology of the esophagus (D. Predescu, S. Constantinoiu), superior digestive endoscopy in esophageal pathology (Daniela Dinu, P. Hoară, Irina Predescu, D. Predescu), esophageal imaging (D. Predescu, S. Constantinoiu) and functional investigations (P. Hoară, Rodica Bârlă).

A textbook on esophageal surgery would not have been complete without a chapter on anesthesia and intensive care (Chapter 6). The first sub-chapter, the one on anesthetic technics, is written by dr. Radu Stoica and dr. Genoveva Cadar. The following well-documented sub-chapters deal with nutrition and intensive care in patients with esophageal pathology. These sub-chapters are written by prof. dr. Ioana Grigoras, dr. Irina Ristescu and dr. Emilia Patrascanu, who co-authored the chapter on intensive care (from the Regional Oncology Institute of Iasi).

Chapter 7 extensively describes esophageal atresia and tracheoesophageal fistulae (R. Drașovean, C. Ciuce).

A complex chapter (Chapter 8), which captures the reader through the novelty of

the information provided, refers to gastro-esophageal reflux pathology, which is so common nowadays. Hiatal hernias (S. Constantinoiu, Cristina Gîndea), signs of reflux disease (GERD) and its complications (A. Constantin, Fl. Achim), functional and imaging investigations in GERD (P. Hoară, Daniela Dinu, A. Caragui) are presented in great detail. Barrett esophagus (Rodica Bîrlă) occupies a central spot, being approached from a multidisciplinary point of view: diagnosis, pathological anatomy, complications, indications for medical and surgical treatment, adeno-carcinoma developed on Barrett esophagus. Next, the chapter also covers medical treatment in GERD, indications of surgical treatment in hiatal hernia and GERD, classical and laparoscopic surgical techniques in hiatal hernia and GERD (Nissen, Dor and Toupet, Belsey - Mark IV operation, surgery for GERD complications, esophageal stenting etc.) (S. Constantinoiu *et al.*), unconventional minimally invasive therapy for GERD (A.E. Nicolau, A. Lobonţiu), reflux disease associated with obesity (C. Copăescu), hiatal ant reflux surgery failures, Roux-en-Y procedure in GERD (I.N. Mateş).

Esophageal motility disorders and their morphological consequences on the surgical approach follow: the Zenker and Killian –Jamieson diverticula, the medial thoracic diverticulum and the epiphrenic diverticulum, each described in a separate chapter (Chapter 9). Due to their extensive experience in this area of esophageal pathology, the team from the Clinical Hospital "Sf. Maria" explains in the chapter on achalasia cardia its multiple etiopathogenetic hypotheses, clinical appearance, diagnostic (esophageal manometry, barium exam and endoscopy). The treatment is multimodal, but mainly surgical – LES myotomy. The latest trend in Heller myotomy is also presented – sectioning LES muscles endoscopically. Other therapeutic methods in achalasia cardia are described as well: medication (calcium channels blockers, nitrates), local injections of botulinic toxin, pneumatic dilation, together with their limitations. Dr. Matias Losano Brotons from South America has described with accurate information the Chagas disease

Chapter 10 is dedicated to post-caustic esophagitis: etiology and diagnosis (D. Predescu), treatment (V. Binţinţan, C. Ciuce), esophageal reconstruction techniques using right ileo-colon and transverse colon, left colon (D. Predescu, S. Constantinoiu).

The following two chapters address esophageal foreign bodies and trauma to the esophagus (perforations and contusions, Boerhaave syndrome).

Chapter 13 treats benign esophageal tumors (I. Cordoş, C. Bolca). All types of esophageal benign tumors are described: the parietal structure they originated from, their ways of development (intraluminal, interparietal, extra parietal), the possibility of compression on neighboring organs (trachea, heart, aorta). The possible surgical approaches are also mentioned: endoscopic, classical or minimally invasive. The main aims are removing the benign tumor and saving the host organ.

An important chapter, extensively developed, is Chapter 14, dedicated to esophageal cancer, with 8 subchapters: epidemiology and etiopathogenesis (S. Constantinoiu, I.N. Mateş), clinical aspects (R. Scurtu, G. Dindelegan, C. Ciuce), imaging and staging of esophageal cancer (V. Scripcariu, B. Filip), pathological anatomy (Cristina Iosif, Simona Enache), radiochemistry in esophageal cancer (Rodica Anghel), stenting in locally advanced stages (G. Dimofte), and esophageal bypass (I.N. Mateş, S. Constantinoiu).

Chapter 15 extensively presents the sur-

gical treatment of esophageal cancer: approaches of the cervical and cervical-mediastinal esophagus (S. Constantinoiu, Fl. Achim), reconstruction of the pharynx and cervical esophagus through free jejunum transfer (C. Ciuce), esophagus approaches via the thorax (I. Cordoș, C. Bolca), Sloan Orringer trans hiatal esophagectomy (S. Constantinoiu), McKeown triple approach esophagectomy, radical esophagectomy (I. Cordoș), three-field lymphadenectomy in esophageal cancer (V. Scripcariu, B. Filip), minimally invasive esophagectomy, robotic esophagectomy (S. Constantinoiu, Fl. Achim), reconstruction of the esophagus with colonic interposition (R. Scurtu, C. Ciuce), or with the stomach (I. Cordoș) in esophageal cancer, postoperative period (Luminița Cociu), complications of esophageal resection (C. Ciuce), quality of life and prognosis in esophageal cancer patients (V. Scripcariu, B. Filip).

Chapter 16 provides new data on esogastric junction adenocarcinoma: the relation between GERD-Barrett's esophagus-EGJ ADK, the classification of EGJ adenocarcinomas, the clinical presentation, imagistic investigations, staging, pathological anatomy, endoscopic treatment (endoscopic mucosal resection, endoscopic submucosal dissection (ESD) and endoscopic ablation techniques – radiofrequency, PDT therapy, APC, laser therapy, cryotherapy), surgical treatment (the Ivor Lewis procedure, total gastrectomy with trans hiatal Hill esophagectomy and Roux- en-Y reconstruction, the triple approach of EGJ adenocarcinomas), radio chemotherapy, palliative treatment, survival and prognosis.

The last chapter presents an exhaustive analysis of GIST in general and of the esophageal ones in particular (M. Gheorghe, S. Constantinoiu) as well as the study of other rare esophageal tumors (I.N. Mates, M. Gheorghe). Both sub-chapters present an update in the study of these tumors.

The treatise, based on a rich Romanian experience and on the study of an exhaustive recent bibliography is easy to go through due to the concise, orderly and clear style and to modern editing. The very rich, good quality, color iconography renders the work an academic feel.

The excellent graphic state honors the Romanian Academy Publishing House, which has published this volume. The treatise is addressed to resident doctors, specialists and consultants of general surgery, thoracic surgery, ENT, oromaxilofacial surgery, but also to residents and specialists in related fields (gastroenterology, anesthesiology and intensive care), as well as to all those who want to add to their knowledge of surgery.

As the renowned thoracic surgery Professor T. Horvat said in his foreword, *"this treatise is the missing granite stone from the edifice of Romanian surgical literature, it is the treatise of the current generation of surgeons, which will only be written once again after a generation will have passed"*.

The publishing of this masterly treatise, the most complete and up-to-date in Romanian literature, represents a special editorial event, and the editor, known for his high professionalism, for the passion and perseverance he puts into everything he accomplishes, is part of the rare category of people who make up the drive and the engine of going forward.

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CLINICAL APPLICATIONS OF MOLECULAR BIOMARKERS IN PROSTATE CANCER DETECTION

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CLINICAL APPLICATIONS OF MOLECULAR BIOMARKERS IN PROSTATE CANCER DETECTION (Abstract): Prostate cancer is a heterogeneous disease with regard to molecular alterations and clinical course. Early diagnosis of prostate cancer can increase the curative success rate for this disease. Because of the recent developments in the field of molecular biology, an increased interest occurred for molecular biomarkers, as tools for early prostate cancer detection, monitoring disease progression, predicting disease recurrence and therapeutic treatment efficacy. Many molecular biomarkers have been discovered in human serum, urine, seminal fluid and histological specimens. **Key words**: PROSTATE CANCER (PCA), BIOMARKERS, BENIGN PROSTATIC HYPERPLASIA (BPH)

THE ACCURACY OF AI-ASSISTED IMAGING DIAGNOSIS *VERSUS* CONVENTIONAL EVALUATION OF DENTAL PROFESSIONALS

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THE ACCURACY OF AI-ASSISTED IMAGING DIAGNOSIS *VERSUS* CONVENTIONAL EVALUATION OF DENTAL PROFESSIONALS (Abstract): The accurate detection of dental caries is crucial for effective treatment planning and prevention of disease progression. Artificial intelligence (AI) has emerged as a valuable tool in odonto-periodontal imaging, offering potential improvements in diagnostic accuracy compared to traditional methods employed by dental professionals. This study **aimed** to compare the accuracy of AI-assisted odonto-periodontal imaging in detecting dental caries with diagnoses established by general dentists, specialists, and senior specialists. **Materials and methods:** A comparative analytical study was conducted on a sample of 60 dentists categorized into three professional groups-general dentists, specialists, and senior specialists-alongside AI diagnostics using the Planmeca Romexis platform. **Results:** High-quality panoramic radiographs (OPG) representing various carious lesions were analyzed independently by all participants. Statistical analysis was performed using *SPSS version 26.0,* employing chi-square and ANOVA tests to assess differences among groups. Senior specialists and AI reported the highest number of detected lesions, with AI demonstrating a lower standard deviation, indicating greater consistency. The ANOVA test confirmed significant differences in caries detection across groups ($F=18.849$, $p<.001$), highlighting the superior diagnostic performance of AI. **Conclusions:** This study demonstrates the significant advantages of AI-assisted diagnostic tools in dental-periodontal imaging, particularly in the detection of carious lesions. **Keywords**: AI, DENTAL CAVITIES, ORAL DIAGNOSIS, DENTAL IMAGING.

INTRODUCTION

The capacity of human readers to evaluate a wide range of qualitative aspects is restricted, and their performance varies, when it comes to interpreting medical images. On the other hand, AI is able to reliably and consistently assess a wide variety of quantitative characteristics. Diagnostics,

prognoses, treatment efficacy, and risk assessment are all areas that can benefit from these imaging biomarkers (1, 2).

The use of AI has revolutionized the dental industry and is now an integral part of patient care. Improved diagnostic imaging, treatment planning, patient management, and workflow optimization are just a few ways it has revolutionized dentistry. Image interpretation, charting automation, and treatment result prediction are all enhanced by it (1). Radiographic and CT scan analysis is made more accurate, allowing for more precise diagnosis of diseases including cavities and bone resorption, thanks to its advanced algorithms. Artificial intelligence (AI) uses predictive analytics to develop individualized treatment plans and optimizes the production of orthodontic devices like aligners. Improving patient outcomes is another benefit of AI's real-time clinical decision assistance and thorough risk evaluations (1-3).

It is now generally acknowledged that radiography may greatly benefit from the integration of AI in order to decrease human error, boost efficiency, and accomplish goals with little to no human intervention (3). Conventional imaging modalities, including X-rays, may not pick up on symptoms of illness that aren't immediately obvious (4). By evaluating radiographs and imaging modalities alone or in combination, AI systems may be able to routinely detect these tiny changes, seemingly with amazing precision (5), enabling prompt intervention (6).

Orthopantomogram (OPG), also known as panoramic radiography, is an essential tool in dentistry and orthodontics, providing a detailed image of the dental and bony structures of the oral cavity. By interpreting an OPG, a specialist can identify a range of dental and bone conditions and abnormalities (7).

Recording the patient's oral health and identifying and diagnosing problematic symptoms, such as cavities, are common recurrent activities in the dental field. Personalized suggestions for preventative and surgical care are the ultimate outcome of this evaluation (8). Visual examination is the gold standard in clinical practice since it is simple to do and yields satisfactory results following dental washing and drying. Despite the fact that educated dentists often have strong intra- and inter-examiner repeatability, inconsistent diagnoses are nonetheless seen frequently in clinical practice, even though diagnostic studies have proved this (3, 9, 10, 11). So, it may be a good idea to use AI approaches for independent verification (12-14).

This study proposes to comparatively evaluate the accuracy of AI-assisted odonto-periodontal imaging diagnosis versus the one established by dentists, specialists and senior specialists.

MATERIALS AND METHODS

The comparative analytical study aimed at assessing the accuracy of AI-assisted dental and periodontal imaging diagnosis versus that established by general dentists, specialists and senior specialists was conducted on a group of 60 dentists selected based on their level of professional training.

Study group:

- **Group 1:** 20 general dentists.
- **Group 2:** 20 specialists
- **Group 3:** 20 senior specialists
- **Group 4:** The artificial intelligence (AI) system used for diagnosis (Planmeca Romexis).

To improve diagnosis accuracy and simplify operations in dental clinics, Planmeca Romexis® incorporates several tools and features into its complete software platform. Using cutting-edge imaging technology and AI, it can identify periodontal diseases and cavities, which are two

of its most remarkable features.

When used with Planmeca Emerald® intraoral scanners, the software is compatible with the scanning tip known as Planmeca Cariosity®. In order to identify dental cavities, this set uses radiation-free transillumination technology. Clinicians can see into tooth structure with near-infrared light, which helps them detect early-stage fractures, approximal, occlusal, and secondary cavities. For additional analysis and patient communication, the recorded photos can be saved in the Romexis database.

Dental practitioners benefit from early diagnosis and treatment planning with the help of Planmeca Romexis® software's superior imaging capabilities, which enable the identification of numerous coronal lesions. The method is able to identify a variety of coronal lesions, including:

- calculus deposits
- signs of various dental pathologies and other treatable conditions found in dental radiographs - including hard-to-detect issues such as early-stage cavities or initial signs of periapical radiolucency
- the percentage of carious involvement in the enamel and dentin

The software can identify potential cavities and assist in determining the best treatment plan, such as a filling, an inlay, an onlay, a crown, endodontic treatment, or an extraction. Findings are highlighted with colors and text, providing an easily understandable, independent opinion that helps build patient trust. This enables timely interventions and offers long-term health benefits by improving patient retention.

Selection of cases. Different dento-periodontal diseases (such as periodontitis, interproximal cavities, alveolar bone loss, and periapical lesions) were depicted in six high-quality radiographic pictures (OPG). To make sure the reference diagnosis was legitimate, a senior specialist and an expert in dental imaging had already verified the instances in question.

Participant Intervention. Without seeing the patient's medical history, each participant independently assessed the collection of photos and made a diagnosis based on their findings. A deep learning system trained on comparable data was used to construct the AI diagnostic.

Assessing Performance. All of the dental teams' and the AI's diagnoses were compared to the "gold standard" reference diagnostic.

Statistical analysis. After data collection was complete, *SPSS version 26.0* (IBM, USA) was used for statistical analysis. Frequencies and mean values were used to display the results. It was determined that there were statistically significant variations in the dental-periodontal assessment between the groups using procedures like the chi-square test and ANOVA analysis.

Multiple comparisons on the same data set were corrected statistically using the Bonferroni test because of the small sample size. This method is designed to lessen the likelihood of getting false positive findings because of all the tests that are run. To rule out the possibility of random effects due to multiple comparisons, the Bonferroni procedure was employed to guarantee that the observed group differences are strong and durable. The reliability of the statistical findings is guaranteed in this way. A significance criterion of $p < 0.05$ was established.

RESULTS

The results of the study provide a comparative analysis of the diagnostic accuracy of AI-assisted odonto-periodontal imaging versus that of dentists with different levels of expertise. The findings are presented in terms of diagnostic performance, highlighting the agreement between AI and human evalua-

tions relative to the established reference standard.

Table I provides an overview of dental health by analysing the total number of decayed teeth, enamel cavities, and dentin cavities. For the total number of decayed teeth, values range from a minimum of 0 to a maximum of 19, with a mean of 8.36 and a standard deviation of 4.83, indicating significant variation between cases. Enamel cavities show a lower mean of 2.64, with a range from 0 to 9 and a standard deviation of 2.75, suggesting less enamel involvement compared to dentin cavities. In the case of dentin cavities, the values are higher, with a mean of 5.73 and a maximum of 12, highlighting a more significant dental concern. The high standard deviations across all three categories reflect considerable diversity in the dental health status within the studied group.

TABLE I.

The average values of decayed teeth, enamel cavities, and dentin cavities

	Minimum	Maximum	Mean	Std. Deviation
Decayed teeth	.00	19.00	8.3636	4.82505
Enamel cavities	.00	9.00	2.6364	2.75478
Dentin cavities	.00	12.00	5.7273	2.87453
Valid N (listwise)				

Analysing the percentage distribution of the number of cavities detected by the participants reveals significant differences between professional categories: senior specialists, specialists, general dentists, and AI and data is represented below (tab. II).

TABLE II.

The distribution of the number of detected cavities cases among participant groups.

		Doctors				Total
		Senior specialist	Specialist	Dentist	AI	
Number of teeth cavities	0.00			5.0%		1.5%
	1.00		10.0%	10.0%		6.1%
	2.00		20.0%	5.0%		7.6%
	3.00			5.0%		1.5%
	4.00		5.0%	15.0%		6.1%
	5.00		10.0%	5.0%		4.5%
	6.00			10.0%		3.0%
	7.00	20.0%	5.0%	20.0%		13.6%
	8.00		15.0%	15.0%	33.3%	12.1%
	9.00		20.0%	5.0%		7.6%
	10.00	15.0%	10.0%	5.0%		9.1%
	11.00	15.0%	5.0%		16.7%	7.6%
	12.00				33.3%	3.0%
	13.00	15.0%				4.5%
	17.00	20.0%			16.7%	7.6%
	19.00	15.0%				4.5%
Total		100.0%	100.0%	100.0%	100.0%	100.0%

For senior specialists, a balanced distribution of detected cavities is observed, with notable peaks at 7 and 17 cavities (20% each). This indicates a moderate involvement in detecting more complex cases, along with a uniform distribution of the number of cavities among their patients.

In the case of specialist dentists, the distribution is more balanced, with a peak of 20% for 9 detected cavities. This suggests that these participants evaluated patients with a wide range of dental issues but did not identify all existing carious lesions.

General dentists stand out with a higher proportion of cases involving a significant number of detected cavities, such as 7 and 8 cavities (20% each). This may reflect greater expertise in identifying cavities or increased exposure to patients with severe dental issues.

The AI system recorded the highest proportion of cases with extreme values, such as 8 and 12 detected cavities (33.3% each). These results indicate an enhanced ability to detect the presence of carious lesions.

Overall, the distribution of the number of detected cavities varies significantly depending on the type of participant, suggesting differences in professional experience, clinical approach, or the specific patient population examined. These differences highlight the importance of professional context in analyzing clinical data.

The Chi-Square analysis conducted to assess the relationship between the number of detected cavities and the type of participant revealed a statistically significant association. The Pearson Chi-Square test yielded a value of 86.075, with 45 degrees of freedom and a significance value of .000, indicating a strong relationship between the variables and rejecting the null hypothesis that there is no connection between the doctors and the number of detected cavities. These results were confirmed by the Likelihood Ratio test, which produced similar values (86.846) and an identical asymptotic significance ($p = .000$) (tab. III).

TABLE III.

The relationship between the number of detected cavities and the type of participant.

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	86.075a	45	.000
Likelihood Ratio	86.846	45	.000
No. of Valid Cases	66		
64 cells (100.0%) have expected count less than 5. The minimum expected count is .09.			

A comparative evaluation of the average values of detected decayed teeth based on the participant groups was conducted (tab. IV).

Senior specialists had the highest average number of detected decayed teeth, 12.75, indicating significant involvement in evaluating more complex cases. The variability within this group is relatively high, with a standard deviation (SD) of 4.31, and the 95% confidence interval for the mean ranges from 10.73 to 14.77. The minimum number of decayed teeth detected in this group is 7, while the maximum is 19, indicating a wide range of case severity.

Specialists, on the other hand, recorded a

lower average number of detected decayed teeth, at 6.10, with moderate variability (a standard deviation of 3.45) and a confidence interval of 4.49 to 7.71. The minimum number of detected decayed teeth in this group is 1, while the maximum is 11.

TABLE IV.
Comparative evaluation of the average number of detected teeth based on participant groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Senior specialist	20	12.750	4.31491	.96484	10.7306	14.7694	7.00	19.00
Specialist	20	6.1000	3.44735	.77085	4.4866	7.7134	1.00	11.00
General dentist	20	5.3500	2.87045	.64185	4.0066	6.6934	.00	10.00
AI	6	11.3333	3.32666	1.35810	7.8422	14.8244	8.00	17.00
Total	66	8.3636	4.82505	.59392	7.1775	9.5498	.00	19.00

General dentists registered the lowest average number of detected decayed teeth, at 5.35, indicating a lower focus on identifying multiple caries. Variability in this group is lower (a standard deviation of 2.87), with a confidence interval for the mean ranging from 4.01 to 6.69. Extreme values in this group range from 0 to 10, reflecting less severe cases compared to other categories.

In the case of AI, the average number of detected decayed teeth is high, at 11.33, compared to that of senior specialists. However, the small sample size for this group (N=6) limits the robustness of the conclusions. The standard deviation is 3.33, and the confidence interval for the mean ranges from 7.84 to 14.82. Extreme values in this group range from 8 to 17, suggesting a high severity level of the analyzed cases.

Overall, the average number of detected decayed teeth across all categories is 8.36, with a standard deviation of 4.83, indicating significant variability between groups. The values range from 0 to 19 decayed teeth, with a confidence interval for the mean ranging from 7.18 to 9.55.

These results reflect the diversity of the analyzed cases and highlight the differences between groups in terms of professional experience and the types of patients evaluated.

The ANOVA test results show statistically significant differences in the number of detected decayed teeth among the participant categories. The high F-value (18.849) and $p < .001$ indicate that the type of participant has a significant effect on the number of detected decayed teeth. To better understand which groups, differ from each other, a post hoc analysis (e.g., Tukey test) would be useful to identify specific group pairs with significant differences.

TABLE V.
ANOVA test for identifying differences in detected caries among participant groups

Decayed teeth					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	721.839	3	240.613	18.849	.000
Within Groups	791.433	62	12.765		
Total	1513.273	65			

The detection of enamel caries can be challenging, as they are often asymptomatic in the early stages and may require a combination of careful visual examination and detailed interpretation of radiographic images, such as orthopantomograms or periapical radiographs, to be accurately identified.

Descriptive analysis indicates significant differences between the participant groups (tab. VI).

TABLE 6**.**
Comparative evaluation of the average values recorded across groups.

	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
Senior specialist	20	4.7500	2.80741	.62776	3.4361	6.0639	.00	9.00
Specialist	20	1.5000	1.73205	.38730	.6894	2.3106	.00	5.00
General dentist	20	.8000	1.23969	.27720	.2198	1.3802	.00	5.00
AI	6	5.5000	2.25832	.92195	3.1300	7.8700	2.00	9.00
Total	66	2.6364	2.75478	.33909	1.9592	3.3136	.00	9.00

Senior specialists and AI reported the highest average number of cases (4.75 and 5.50, respectively), while specialist and general dentists recorded lower averages (1.50 and 0.80). The confidence intervals for the means of AI and senior specialists partially overlap, suggesting similarities in case detection. However, AI had the highest overall average and moderate variability, indicating greater consistency in reporting compared to senior specialists.

The dispersion of data varies significantly among the groups. Specialist and general dentists show low variability, indicating greater consistency in their results but also a possible underestimation of cases. In contrast, senior specialists exhibited a higher standard deviation (2.81), reflecting greater variability in caries detection. AI, with a standard deviation of 2.26, demonstrated relatively consistent detection, comparable to that of senior specialists in terms of maximum values.

Based on the minimum and maximum values, AI and senior specialists detected the most severe cases (up to 9 enamel caries), whereas specialists and general dentists reported a maximum of 5 cases. These findings suggest that AI and senior specialists are more sensitive in identifying extreme

cases, while specialists and general dentists tend to have a more conservative approach. The differences between groups highlight AI's potential to complement clinical diagnosis, especially in complex cases.

The ANOVA test results show statistically significant differences between the participant groups) (tab. VII).

TABLE VII.

Association of periodontal disease with the domicile of the patients

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	231.823	3	77.274	18.325	.000
Within Groups	261.450	62	4.217		
Total	493.273	65			

The F-value of 18.325 and the significance level of p=0.000 indicate that at least one group differs significantly from the others in terms of the average number of reported cases. The variability between groups (Sum of Squares = 231.823) accounts for a significant portion of the differences, while the within-group variability (Sum of Squares = 261.450) reflects individual variations within each group.

These results suggest that the observed differences are not random and confirm the existence of distinct reporting patterns among the groups. It is evident from the context that AI and senior specialists tend to report more cases, while specialists and general dentists have reported fewer cases.

DISCUSSION

The findings of this study highlight significant differences in the detection of carious lesions among the different participant groups, namely senior specialists, specialists, general dentists, and AI-assisted diagnosis.

Senior specialists and AI reported the highest number of detected carious lesions, suggesting that experience and technological support play a crucial role in caries detection. AI's ability to consistently identify carious lesions with high accuracy can be attributed to its advanced image processing algorithms and pattern recognition capabilities, which minimize the risk of oversight commonly encountered in human evaluations.

General dentists and specialists reported a lower number of detected lesions, potentially due to differences in diagnostic approach, reliance on clinical examination, and familiarity with radiographic analysis. The statistical analysis revealed a significant difference between these groups, emphasizing the variability in diagnostic accuracy based on professional experience and specialization.

The AI system exhibited a high level of consistency, with a relatively low standard deviation in the number of detected caries. This suggests that AI offers a standardized approach to diagnosis, reducing inter-observer variability that can arise in human assessments. Furthermore, AI's ability to detect caries in their incipient stages allows for earlier intervention, potentially improving long-term oral health outcomes and reducing the need for extensive treatments.

Consistent with our findings, recent research has thoroughly investigated the use of artificial intelligence (AI) for the identification and diagnosis of dental cavities. Khanagar *et al*. (15) conducted a comprehensive evaluation of artificial intelligence (AI) models developed for the purpose of

detecting, diagnosing, and predicting dental caries. Researchers found that by incorporating these models into clinical practice, diagnostic accuracy and patient outcomes might be significantly improved.

Also, Al-Khalifa *et al.* (16) reviewed the literature on dental caries and found that AI might be a useful tool for detecting the condition early and accurately. Deep learning models, such as convolutional neural networks (CNNs), can interpret several imaging modalities to aid in the identification and diagnosis of dental caries more efficiently and accurately than conventional approaches, according to the reviewed research.

Furthermore, Frenkel *et al.* (17) conducted an external validation of an AI-based model for caries detection using dental photographs. The study demonstrated that the AI model could detect, classify, localize, and segment carious lesions with high diagnostic accuracy, indicating its potential utility in clinical settings

Despite the promising performance of AI in cavities detection, certain challenges persist. The reliance on high-quality radiographic images is essential for optimal AI performance, and variations in image quality may impact detection accuracy (18). Additionally, AI algorithms are limited by the data they are trained on, which may introduce bias if the training dataset lacks diversity (19).

The integration of AI into routine dental practice offers numerous benefits, including enhanced diagnostic accuracy, improved workflow efficiency, and better patient communication through visual representation of detected lesions (20, 21).

These studies align with our findings, underscoring the significant advantages of AI-assisted diagnostic tools in dental imaging, particularly in the detection of carious lesions. The integration of AI into dental practice offers the potential for earlier intervention, improved diagnostic accuracy, and enhanced patient outcomes.

However, AI should be viewed as a complementary tool rather than a replacement

for clinical judgment, with final treatment decisions remaining under the purview of dental professionals

CONCLUSIONS

The study underscores the potential of AI-assisted diagnosis in enhancing the detection of carious lesions, particularly in cases that may be challenging for human evaluators. Future research should focus on refining AI algorithms, expanding training datasets, and exploring the integration of AI with other diagnostic modalities to further improve diagnostic accuracy and clinical decision-making.

This study demonstrates the significant advantages of AI-assisted diagnostic tools in dental-periodontal imaging, particularly in the detection of carious lesions. The results highlight AI's potential to support dental professionals in achieving higher diagnostic accuracy, reducing variability, and ensuring earlier intervention for better oral health outcomes.

Despite its strengths, AI should be integrated into dental practices with caution, ensuring that it complements rather than replaces the clinical expertise of dental practitioners. Further advancements in AI technology, along with continued training for professionals, will be essential to fully harness its benefits and address its limitations effectively.

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PROMISING BIOLOGICAL ACTIVITIES OF SESQUITERPENE LACTONES

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PROMISING BIOLOGICAL ACTIVITIES OF SESQUITERPENE LACTONES (Abstract): Sesquiterpene lactones (SQLs) are an important group of bioactive compounds that are usually found in the *Asteraceae* family, where they seem to be some of the most important constituents for defense against microorganisms. These secondary metabolites have a skeleton of 15 carbon atoms and are derived from farnesyl diphosphate. It is generally believed that they might present certain biological actions in humans. Some of these actions are worth extended research: anti-inflammatory, antitumor, antibacterial, antifungal, antinociceptive, antiulcer and hepatoprotective. It seems that the presence of the α-methylene-γ-lactone group is correlated with the existence of many pharmacological actions. Due to promising results on human cancer cell lines, the research was expanded to clinical trials investigating the antitumor potential. Sesquiterpene lactones inhibit the expression of the nuclear factor kappa-light-chain-enhancer of activated B cells, which could explain the anti-inflammatory effect. They also have good activity against Gram positive bacteria. As a result, some of these compounds show promise for practical use in the treatment of cancer, different inflammatory diseases and even as antibacterial or antifungal agents, while some have already been introduced in therapy as antimalarial agents (artemisinin and its semisynthetic derivatives). However, more studies regarding their biological activities and toxicity mechanisms are still to be done. **Keywords:** SESQUITERPENE LACTONES, BIOLOGICAL ACTIONS, ANTITUMOR, ANTI-INFLAMMATORY.

INTRODUCTION

Medicinal plants have always been an important source of therapeutic agents, having a large variety of pharmacological activities (1). Nowadays, it is estimated that secondary metabolites extracted from plants still play an important role in the production of medicines and dietary supplements, covering around half of their total number (2, 3). Taking into consideration the need for discovering new agents for existent diseases, more and more researchers are focusing on the plant kingdom in the hope of discovering new efficient treatments or confirming new actions described by traditional medicine (4).

Around 30,000 compounds have been classified as terpenoids, which show a remarkable role in the defense mechanism of the plant. Many of these exert various biological actions and are currently used in the pharmaceutical industry, which explains the constant search for new similar structures and the tests carried out for dif-

ferent types of activities (3). Some terpenoid compounds represent constituents of volatile oils that produce the specific aroma of plants (4).

Sesquiterpenes are an important and complex group of natural terpenoids, in which have been included around 11,000 structures, with almost half of them representing the subclass of sesquiterpene lactones (5).

They are biosynthetically derived from farnesyl diphosphate and their name is related to the chemical structure, which contains a 15-carbon atoms skeleton formed by three isoprene units. Sesquiterpenes may or may not contain cycles in their structure, which leads to the following classification: acyclic, monocyclic, bicyclic, tricyclic and tetracyclic sesquiterpenes (3).

Sesquiterpene lactones (SQLs) represent a highly diversified group of bioactive compounds that can be found almost exclusively in the *Asteraceae* family, but have also been identified in species belonging to the other families such as *Cactaceae* and *Solanaceae* (2, 6). These compounds are stable secondary metabolites with bitter taste and a relatively high lipophilicity, lacking color (7). They are components of the plant latex, showing good antibacterial activity, but can also be synthetized in other types of cells, especially in case of plant injury (6). Sesquiterpene lactones can be extracted with different solvents, such as methanol, acetonitrile, n-hexane, chloroform and dichloromethane or by using supercritical carbon dioxide (8). The analysis of this type of compounds can be made using chromatographic methods, such as High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC) or hyphenated methods, such as HPLC-UV and Gas Chromatography-Mass Spectrometry (GC-MS) (9, 10).

Sesquiterpene lactones can be classified according to their carbocyclic skeleton in germacranolides, which represent the largest group and are precursors for other types of SQLs, guaianolides, pseudoguaianolides, eudesmanolides, eremophilanoides and hypocretenolides (6, 7).

Supposedly, SQLs present certain therapeutic actions in humans and some of these, including the anti-inflammatory, antitumor, antibacterial, antiprotozoal, antinociceptive, antiulcer and hepatoprotective properties, are currently being investigated (2, 6, 11). Some compounds derived from artemisinin, thapsigargin and parthenolide have already been included in cancer clinical trials, given their significant cytotoxic activity (7). However, new studies have revealed other potential activities of this type of compounds (e.g. histamine H1 receptor antagonism in the gut), proving the importance of research on sesquiterpene lactones (12). However, SQLs might be responsible for a series of conditions in humans, such as contact dermatitis, and could also present genotoxic activity, reproductive toxicity and teratogenesis (13, 14).

ANTITUMOR ACTIVITY

It is believed that three major characteristics are responsible for the antitumor and anti-inflammatory activities of SQLs: alkylating properties, high lipophilicity which facilitates the passage through membranes and conformational flexibility (7). Research has shown that the α-methylene-γ-lactone moiety is of major importance regarding most biological actions exerted by these compounds (15). This structure reacts with nucleophilic groups, such as sulfhydryl groups that can be found in enzymes and proteins, leading to the disruption of different functions (2, 13). Therefore, certain SQLs have the capacity

of modifying the redox state, inducing oxidative stress and apoptosis in cancer cells in a relatively selective manner (5).

There are several ongoing cancer clinical trials in which SQLs are investigated as potential antitumor agents. Artesunate, a well-known antimalarial drug, is being investigated regarding its safety and efficiency in stage II/III Colorectal Cancer (ClinicalTrials.gov Identifier: NCT02633098), as treatment in patients with cervical dysplasia (ClinicalTrials.gov Identifier: NCT02354534) and as treatment of HPV-associated anal intraepithelial neoplasia (ClinicalTrials.gov Identifier: NCT03100045). A phase one study was also conducted to evaluate its safety and pharmacokinetic properties when administered orally in patients with advanced hepatocellular carcinoma (ClinicalTrials.gov Identifier: NCT02304289). Artemether, which is currently used to treat malaria, has also been included in a phase 1/2a study that will assess its potential use in treating cancer (ClinicalTrials.gov Identifier: NCT02263950). Another phase II trial studies how well a thapsigargin prodrug works in treating patients with recurrent or progressive glioblastoma (NLM Identifier NCT02067156) (16). The activity of some SQLs has been examined on cancer cells, revealing potential benefits in different types of cancer (tab. I).

TABLE I.
Examples of Sesquiterpene Lactones and Their Anticancer Activity Based on Research on Human Cell Lines

SQL	Type of human cancer cell line	Reference
Parthenolide	Multiple myeloma cell lines	17
	Colorectal cancer cells	18
	Non-small cell lung cancer cells	19
	Pancreatic cancer cells	20
	Cervical cancer cells	21
	Osteosarcoma cancer cell	22
	Bladder cancer cells	23
	Breast cancer cell line	24
	Prostate cancer cell lines	25
	Melanoma cells	26
	Leukemic monoblastic U937 cell line	27
Costunolide	Osteosarcoma cells	28
	Esophageal cancer cells	29
	Colon cancer cell	30
	Lung squamous carcinoma cells	31
	Breast cancer cells	32,33
	Prostate cancer cells	34
	Ovarian cancer cells	35
	Promonocytic leukemia U937 cells	36
Alantolactone	Lung Squamous Cancer Cells	37
	Breast cancer MDA-MB-231 cells	38
	Colorectal cancer cells	39
	Hepatoma cells	40
	Leukemia HL-60 cells	41
	Human glioblastoma cell lines	42

ANTI-INFLAMMATORY ACTIVITY

It is believed that SQLs can prevent the production of mediators involved in the inflammatory response, therefore modulating functions present in both acute and chronic inflammation (43).

The Nuclear Factor kappa-light-chain enhancer of activated B cells (NF-κB) represents a protein complex that plays an important part in a series of processes such as inflammation, immune regulation, cell death and proliferation (44). However, it is involved in pathological processes as well, given the fact that it controls some mechanisms through which apoptosis and metastasis develop (6). Normally, NF-κB can be found in the cytoplasm tied to its inhibitory subunit (IκBα). The existence of proinflammatory stimuli leads to the activation of IκB kinase, which causes the release of NF-κB from IκBα and its displacement into the nucleus, where it initiates the transcription of genes associated with inflammation (43). Therefore, the inhibition of this transcription factor by SQLs could reduce the inflammatory response and block the multiplication of tumor cells (6).

Nevertheless, the inhibition of 5-lipoxygenase and cyclooxygenase has also been considered as another possible mechanism of action. For example, rudbeckolide from *Rudbeckia hirta* and ziniolide from *Xanthium spinosum* show good inhibitory activity on these enzymes (45, 46).

ANTIBACTERIAL AND ANTIPROTOZOAL ACTIVITIES

To resist the attacks of fungi, bacteria and viruses, plants can produce several chemical substances, such as phenolic compounds, tannins, alkaloids and terpenoids (4). In the *Asteraceae* family, sesquiterpene lactones seem to be some of the most important constituents for defense against microorganisms (6).

The antimicrobial activity of these secondary metabolites is correlated with the presence of the α-methylene-γ-lactone functional group and of a double α, β-unsaturated carbonyl group. It is assumed that SQLs induce alteration of the phospholipidic wall of membranes and modifications of protein synthesis (47).

Sesquiterpene lactones seem to have a better activity against Gram positive bacteria than against Gram negative bacteria (48). Examples of sesquiterpene lactones with good antibacterial activity include alantolactone and isoalantolactone, which are active against *Staphylococcus aureus*, but also on *Mycobacterium tuberculosis* (48, 49). A study focusing on germacranolide shows that parthenolide was the most active of the tested substances against both *Mycobacterium tuberculosis* and *Mycobacterium avium*. The same study highlights that the α-methylene-γ-lactone moiety, the presence of another alkylating site and a good lipophilicity seem to be responsible for the antimycobacterial activity (50).

Some of these secondary metabolites possess antiprotozoal activity, artemisinin being currently used as an antimalarial agent. Its discovery in the 1970s followed by clinical observations of its potent activity against *Plasmodium falciparum* led to its introduction in therapy along with other semisynthetic derivatives (4). It is believed that the compound acts in two phases. The intra-parasitic iron is thought to be responsible for the breakage of the endoperoxide moiety, followed by production of free radicals, which will eventually form stable bonds with the parasitic proteins (1).

SQLs are also active on other parasites such as *Trichomonas vaginalis* and *Entamoeba hystolitica* (51, 52).

ANTIFUNGAL ACTIVITY

Sesquiterpene lactones from *Centaurea thessala* and *Centaurea attica* have been investigated for their antifungal action, showing good activity against fungi from the *Aspergillus* and *Penicillium* genera (53). A study that included 36 SQLs emphasized the good antifungal activity of some SQLs such as dehydrozaluzanin C, dehydrocostus lactone, costunolide and zaluzanin C against *Colletotrichum acutatum* and *C. fragariae* (54).

In a research conducted by Picman, almost half of the 45 sesquiterpene lactones considered showed activity against *Microsporum cookei, Trichophyton mentagrophytes*, while only a few demonstrated activities against *Fusarium* spp., highlighting that eudesmanolides seem to be the most active (55). It was also proved that some guaianolides can inhibit the growth of *Candida albicans* (56). However, the research done to study the antifungal properties of this class of secondary metabolites is still insufficient.

CONCLUSIONS

Sesquiterpene lactones are a large class of constituents of the *Asteraceae* family with many potential therapeutic actions due to the α-methylene-γ-lactone group and other characteristic functional groups.

Some of these compounds show promise for practical use in the treatment of cancer, different inflammatory diseases or even as antibacterial agents, but extensive research on pharmacological actions and toxicity is still to be done.

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NEWS

BLOCK COPOLYMER-BORON CLUSTER CONJUGATE FOR EFFECTIVE BORON NEUTRON CAPTURE THERAPY OF SOLID TUMORS

The cancer is considered a major health problem worldwide and a big socio-economic burden which increases day by day. The mainstream treatment modalities in treating this disease are surgery, chemotherapy and radiotherapy. Boron neutron capture therapy (BNCT) is a selective tumor radio-therapeutic technique which is emerging as a hopeful tool in treating of cancer. This method is based on the administration of 10B-compounds in selectively tumor tissues and further thermal neutron irradiation of these to kill the cancer cells. Until now, the boron derivatives of sodium borocaptate and borophenylalanine presented an important potential for cancer therapy, especially in the cases where the traditional methods are difficult to be applied. In this study, the authors developed new block copolymer boron cluster conjugates, using sodium borocaptate (BSH) and poly(ethylene glycol)-b-poly(glutamic acid) copolymer in order to obtain a safe and efficient boron neutron capture therapy (an homogenous delivering of the boron clusters into the tumor cells and an effectively penetration in cancer tissues). The both synthetized conjugates showed a high cellular uptake by cancer cells and a high tumor accumulation, the most important elements for an efficient BNCT. The PEGylated BSH-polymer conjugate demonstrated a better profile in comparison with the non-PEGylated one. The obtained results are promising in the cancer therapy using PEG-modified clusters to develop new drug delivery systems (Mi P, Yanagie H, Dewi N, Yen H-C, Liu X, Suzuki M, Sakurai Y, Ono K, Takahashi H, Cabral H, Kataoka K. Block copolymer-boron cluster conjugate for effective boron neutron capture therapy of solid tumors. *Journal of Controlled Release* 2017; 254: 1-9).

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COLORECTAL CANCER: A SMALL REVIEW OF CURRENT AND FUTURE MOLECULAR SCREENING MARKERS AND THE FIRST STEPS TO PERSONALIZED MEDICINE

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COLORECTAL CANCER: A SMALL REVIEW OF CURRENT AND FUTURE MOLECULAR SCREENING MARKERS AND THE FIRST STEPS TO PERSONALIZED MEDICINE (Abstract): Colorectal cancer is the third most common cancer, the fourth cause of death globally and represents 9.4% of all incident cancer in men and 10.1% in women, with high incidences reported in North America, Oceania and Europe, and low incidences in Asia and Africa. The improved prognosis in the past decades, and a 5-year survival rate which exceeds 65% in developed countries, demonstrate that colorectal cancer is a curable disease. Colorectal cancer is a very heterogeneous condition, in which early diagnosis and specific treatment project it in the curable area of disease, therefore a screening process must ensure effectiveness in reduction of disease mortality and cost. Serological markers have some limitations, but molecular markers, that explore chromosomal and microsatellite instability and mutations, are gaining ground to become the golden standard and will lead the way in new screening methods and personalized medicine guidelines. A major future challenge for all cancer biomarkers will be the integration of pharmaco-genomics, proteomics and metabolomics functional data, with epidemiological and clinical data. **Keywords**: COLORECTAL CANCER, MOLECULAR MARKERS, SINGLE NUCLEOTIDE POLYMORPHISM, PERSONALIZED MEDICINE.

Colorectal cancer is classified as the third most common cancer and the fourth cause of death globally, accounting for roughly 1 to 2 million new cases and 600,000 deaths per year in the USA (1-3). Although it's incidence is low at ages younger than 50 years, median age at diagnosis is about 70 years in developed countries (4). Worldwide colorectal cancer represents 9.4% of all incident cancer in men and 10.1% in women, however, it is not uniformly common throughout the globe with different populations experiencing different incidence rates, with high incidence reported in countries from North America, Oceania and Europe, and low incidence in countries of central Asia and Africa (5, 6). With improved, slow but steady, prognosis in the past decades, and a 5 year survival rate that exceeds 65% in developed countries, colorectal cancer is being seen as a curable disease with new molecular technologies used in prediction, diagnosis, and recurrence risk (4, 7-9). Unlike other types of cancer, no single risk factor can be pinpointed as having a high

prevalence, and a combination of multiple factors are highlighted in epidemiological studies. Of those cited we remind family history of colorectal cancer (10), diabetes (11), inflammatory bowel disease (12), smoking (13), excessive alcohol consumption (14), obesity (15), and high consumption of red and processed meat (16). The risk increase is strongest for people with first-degree relatives with colorectal cancer (those with relatives diagnosed at young ages or multiple affected relatives) and people with inflammatory bowel disease, and according to a large twin study, 34 35% of colorectal cancer risk may be attributed to heritable factors (17). Apart from hereditary forms, such as hereditary non-polyposis colon cancer like Lynch syndrome and familial adenomatous polyposis, which are understood as being determined by well-known genetic aberrations, that represent less than 5% of all colorectal cancer, genetic risk determining factors are still not yet understood (18). It is believed that the cause of colorectal cancer may lie in a cascade of genetic mutations that describes the adenoma-carcinoma sequence, in which normal epithelial mucosa gradually progresses to adenoma and then to carcinoma as a direct result of these changes (19, 20). Increased screening would most likely result in cost savings to the healthcare system, since more CRCs would be detected at an earlier stage and newer, more expensive chemotherapies could be avoided (21).

Molecular pathogenesis

Colorectal cancer molecular pathogenesis is very heterogeneous and it result is the loss of genomic stability , which needs to be understood if prognosis and treatment response are to be optimal (22-25). Several forms of genetic instability have been identified and include epigenetic gene instability, microsatellite instability(MSI), and chromosomal instability(CIN), of which the ladder includes 80-85% of all colorectal cancers through oncogene activation and loss of heterozygosity of tumor suppressor genes, and it is suggested that chromosomal instability induces carcinoma through the mutation or loss of the TP53 and APC genes, and activation of the KRAS, with a poor prognosis (19, 26, 27).

Microsatellite instability

Representing about 15% of CRC, microsatellite instability consists of deletions and insertions of short repetitive DNA sequences in a given gene, a consequence of DNA mismatch repair deficiency, where 3% of these are associated with Lynch syndrome and the other 12% are caused by sporadic, acquired hypermethylation of the promoter of the MLH1 gene, which occurs in tumors with the CpG island methylator phenotype (19, 28, 29). Although any single MSI in a gene may not have a significant subclinical or clinical impact, their accumulation can give rise to tumor progression.(19) They are frequently found in tumor suppressors such as activin receptor type 2 (83%) and transforming growth factor βR2 (69%) (19). MSI has a significant impact on tumor biology and is reflected by a more favorable prognosis of this molecular CRC subtype and a lacking response of MSI-H CRCs to 5`fluorouracil monotherapy (30). MSI status is determined in poorly differentiated cancers according to conventional histologic grading.

Epigenetic gene silencing

All stages of tumor formation to progression have been associated with chromatin alteration consisting in DNA methyla-

tion. Cancers that present a great degree of DNA methylation can be considered CpG island methylation phenotype (CIMP)(31). In normal cells CpG islands are found in regions close to promoters, and if there is an aberrant hypermethylation tumor suppressor genes can be sliced repressing gene expression (19, 31, 32). Reversing gene silencing may be useful in cancer prevention and therapy.

Genome-wide association studies

Genome-wide association studies (GWAS) have evolved over in the last years into a powerful tool for investigating the genetic architecture of human disease (33). They analyze and measure the variation in the genome's DNA sequence to identify genetic disease risk factors that are common in each population and rely on the identification of SNPs. The modern unit of genetic variation is the single nucleotide polymorphism or SNP. SNPs are single base-pair, high frequency occurring changes in the human DNA genomic sequence (34).

SNPs

They are best described as a variation at a single position in a DNA sequence of four nucleotide bases: A, C, G, and T. If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP(35). If a SNP occurs within a gene, then the gene is described as having more than one allele in which case, SNPs may lead to variations in the amino acid sequence. However, SNPs, are not just associated with genes as they can also occur in noncoding regions of DNA.

Most SNPs have no effect whatsoever on health and development, but some of these genetic differences, however, have proven to be quite important in the study of human health. SNPs may help predict the individual's response to certain drugs, susceptibility to environmental factors, and risk of developing a particular disease. SNPs can also be used to track the inheritance of disease genes within families. Typically SNPs are used as *markers* of a genomic region, relatively even distribution in the human genome, with the large majority of them having a minimal impact on biological systems but can have functional consequences especially it they are found in coding regions (36-38). SNPs are increasingly becoming the DNA marker system of choice due to their prevalence in the genome and their ability to be used in highly multiplexed genotyping assays.

Identifying genes involved in the development of cancer is crucial to fully understand cancer biology, for developing novel therapeutics for cancer treatment and for providing methods for cancer prevention and early diagnosis. The use of polymorphic markers, in particular single nucleotide SNPs, promises to provide a comprehensive tool for analyzing the human genome and identifying those genes and genomic regions that contribute to the cancer phenotype (9, 39).

Whole genome sequencing

Today whole genome sequencing is the best method to obtain all an individual's genetic information, practically drawing a comprehensive genetic map, and even though the cost of sequencing is constantly falling, new technological advancements are required to make this method a cost-effective screening process

Clinical application of molecular markers

Many CRC remain asymptomatic years

before they are diagnosed due to the time needed for the genetic mutation to take place. CRC has two gastrointestinal tumor markers used for screening carcinoembryonic antigen (CEA) and carbohydrate antigen (CA). Although elevated levels are associated with disease progression, they can be under the detection range until advanced disease stages are present, and postoperative they are important in disease recurrence.(40). Molecular markers have the advantage that they can be an early warning system not only raising awareness, but by their non-invasive nature be more appealing with a far greater compliance than standard screening methods such as colonoscopy and fecal occult blood test(FOBT).They can encourage more patients to undergo screening, and could significantly decrease CRC mortality.

Current molecular markers used in diagnosis

CIN markers include the RAS family inner surface plasma membrane protein encoders (HRAS, NRAS, KRAS) of which KRAS plays the leading role(26). It encodes GTP binding proteins that act like molecular switches, inactivating intracellular signal transducers for surface receptors such as EGFR (epidermal growth factor receptor), being an intracellular downstream component of the EGFR signaling cascade. Once activated the RAS gene suppresses apoptosis and promotes cell survival. From an oncogenic point of view the RAS family mutation is an early CRC event and as many as 37% of CRCs and 50% of adenomas larger than one centimeter exhibit this mutation(27). In 2008, exclusion of KRAS hot spot mutations in exon 2 has become mandatory for the application of EGFR-targeting antibodies in the first-line therapy of advanced colon cancer. The list of genetic alterations to be excluded for EGFR antibody therapy has been extended to KRAS exons 3,4 and NRAS exons 2–4 in 2013 after no responsiveness has also been found for these alterations(41). Other genetic changes like BRAF and PI3K mutations have been described to be associated with no, to some, EGFR antibody response, but the data generated in a different study is too controversial to include these markers in routine predictive molecular testing and therapy decision making(41-43). It is foreseeable, however, that the number of markers predicting therapy response in CRC will rise with new data being generated from larger CRC cohorts.

MSI markers identification relies on two main methods of detection that highlight genetic mechanism causing the disease, and are represented by polymerase chain reaction (PCR) and immunohistochemistry. MSI-H is a trademark alteration of hereditary nonpolyposis colon cancer (HNPCC)/Lynch syndrome-associated tumors, but is also found in sporadic colon cancers (44, 45). With the revised Bethesda guidelines in mind, colon cancer is tested for MSI to evaluate the possibility of Lynch syndrome (46). In combination with immunohistochemical analysis of the mismatch repair proteins (MLH1, MSH2, PMS2 and MSH6) and EPCAM40 the molecularly determined MSI status is the first step in genetic determination and counseling. Among the tested regions, when MSIs are present in two or more regions, the tumor is classified as MSI-High, otherwise, the tumor is considered as MSI-Low (MSI in one region) or MSI-Stable (no MSI) (28).Only in the absence of MSI-H, histological poor /high-grade differentiation (G3–4) is considered a prognostic marker while MSI-H cancers are considered low

grade(41).

Plasma methylated Septin 9 (SEPT9) DNA is a marker of colorectal cancer(3). SEPT9 gene encodes the Septin 9 protein, which is a member of GTP-binding proteins that involves in many cellular processes such as cell cycle (47, 48). Disruption of SEPT9 gene could result in tumor formation. In comparison with normal individuals the biomarker septin-9 has been found to be hyper methylated in nearly 100% of tissue neoplasia specimens and detected in circulating DNA fractions of CRC patients(49). In a study of 8000 patients that underwent routine screening colonoscopy, blood was collected and the results from the septin-9 test were compared to those of colonoscopy with a 67% CRC detection rate and 11% false positive rate (47). It is a sensitive screening test that can detect the majority of CRC, and can be combined with colonoscopy to ensure (50) .

In the past few years GWAS have found some heterogeneity in colorectal cancer risk, and many polymorphisms have been showed to have a link in risk assessment, early diagnosis, evolution, and recurrence of CRC. As databases like SNPedia or DBsnp are continuously updating, there isn't yet a SNP or SNPs that can make up a screening panel that will have a good sensibility and specificity. Although studies have shown an association between the presence of a certain SNP and CRC there is still little data that can standardize a single nucleotide polymorphism to a given condition, and with hole genome sequencing still at an excessive cost, modern technology is needed to ensure a cost-effective reliable method

CONCLUSIONS

Colorectal cancer is a very heterogeneous condition in which early diagnosis and specific treatment project it in the curable area of disease, therefore a screening process must ensure effectiveness in reduction of disease mortality and cost. Serological markers like CEA and CA, have some limitation and in many cases unreliable in early detection, as well as imaging and invasive methods where the patient's compliance is key. Molecular markers that explore chromosomal and microsatellite instability as well as other mutations are steadily gaining ground and will most likely be the golden standard soon an open mind must be had and focused placed on molecular markers which show a great promise and will lead the way in new screening methods and personalized medicine guidelines.

Also, with every human genome sequenced and analyzed more and more information is gained and if the evolution of technology and cost is constant, hole genome sequencing will be the only screening test an individual will have to do. A major future challenge not only for CRC, but for cancer biomarkers in general will be the integration of pharmaco-genomics, proteomics and metabolomics functional data, with epidemiological and clinical data (51).

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PHENOTYPIC VARIABILITY IN EDWARDS SYNDROME: SYNOPSIS OF 19 CASES WITH TRISOMY 18

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PHENOTYPIC VARIABILITY IN EDWARDS SYNDROME (Abstract): Edwards Syndrome is the second most common autosomal trisomy syndrome after trisomy 21, with a liveborn prevalence of 1/10,000. ES is characterized by variable clinical manifestations, with involvement of multiple organs and systems. ES is produced by trisomy 18, and most cases present a complete and homogeneous chromosomal aneuploidy. **Aim:** To identify the clinical suggestive features of ES. **Material and methods:** We performed in Iasi Medical Genetics Center a retrospective study on 12 years (2003-2015) and we discovered 19 patients with trisomy 18. **Results:** 18 patients had a complete trisomy and 1 case presented a partial trisomy. The frequent clinical features were: intrauterine growth retardation, micrognathia, dysplastic ears, dolichocephaly, heart defects, prominent calcaneus, overriding fingers and preterm birth. Also, we identified an increased frequency of caesareans procedures. The common visceral anomalies were cardiac and most frequent such anomalies were: ventricular septal defect and patent ductus arteriosus. Other visceral anomalies were: horseshoe kidney, esophageal atresia and hydrocephalus. Breech presentation was a common feature. **Conclusions:** Edwards syndrome is suspected in patients with the associations of the following features: growth retardation, typical craniofacial dysmorphism, heart defects, prominent calcaneus, overriding fingers and preterm birth. Gastrointestinal and renal anomalies are common features. **Keywords**: EDWARDS SYNDROME, 18 TRISOMY, GROWTH RETARDATION, CRANIOFACIAL DYSMORPHISM, HEART DEFECTS.

Edwards syndrome (ES) is caused by trisomy 18, the second most common autosomal trisomy syndrome after trisomy 21 (1). The first reported cases were described in 1960 by Edwards *et al* and Smith *et a*l (2, 3). ES pregnancies have a high risk of fetal loss and stillbirth, so overall prevalence (1/2,600) is higher than liveborn prevalence (1/10,000) (4). The probability of survival to term increases with the increase of gestational age: 28% at 12 weeks, 35% at 18 weeks and 41% at 20 weeks (5). The prevalence at birth is higher in females compared to males (♀:♂

= 6:4) due to higher rate of fetal loss in males (6).

The cytogenetic changes in ES are: homogeneous complete trisomy (94% of cases), mosaics (about 5% of cases) or partial 18q trisomy (about 1% of cases) (7). The extra chromosome 18 has in majority of cases a maternal origin, being generated by a meiotic non-disjunction. About 50% of the maternal errors occur in meiosis II, unlike other human trisomies where the malsegregation is more frequent in meiosis I. The frequency of non-disjunction increases with advancing maternal age. In the rare cases with paternal origin, the extra chromosome is the result of a postzygotic error (8-11).

ES is a plurimalformative syndrome whose main features are: prenatal growth deficiency, characteristic craniofacial features (dolichocephaly, short palpebral fissures, micrognathia, dysplastic ears), distinctive hand posture (clenched fists with overriding fingers), nail hypoplasia, posterior prominence of calcaneus (rocker-bottom foot), short sternum and visceral major malformations (particularly cardiac) (12). The phenotype is variable: more than 130 different anomalies have been reported in the literature, which may affect all organs and systems (13, 14).

MATERIAL AND METHODS

We made a clinical and cytogenetic retrospective study on a group of 19 cases of ES. The cases were identified between 2003 and 2015 in Iasi Medical Genetics Center and all were confirmed by chromosomal analyze.

RESULTS

In our group 11 patients were males and 8 females (♂:♀ = 1.37). The most frequent clinical features (tab. 1) were: intrauterine growth retardation (IUGR) (19 cases), cranial dysmorphism (fig. 1) with micrognathia (19 cases), dysplastic ears (19 cases), dolichocephaly (18 cases), heart defects (18 cases), prominent calcaneus (17 cases) (fig. 2) overriding fingers (13 cases) (fig. 3) and preterm birth (13 cases). An increased frequency of caesareans procedure was noticed (14 cases). The visceral congenital anomalies were represented by: cardiac (18/19 cases) gastrointestinal (8/19) renal (7/19) and centrals nervous system (5/19). The most common anomalies in descending order of frequency were: ventricular septal defect (12 cases) patent ductus arteriosus (11 cases), horseshoe kidney (4 cases), esophageal atresia (4 cases), hydrocephalus (4 cases) pentalogy of Fallot (3 cases) and Meckel diverticulum (3 cases).

The obstetrical history revealed IUGR (all cases) associated with polyhydramnios in 5 cases. Breech presentation was noticed in 8 cases and a advanced maternal age was found in 6 cases.

The prognosis was bad in majority of cases. From 16 patients with known period of survival 10 died in first month of life, 5 lived longer than 1 month and only one girl survived beyond the first year.

Clinical diagnosis was confirmed by chromosomal analysis in all cases: a complete and homogeneous 18 trisomy (fig. 4) – 47,XX(XY),+18 (18/19 cases) and a partial 18 trisomy associated with a complex abnormality
46,X,Yqh+,1qh+,+18,der(13;18)
(q12;p11.2) (fig. 5) (1 case). The patient (C19) with complex abnormality (partial 18 trisomy and a very small partial 13 monosomy) had suggestive features of ES and diaphragmatic hernia.

TABLE I

The features of ES patients

Signs	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19
Preterm birth	+	-	+	+	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+
Cesarean	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-
Breech presentation	+	+	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-
IUGR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyhydramnios	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-
Dolichocephaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Micrognathia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dysplastic ears	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Overriding fingers	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	-	-	-
Nail hypoplasia	+	+	-	-	+	+	+	-	+	+	-	-	+	-	+	+	-	-	-
Prominent calcaneus	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Heart defects	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kidney anomalies	-	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+	-	-	-
Gastrointestinal defects	-	-	+	-	-	+	+	-	+	-	-	+	+	-	-	+	+	-	-
Cleft lip/ palate	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Central nervous system anomalies	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-
Diaphragmatic hernia	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limb anomalies	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	+	+	-

Fig. 1. Cranial dysmorphism

Fig. 2. Prominent calcaneus

Fig. 3. Overriding fingers

Fig. 4. Karyotype 47,XY,+18

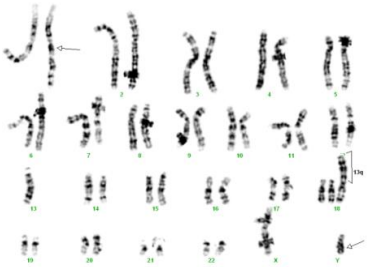

Fig. 5. Karyotype 46,X,Yqh+,1qh+,+18,der(13;18) (q12;p11.2)

DISCUSSION

ES is characterized by variable clinical manifestations, with involvement of multiple organs and systems. There are key features present in patients, but there is also a wide heterogeneity of clinical manifestations. Some features overlapping with other syndromes can sometimes complicate the diagnosis. To facilitate accurate diagnosis, we tried to identify the most suggestive combinations of clinical features.

IUGR is one of the most frequent finding in ES; the mean birth weight is 1700-1800 g at a mean gestational age of 37 weeks (15). The study of Kroes *et al* also confirms that IUGR is a specific prenatal feature of trisomy 18, feature that is not characteristic for trisomy 13 (17). In our study IUGR was present in all cases, consistent with literature data. The growth deficiency continues after birth for all major anthropometric parameters – weight, height and cranial circumference – but with major implication on the weight (18). Gastrointestinal anomalies and orofacial clefts may contribute to feeding problems (13).

The triad of craniofacial features: prominent occipital bone, low-set, dysplastic ears ("faun ears") and micrognathia was highly suggestive for ES (18/19 cases). Rosa *et al* in a study on 50 patients reported as main craniofacial abnormalities: micro retrognathia (76%), abnormalities of the ear helix/ dysplastic ears (70%) and prominent occipital bone (52%) (19). Cleft lip/palate was present in 3/19 of our cases (one bilateral form, one unilateral form and one palate cleft). Our result is like literature data; orofacial clefts are considered a less frequent (5-25%) anomalies in ES (1, 12, 18), but is more frequent associated with trisomy 13: 60-70% (20-21).

Previous studies show that 80-100% of ES patients have congenital heart defects (CHD); the most common cardiac anomalies are ventricular and atrial septal defects (VSD, ASD), patent ductus arteriosus (PDA) and polyvalvular disease (13, 22, 23). We found a similar situation and we identified heart defects in 18/19 of cases. VSD (12/18) and PDA (11/18) were the most common cardiac anomalies. Other detected CHD were: ASD (4/18), polyvalvular disease (4/18), pentalogy of Fallot (3/18), coarctation of aorta (2/18), anomalous origin of coronary arteries (2/18), bicuspid aortic valve (2/18), single ventricle (1/18), dextrocardia (1/18). The complex heart defects were detected in 3/19 of cases, like literature data (10%) (13).

In our study, 17/19 of the patients had prominent calcaneus ("rocker bottom foot"). This abnormality has also been reported in the literature as occurring from 48 to 90% of the cases (24, 25). Other features concerning the lower limb identified in our study were: syndactyly of the toes – 4 cases (I-II/II-III/III-IV cutaneous), short hallux – 3 cases, and clubfoot – 2 cases. In the literature, these findings are cited with a highly variable frequency (18, 24, 25).

The most frequent abnormality of upper limbs in our study was clenched fists with overriding fingers: 13/19 of cases. In other reports the frequency was from 58 to 96% of the cases (25-27). Many authors consider this feature one of the important diagnostic clues, and it is often detected sonographically in the prenatal period (especially in third trimester of pregnancy) (1, 13).

In the literature, a major malformation of limb occurs in 5-10% of patients, including radial aplasia and other preaxial limb defects (1, 18, 24). We detected the radial aplasia in one case and agenesis of the thumb in other case. Nail hypoplasia is cited in the literature with a highly variable frequency (13-63%) (24, 25). In our study this feature was detected in 10/19 of cases.

Gastrointestinal anomalies are cited among patients with ES. In the literature,

the frequency of esophageal atresia/ tracheo-esophageal fistula among the patients with trisomy 18 ranges from 16 to 20% (25, 28). In our study the frequency was similar 4/19. Omphalocele have been described in less than 10% of the cases, which is compatible with the rate in our study – 1/19 (18, 29). Meckel diverticulum was detected in 3 cases in our study, like literature data (less than 25%) (18). In our study the global frequency of gastrointestinal anomalies (42.10%) was higher than reported in the literature (less than 25%) (12, 18).

Horseshoe kidney (HK) is a common congenital anomaly of the kidney with a prevalence of 1 in 500 (30). HK is reported in 20-66% of patients with ES in the literature data (30, 18). In our study, only 4 cases had this anomaly. Other renal anomalies found by us were: renal hypoplasia – 1 case, duplication of ureter – 1 case and renal cysts – 1 case.

Several structural abnormalities of the central nervous system (CNS) have been reported in trisomy 18; the most frequent are cerebellar hypoplasia, agenesis of corpus callosum, hydrocephalus and myelomeningocele. These anomalies are present in about 5-25% of infants (1, 18, 26). CNS abnormalities detected in our study were consistent with literature data: hydrocephalus – 4 cases, agenesis of corpus callosum – 1 case and myelomeningocele – 1 case.

Yamanaka et al reported a higher preterm birth frequency (35%) compared to general population. In our study the frequency of preterm birth was even higher 68.42%. An increased incidence (64%) of caesareans has been reported (15). In our study 14/19 (73.68%) of patients were born by cesarean.

We found a sex ratio ♂:♀ of 1.37. This finding is discordant with other studies that showed a sex ratio of prenatally diagnosed cases of 1:1, but for the live-born sex ratio ♂:♀ was between 0.63 (Huether, 1702 cases) and 0.56 (Niedrist, 161 cases) (32, 33). This discrepancy could be related to small size of our study group.

Management of neonates with trisomy 18 is controversial, due to the bad prognosis (lethality and intellectual disability) and the lack of precise information concerning efficacy of treatment (1, 15, 16).

CONCLUSIONS

ES is a chromosomal syndrome with variable clinical features. The most suggestive clinical findings were: IUGR, typical craniofacial dysmorphism (dolichocephaly, dysplastic ears, micrognathia), heart defects, prominent calcaneus, overriding fingers and preterm birth. Gastrointestinal and renal anomalies are also common features. Early diagnosis is important for appropriate management decisions regarding neonatal care and invasive procedures.

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CONSIDERATIONS OF PLATELET-RICH FIBRIN USE IN ORAL SURGERY

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CONSIDERATIONS OF PLATELET-RICH FIBRIN USE IN ORAL SURGERY (Abstract) Nowadays, tooth extraction has become a frequently performed surgical procedure whose most common postoperative outcome is alveolar bone atrophy. To reduce the bone dimensional changes, localized alveolar ridge augmentation or socket preservation with bone grafting is required. The socket may be filled with synthetic grafts, xenografts, autografts, allografts, autologous platelet concentrate. The use of platelet concentrates for healing improvement and regeneration of the soft and hard tissues in oral cavity is one of the latest achievements in dentistry. One of the newest practices in bone augmentation is the use of platelet rich fibrin, derived from the patient's own blood and can be very easily accepted by patients due to cost effectiveness, low donor morbidity and low rate of postoperative complications or infection. This biomaterial might be a solution for patients that have strong beliefs regarding the use of allografts or xenografts, or which are afraid of complications during or after autografting procedure. One cannot say that there is an ideal grafting material and must consider a person's religious affiliation or beliefs when performing bone augmentation. **Keywords:** PLATELET-RICH FIBRIN, ETHICS, BONE AUGMENTATION.

A.B., a dental implant patient, asks: For personal religious reasons, I do not want animal or human bone graft material to be placed in my body. I should do an extraction and my dentist says I will need a bone graft. Now, he cannot offer me a bone graft that does not include animal or human graft products. What are my alternatives? Thanks!

Nowadays, tooth extraction has become a frequently performed surgical procedure whose most common postoperative outcome is alveolar bone atrophy (1). Hard and soft tissue preservation following the extraction of one or more teeth is one of the most challenging objectives of dental procedures (2). Sufficient alveolar bone volume and favorable architecture of the bone are essential for aesthetic appearance, especially when anterior sites are involved, and for the placement of dental implants in a prosthetic ideal position. To reduce the bone dimensional changes, localized alveo-

lar ridge augmentation or socket grafting is needed (1, 2). The socket may be filled with (2, 3): Synthetic grafts (hydroxyapatite, tricalcium phosphate, etc.); Xenografts (bone substitute of animal origin, such as bovine or porcine bone); Autografts (bone harvested from the patients' own body - the donor site can be the menton region, retromolar area, maxillary tuberosity, iliac crest, calvarias, tibia (4)); Allografts (cadaveric bone, usually obtained from a bone bank); Autologous platelet concentrate (platelet-rich plasma, platelet-rich fibrin).

The use of platelet concentrates for healing improvement and regeneration of the soft and hard tissues in oral cavity is one of the latest achievements in dentistry (5). This platelet concentrates are obtained by centrifugation fresh venous blood drawn from the patient's vein (6). Platelet-rich plasma (PRP) is a first generation concentrate, the blood is collected with anticoagulant and needs chemical additives, such as Calcium Chloride and bovine thrombin to make gel condition (7). But the latest studies show that PRP has a low effect on hard tissue regeneration (8). Thus, a second-generation aggregate was introduced: platelet-rich fibrin (PRF). The blood is collected without any anticoagulant, immediately centrifuged and the PRF protocol doesn't need biochemical additives to make gel condition (6). The PRF clot is obtained through a natural polymerization process during centrifugation and its natural and stable fibrin architecture seems responsible for tissue regeneration (5). The PRF can be used as a filling material in extractions, alone or mixed with a bone substitute. As the number of available biomaterials increases, the doctor has the obligation to explain the nature, purpose and the benefit/risk ratio of a proposed bone grafting material. According to The General Medical Council, „when providing information, the physician must do his best to find out about patients' individual needs and priorities": religious affiliation and beliefs, culture or other factors that can influence his decision (9). Valid consent to treatment is therefore absolutely central in all forms of healthcare, from providing personal care to undertaking major surgery." The DoH states that "different patients will make different choices in apparently similar situations" (9).

Until now in Romania there hasn't been developed any standard informed consent form that specifies the type of biomaterial that is being used. The Romanian College of Dentists developed in 2013 a compulsory informed consent form for surgical procedures in the dental office, without making any reference to graft types and their origin. Therefore, providing a treatment plan without taking in consideration the ethical, legal or social implications, the treatment may not reflect the, best interest'of the patient. When using xenografts or PRP combined with bovine thrombin or autologous platelets concentrate which needs blood drawing, the doctor should know that certain religious groups have restraints regarding the dietary use of porcine and bovine products or don't accept blood products, limiting treatment options (10). When planning a bone augmentation, the doctor should bear in mind the religious beliefs, explain the surgical act, present the biomaterials that are being used and act accordingly.

One of the newest practices in bone augmentation is the use of platelet rich fibrin, derived from the patient's own blood and can be very easy accepted by

patients due to cost effectiveness, low donor morbidity and low rate of postoperative complications or infection. This technique might be an alternative to autografts, which are harvested from the patient's body, so the patient could avoid the postoperative evolution and possible complications: hematoma, edema, infection, vascular and nerve lesion, morbidity of the donor area and chronic pain (10). Up to 15% of patients having an iliac graft harvested have been complain of pain for more than 3 months in the donor site (10). Furthermore, the PRF technique obviates immunological reactions and the risk of disease transmission associated with allograft bone, taken from a tissue bank (11). Allografts are harvested from dead human donors, whose families have consented to tissue donation, respecting a certain protocol, but there is still a minimal risk of immunogenic reactions, transmission of disease from donor to recipient or recipient site infection given by a potentially contaminated product (12, 13).

Platelet-rich fibrin (PRF) was developed in 2001 in France by Prof. Dr. Joseph Choukroun (MD, Anesthesiologist, Private Practice, Pain Clinic Center, Nice, France) (14) and it has been used in Europe for the past decade. This biomaterial is being prepared by the surgeon, from the patients' blood and needs a very simple protocol. Oral surgeons have adopted this technique in their private dental practice and the PRF preparation requires an adequate centrifuge, 10 ml blood collection tubes (14), winged infusion sets, also known as „butterfly" sets for venipuncture and a PRF box kit. PRF is an autologous fibrin matrix rich in platelets, leucocytes and monocytes that induces a cascade of multiple growth factors release (15). It is a simple technique, blood is collected from the patient, centrifugated and the resultant product is formed of three layers (fig.1a): the top layer is represented by platelet poor plasma, in the middle is the platelet-rich-fibrin clot (fig. 1b), which it is used clinically and red blood cells at the bottom, that are easily discarded (16).

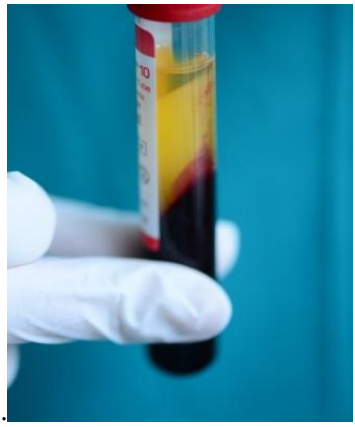
a.  b.

Fig 1. a. The resultant product and is formed of three layers: top is the PPP, middle is the PRF clot and bottom are the red blood cells; **b.** The PRF clot (personal cases)

To obtain a viable product for the patient's benefit and at the same time to assure his safety, the doctor must know and respect the PRF protocol, yet only a few

practitioners know it. It is known that PRF is derived from the patient's blood and no anticoagulant, no chemical additives, such as Calcium Chloride or bovine thrombin are used, thus is 100% natural to the patient. Because of the absence of an anticoagulant, blood begins to coagulate as soon as it encounters the glass surface (14), so the drawing must be fast. An important issue is the blood drawing, because the dental surgeon isn't trained in phlebotomy procedures. Therefore, when choosing for bone augmentation the PRF technique, the doctor must explain the procedure to the patient, and the reason for the blood sample being taken. Also, the patient should be informed about the complications or accidents of a phlebotomy: hematoma, edema, syncope or the doctor can't find the vein. Today, after long research, Choukroun et al. approved one type of centrifuge, with its own protocol: The PRF-Duo centrifuge. Surgeons should know that they can use this autologous product even in patients with anticoagulant therapy, but the time of centrifugation must be changed. The tubes that are used for collecting blood must be labelled with CE 0197, a certification of medical quality used by the European community, analogous to FDA certification in the United States (15). The blood drawing and the centrifugation represent only half of the PRF protocol. The other half is the handling of the PRF clot and properly preparing it for clinical use by using the PRF box kit. After centrifugation, the PRF must be extracted immediately from the tube. A tweezer is inserted into the tube, gently grab the fibrin clot with attached red blood cells and with a scissor cut through the RBC's (14, 15). The fibrin clot is transferred to sterile metal surface and the red blood cells are scraped away and discarded (fig. 2) (17).

Fig. 2. Handling of the PRF clot (personal cases)

The PRF box kit (fig. 3a) was created for the fabrication of plugs (fig. 3b) and membranes. PRF can be used as a plug, that contains a high concentration of platelets enmeshed in a fibrin matrix, which releases a high concentration of growth factors over a long time or as a fibrin membrane acting as a matrix to promote healing (18). The PRF Box is a necessary instrument used to automatically produce high qualities membranes of constant thickness and size (17).

a. b.

Fig. 3 a. The PRF Box (courtesy of Prof. J. Choukroun, France);
b. Obtaining PRF plugs, used in postextractional sockets (personal cases)

Clinically, the membrane displays excellent working properties. It is resilient, strong and pliable, making it easy to manipulate, cut and suture. When preparing membranes, the box recovers the exudate from the PRF clot that can be used in hydration of the biomaterial (17). Also, the PRF box it is useful during surgery to preserve the membranes. Essentially, the doctor should create the PRF by following a precise protocol for centrifuging a given quantity of the patient's blood so the results are in the interest of the patient. This biomaterial might be a response for patients that have strong beliefs regarding the use of allografts or xenografts, or which are afraid of complications during or after autografting procedures. Using this technique, it is imperative that surgeons understand the indications and limitations of the PRF and they must know the proper protocol for PRF synthesis. We cannot say that there is an ideal grafting material and we must consider that bone augmentation must be carried out accordingly to everyone's beliefs.

CONCLUSIONS

Platelet-rich fibrin, unlike other methods of bone augmentation, is entirely autologous and there are limited ethical concerns and no toxicity implication related to this stable and natural blood clot and therefore should be included in the treatment plan of small/medium bone defects in the oral cavity.

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BAROREFLEX DYSFUNCTION IN CHRONIC KIDNEY DISEASE

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BAROREFLEX DYSFUNCTION IN CHRONIC KIDNEY DISEASE (Abstract): Chronic Kidney Disease (CKD) is a globally public health problem taking into account its direct role in morbidity and mortality. The essential risk factors that contribute to the development and progression of CKD are hypertension, diabetes and dislipidemy. Besides hypertension, variations in blood pressure caused by baroreflex control dysfunction can lead to chronic kidney disease progression. The causes of impaired baroreflex dysfunction control in chronic kidney disease are not fully known. Understanding the mechanisms that contribute to increased blood pressure variability due to baroreflex dysfunction in chronic kidney disease may represent a key element in diminishing the physio pathological processes that contribute to the development and progression of renal injury. **Keywords**: VARIABILITY, BAROREFLEX, CHRONIC KIDNEY DISEASE.

Chronic kidney disease involves a complex physio pathological process with multiple etiology, characterized by irreversible alteration in structure and function of the nephrons. Epidemiological studies have shown a prevalence constantly increasing worldwide, more pronounced in the last two decades. The cardiovascular disorders are complications related to chronic kidney disease, both diseases representing a public health problem with multiple social and economic implications. In early stages, chronic kidney disease still remains underdiagnosed and untreated. Early identification of chronic kidney disease, followed by the implementation of therapeutic strategies to prevent the occurrence of cardiovascular complications, would be the key elements in order to reduce morbidity and mortality, improving quality of life, to decrease economic costs and to increase survival rate of the patients.

Arterial hypertension – chronic kidney disease axis.

It is well known that the link between hypertension and renal dysfunction in chronic kidney disease is characterized by a highly association coefficient. Thus, elevated blood pressure can be found in over 90% of patients with renal impairment, which represents both cause and effect of chronic kidney disease. Clinical and experimental studies prove that renal hemodynamic changes resulting in increased glomerular capillary pressure contribute to progression of chronic kidney disease. The afferent arteriole vasodilatation results in an increase in glomerular filtration rate (GFR), renal plasma flow and also filtra-

tion fraction (1,2). An abnormal increase in systemic blood pressure transmitted to dilated afferent arteriole causes an increase in glomerular pressure. Glomerular hypertension and hyperfiltration are the main pathogenic mechanisms leading to glomerular injury (3). These hemodynamic changes are accompanied by enlargement of Bowman capsule, mesangial matrix proliferation and impairment of glomerular basement membrane integrity. Thus, an increase in systemic blood pressure transmitted to the glomerular apparatus plays an important role in the progression of chronic kidney disease.

Renal autoregulation.

Under normal conditions, transitory or sustained increases in blood pressure values are not transmitted to glomerular capillary due to autoregulation mechanism that causes preglomerular vessels constriction (4). For this reason, the glomerular filtration rate and renal plasma flow are constant in the presence of systemic blood pressure variations. Renal autoregulation remains a protective mechanism for glomerular diseases induced by increased blood pressure and also for maintaining normal excretory function (5). If renal autoregulation mechanism is impaired or if blood pressure values exceed the limits of autoregulation, renal microvasculature is exposed to increased pressure, which leads to renal injury. After reduction of functional renal mass or calcium blockers administration (6, 7, 8), there is a decline in kidney function autoregulation, even if systemic blood pressure remains within normal limits (9). Renal autoregulation is mediated by two interacting and synergistic control mechanisms: the intrinsic myogenic response of preglomerular vessels and tubulo-glomerular feedback mechanism. Myogenic mechanism involves direct vasoconstriction as reaction to increased local intravascular pressure. Tubulo-glomerular feedback mechanism is triggered when the concentration of sodium chloride in the distal convoluted tubule cells increases which stimulates the release of adenosine from *macula densa* cells. Adenosine initiates a cascade of events leading to afferent arteriole vasoconstriction in order to maintain a constant glomerular filtration rate (5). The stimulus which causes the activation of renal autoregulation is represented by modification of renal perfusion pressure.

Blood pressure variability.

Blood pressure variability as a mechanism of renal injury progression plays a central role in the physiopathology of this disease. The clinical and experimental studies have revealed that an increase in blood pressure variability causes organ damage (heart, brain, kidney), independent of its mean values (10). For instance, Ohasama study - a long-term prospective study regarding ambulatory monitoring of blood pressure - indicated that an increase in systemic blood pressure variability is an important indicator of cardiovascular mortality (11). A cause-effect relation between increased blood pressure variability and target organ damage is suggested by experimental studies showing that sino-aortic denervation increases blood pressure variability, without impairing mean values of arterial pressure (12, 13). In normotensive rats, sinoaortic denervation suppresses the changers induced by hypertension in heart, kidney and small arteries (14). A high variability in blood pressure is associated with frequent episodes of increased and decreased blood pressure values compared to mean arterial pressure. Frequent transitory elevations of blood pressure represent the

mechano-sensitive trigger of intracellular signalling mechanisms involved in target organ damage in hypertension (15). Besides that, even temporary reduction of blood pressure during episodes of hypo perfusion may have unfavourable effects on target organs.

Baroreflex system.

A short term control mechanism of blood pressure variability is the baroreflex system, whose main role is the short-term correction of blood pressure oscillations during everyday life. This system is composed of specialized mechano-sensory nerve endings known as baroreceptors, which are activated by vascular and/or heart distension during intraluminal blood pressure increasing. Arterial baroreceptors activity innervating the aortic arch and carotid sinus is increased or not according to blood pressure values. Thus, if arterial blood pressure is high, the activity of these receptors is significant and contrary it decreases if blood pressure values are low. Changes in arterial baroreceptor activity occur constantly, due to their efficiency and increased sensitivity, in order to a rapid correction of blood pressure variations, with a very low latency through an efficient negative feedback mechanism. Decreased baroreflex sensitivity occurs when the compensatory autonomic regulation is not appropriate (16). Epidemiological observations suggest that baroreflex dysfunction, manifested mainly by increasing of blood pressure variability associated to decreasing of heart rate variability, is correlated to a high risk of cardiovascular damage (17). Based on existing literature, we suggest that in chronic kidney disease there is a decrease in short-term control of blood pressure variability baroreflex-mediated.

Inflammation in chronic kidney disease: impact on autoregulation mechanism and baroreflex sensitivity.

Clinical studies show that chronic inflammation represents a risk factor for cardiovascular and renal pathology (18). Inflammatory biomarkers such as TNF-alpha (tumour necrosis factor-alpha), IL-6 (interleukin-6) and CRP (C-reactive protein) are predictive factors of mortality in patients with chronic kidney disease (19, 20, 21). Inflammatory cytokines (IL-6 and TNF-alpha) have both local and systemic action. IL-6 plays a major role in the immune response regulation in inflammation and has a very long serum half-life. Data obtained so far suggest that inflammatory cytokines are not only markers for the disease, but also its mediators (22). IL-6 promotes atherosclerosis and together with TNF-alpha induces structural changes in endothelial cells (23). The mechanisms by which inflammatory cytokines contribute to renal disease are not fully understood. At vascular level, cytokines induce the expression of some genes related to a number of autacoids (prostaglandins, leukotrienes, nitric oxide, reactive oxygen species and bradykinin), which affects vascular function. The vascular response depends on the balance between released autacoid type and degree of vascular damage. Minghini et al., have shown that exposure to IL-6 leads to vasodilatation of rat cremaster arterioles (24), and TNF-alpha determines arteriolodilatation during the exposure to endotoxin. If this effect occurs in the renal microvasculature, it can reduce myogenic response and the autoregulation capacity. Recent studies have suggested that circulating proinflammatory cytokines may have a direct effect on the central nervous system by stimulating the sympathetic nervous system flow (25). Consequently, elevated levels of TNF-alpha and IL-6 can deter-

mine an imbalance between autonomic baroreflex response stimulation and decrease of baroreflex sensitivity in chronic kidney disease. TNF-alpha and IL-6 promotes the development of atherosclerotic lesions in large vessels that could directly affect baroreceptor sensitivity to the pressure (26).

CONCLUSIONS

Summarizing the above data, we can conclude that understanding the mechanisms by which inflammation in chronic kidney disease triggers the baroreflex dysfunction can open new possibilities of therapeutic approach of this disease.

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NEWS

HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY – A CUTTING EDGE THERAPHY FOR ADVANCED OVARIAN CANCER?

Nowadays, it is well known the importance of hypertermic intraperitoneal chemotherapy in colo-rectal cancer, pseudomyxoma peritoneii and malign mesothelioma, but it's significance in advanced ovarian cancer isn't so certain so far. There were published the results of a phase II study performed at the Unit of General, Emergency and Transplant Surgery at Sant'Orsola-Malpighi Hospital, University of Bologna, Italy, the Unit of General Surgery and Gynaecologic Surgery of Papa Giovanni XXIII Hospital, Bergamo and the Unit of Gynaecology of the Jena University Hospital, Germany. Patients with primary, advanced (FIGO stage IIIC to IV), or recurrent EOC were eligible for the present study to be treated with cytoreductive surgery and HIPEC with Cisplatin (100 mg/m^2) and Paclitaxel (175 mg/m^2). It seems that using cytoreductive surgery and HIPEC, using cisplatin and paclitxel it is feasible and comparable with the other drugs regimens with similar mortality and morbidity rate. The benefit for the overall survival and for the morbidity and mortality rate, it seems that it's a statement for the treatment in ovarian cancer (Federico Coccolini, Luca Campanati, Fausto Catena et al. Hyperthermic intraperitoneal chemotherapy with cisplatin and paclitaxel in advanced ovarian cancer: a multicenter prospective observational study. *J Gynecol Oncol*. 2016 Jan;26(1):54-61).

Mihaela Buna-Arvinte

SURGICAL TREATMENT OF POSTPARTUM ILIOFEMORAL DEEP VEIN THROMBOSIS - CASE REPORTS

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SURGICAL TREATMENT OF POSTPARTUM ILIOFEMORAL DEEP VEIN THROMBOSIS -CASE REPORTS (Abstract): The writing committee for Antithrombotic Therapy for Venous Thromboembolic Disease of the 2008 ACCP guidelines made the following recommendations for thrombus removal strategies in patients with deep vein thrombosis (DVT): open surgical thrombectomy is recommended in patients with acute iliofemoral DVT to reduce symptoms and post-thrombotic morbidity; whenever available, catheter-directed thrombolysis is preferred to surgical venous thrombectomy, the risk of hemorrhage being diminished; surgical venous thrombectomy is recognized to be efficient in cases where catheter-directed thrombolysis is unavailable or the patients are not suitable candidates for such a procedure. Randomized studies comparing surgical thrombectomy and anticoagulant therapy in patients with iliofemoral DVT (IFDVT) showed that at 6 months, 5 years, and 10 years the patients in the thrombectomy group presented increased permeability, lower venous pressure, less edema, and fewer postthrombotic symptoms compared to the patients receiving anticoagulant therapy. In this article we present 3 cases of IFDVT in postpartum patients diagnosed by Doppler ultrasound of the deep venous system. The 3 patients received anticoagulant therapy prior to surgery. Surgery consisted in thrombectomy of the common, superficial and deep femoral veins, external and internal iliac veins, and femoral-saphenous arteriovenous fistula. The patients received postoperative antithrombotic therapy and were followed-up at 3,6 and 9 months by Doppler ultrasound of the deep venous system. **Keywords:** DEEP VEIN THROMBOSIS, DOPPLER ULTRSOUND, VENOUS THROMBECTOMY, ARTERIOVENOUS FISTULA, ANTICOAGULATION, POSTPARTUM.

Deep vein thrombosis (DVT) is clotting of blood in the deep venous system of the limbs to which a venous wall inflammatory response can be associated. The factors that predispose to venous thrombosis are the ones described by Virchow in 1856 and include venous stasis, vein wall injury, and hypercoagulability(1).The writing committee for Antithrombotic Therapy for Venous Thromboembolic Disease of the 2008 ACCP guidelines made the following recommendations for thrombus removal strategies in patients with DVT: open surgical thrombectomy is recommended in patients with acute iliofemoral DVT to reduce symptoms and post-thrombotic morbidity; whenever available, catheter-directed thrombolysis is preferred to surgical venous thrombectomy, the risk of hemorrhage being diminished; surgical venous thrombectomy is recognized

to be efficient in cases where catheter-directed thrombolysis is unavailable or the patients are not suitable candidates for such a procedure(2,3).The patients who underwent venous thrombectomy had greater permeability, lower venous pressure, less edema, and fewer postthrombotic symptoms compared to the patients treated with anticoagulants alone (4).

CASE REPORTS

We present 3 cases of DVT in postpartum patients.

Case 1.

A 28-year-old female with onset of DVT 12 postpartum. The patient was transferred from the Obstetrics Clinic to the Vascular Surgery Clinic where anticoagulant therapy with heparin 25,000/24h was initiated and then continued with oral anticoagulation with Sintrom 4mg/day, on admission the patient having an INR of 2.3.With this treatment the patient showed a slow improvement of symptoms. Doppler ultrasound of the deep venous system on admission showed cardiac and respiratory modulation of inferior vena cava blood flow, left iliofemoral-popliteal thrombosis, thrombosis at the arch of the internal saphenous vein (VSI) with collateral flow 11 cm from the valve, the remaining VSI being depressible. The patient continued to receive anticoagulant therapy with heparin 1000 UI/24h until surgery. Surgery consisted in thrombectomy of the common femoral vein (VFC), common iliac vein (VIC), external iliac vein (VIE), and a femoral-saphenous arterio-venous fistula (fig. 1, 2).

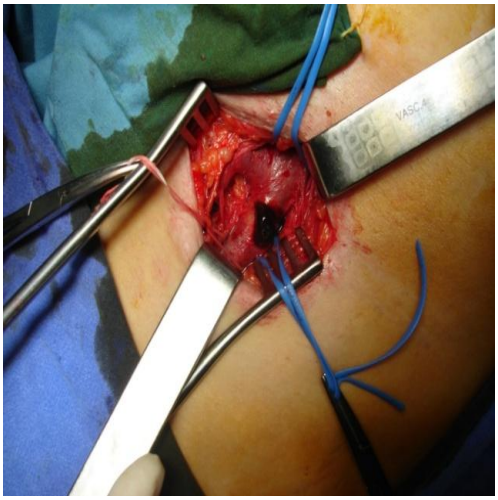

Fig. 1. VFC trombus

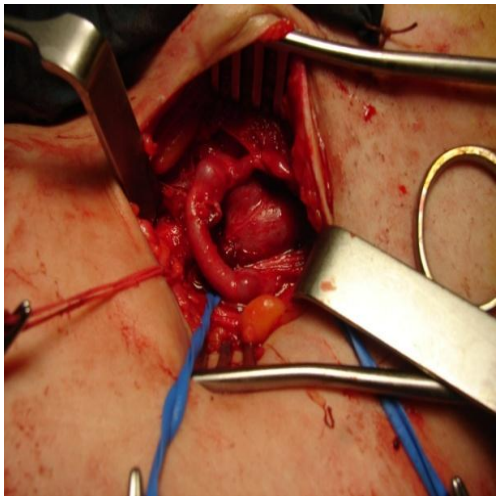

Fig. 2. Femoral-saphenous arteriovenous fistula

The immediate surgical outcome was favorable. Heparin therapy was continued for 7 days postoperatively followed by low molecular weight heparin for 4 days associated in the last 2 days with Sintrom, an INR of 2-3 being obtained. The anticoagulant therapy was associated with the elevation of the lower leg and elastic compression (18-21 mmHg). The long term follow-up (at 3, 6 and 9 months) showed favorable results with the remission of symptoms and permeable deep venous system demonstrat-

ed by deep vein ultrasound.

Case 2.

A 36-year-old female with DVT detected at 24h postpartum. The patient presented severe, continuous pain in the left thigh and calf, more intense during mobilization, significant edema of the left thigh and calf, positive Homans sign, imperceptible left anterior and posterior tibial artery pulse due to the edema but positive Doppler signal at the same level. Doppler ultrasound showed thrombosis of the common, superficial and deep femoral veins, external iliac vein, and partial thrombosis of the common iliac vein with blood flow in the inferior vena cava. Preoperatively, anticoagulant therapy with heparin 1000 UI/hwas administered via syringe pump. Surgery consisted in iliofemoral venous thrombectomy and left femoral-saphenous arterio-venous fistula. The postoperative outcome was unfavorable with recurrent common femoral vein thrombosis. Reoperation consisted in femoro-popliteal venous thrombectomy and left popliteo-popliteal fistula, with favorable surgical outcome. The follow-up Doppler ultrasound at 3, 6, and 9 months showed permeability of the iliac veins, common and deep femoral veins, popliteal vein, and recurrent thrombosis in the superficial femoral vein. Postoperatively the patient continued the anticoagulation therapy with Sintrom 4 mg per day, with the INR maintained at 2.5-3.5.

Case 3.

A 31-year-old woman who developed DVT detected by Doppler ultrasound 7 days postpartum. Anticoagulant therapy with heparin 1000 UI/h via syringe pump was initiated preoperatively. Surgery consisted in ilio-femoral venous thrombectomy aimed at recanalization of the right popliteal vein and femoro-femoral arteriovenous fistula. The immediate surgical outcome was favorable, ultrasound showing a permeable ilio-femoral venous axis. The patient continued the anticoagulation therapy postoperatively. The long term follow-up at 3, 6, and 9 months showed the recurrence of thrombosis in the superficial femoral vein but with permeable right iliac and popliteal veins.

DISCUSSION

In patients with ilio-femoral deep vein thrombosis Rutherford recommends the use of the techniques designed for thrombus removal: open surgery, thrombolysis or endovascular techniques, because of the increased risk of pulmonary embolism and post thrombotic syndrome due to the impaired valvular function (1).

Plate G. et al. demonstrated in 3 studies that patients who underwent venous thrombectomy show improvement in iliac vein permeability, reduced venous pressure, reduction of edema, fewer symptoms due to post thrombotic syndrome than patients who received oral anticoagulation alone. Also, in these patients the valvular function of the femoral-popliteal venous segment is preserved compared to those treated with anticoagulants (4, 10). This was also found by Killewichet al (5).

Rutherford suggests that before choosing the thrombus removal procedure, the risk of pulmonary embolism associated with that procedure has to be taken into account. Patients with free-*floating thrombus* in the inferior vena cave are at the highest risk of developing pulmonary embolism. Therefore, it is necessary to know the upper and lower limit of thrombosis and most particularly the level of involvement of the inferior vena cava (1,6,7).

The writing committee for Antithrom-

botic Therapy for Venous Thromboembolic Disease of the 2008 ACCP guidelines made the following recommendations for thrombus removal strategies in patients with DVT: open surgical thrombectomy has to be performed in patients who are not candidates for catheter-directed thrombolysis (high bleeding risk) or reside in communities where this method is not available.

CONCLUSIONS

Since catheter-directed thrombolysis is not available in our clinic we opted for surgical venous thrombectomy. During this procedure an arterio-venous fistula should be created to help maintain blood flow in the deep venous system, without it the rethrombosis rate being very high (8,9,10). Surgery has to be preferably performed within the first 14 days but not later than 3 weeks. Any aggressive method of thrombus removal or lysis is better than no interventional treatment. Even though some authors are strong supporters of anticoagulant therapy, interventional strategies (endovascular or surgical) allow early mobilization as soon as possible, thus meeting the wishes of some patients, particularly younger patients.

Postoperatively, the patients received anticoagulation therapy with vitamin K antagonists, INR being maintained between 2.0 and 3.0.

In all these 3 cases, the symptoms remitted with preservation of permeability of the iliac vein, deep common femoral vein and popliteal vein, rethrombosis in the superficial femoral vein occurring in 2 cases.

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INTRAUTERINE GROWTH RESTRICTION – PREDICTIVE SERUM MARKERS

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INTRAUTERINE GROWTH RESTRICTION – PREDICTIVE SERUM MARKERS (Abstract) **Aim:** To determine during the first trimester of pregnancy some serum and ultrasound markers that could improve perinatal morbidity and mortality in women with intrauterine growth restriction (IUGR). **Material and methods:** Prospective study of pregnant women gestational age 11 weeks and 0 days - 13 weeks and 6 days, consisting in the determination of pregnancy associated plasma protein (PAPP-A), mean platelet volume (MPV), and ultrasound appearance of placenta. **Discussion and Conclusions**: This study suggests that screening by detailed history and PAPP-A and MPV determination during the first trimester of pregnancy in women at risk for IUGR makes possible the prophylactic treatment and monitoring of pregnancy according to a given protocol and thus neonatal morbidity and mortality to be reduced. **Keywords:** INTRAUTERINE GROWTH RESTRICTION, PREGNANCY-ASSOCIATED PLASMA PROTEIN, MEAN PLATELET VOLUME.

Detection of IUGR risk in pregnant women in the first trimester is an important goal of research in modern obstetrics. It is known that fetuses diagnosed with IUGR are at high risk of perinatal morbidity and mortality by the complications that may occur (1,2,3).

In this context the early determination of the predictive markers for the risk of IUGR is essential. First trimester screening consists in the monitoring of pregnant women at risk according to a secondary prevention protocol and monitoring specific to each associated disorder so that the following two situations that affect fetal prognosis to be avoided:

- ✓ premature, unnecessary extraction of a healthy fetus
- ✓ delayed extraction due to fetal distress undetected on time and which can result in intrauterine fetal death

Evaluation of the predisposing/causative factors for IUGR is the first step and consists in history taking, thorough physical examination, ultrasound, and specific laboratory tests, all aimed at ultimately determining the presence or absence of the risk for IUGR (4-16)

PAPP-A is a protein secreted by the syncytiotrophoblast cells and maternal decidua, thus low PAPP-A levels are associated with abnormal placentation and fetal growth (17-22).

MPV – Increased MPV reflects en-

hanced platelet activation, which may be caused by impaired uteroplacental circulation, and inadequate trophoblastic invasion, associated with an implicit risk of preeclampsia (PE) and IUGR (23-25)

MATERIAL AND METHODS

This prospective study was conducted in 70 women 11 weeks and 0 days -13 weeks and 6 days gestational age monitoring their pregnancy at the Neamt County Emergency Hospital between August 1, 2014 and September 1, 2015.

Throughout their pregnancy the women included in the study received prenatal care:

- ultrasound examinations (at 12 -13 weeks - fetal morphology, fetal development and placental appearance, at 22-24 weeks – fetal morphology, uterine artery Doppler study, at 32-34 weeks – fetal well-being study), repeated depending on fetal status and pregnancy-related disease;

- laboratory tests according to the protocol: complete blood count, MPV, blood glucose level, urea, creatinine, uric acid, transaminases, anti-toxoplasma antibodies (Ab), rubella Ab, anti-herpes Ab, cytomegalovirus (CMV), urinalysis, repeated depending on pregnancy-related disease.
- double test as part of biochemical screening (PAPP-A and beta-human chorionic gonadotropin (B-HCG) hormone.

The study aimed to determine the PAPP-A, MPV and ultrasound placental changes, as potential predictors of the risk for IUGR.

Each participant signed and informed consent form approved by the ethics committee.

Inclusion criteria for participation in the study were:

– 11 weeks and 0 days -13 weeks and 6 days pregnant women at a craniocaudal length (LCC): 44,5 mm – 84.5 mm)

– Normal fetal morphology

Exclusion criteria:

– Double tests with high risk for trisomy 13, 18, 21

– Fetal abnormalities diagnosed with ultrasound in the first trimester

– Genetic abnormalities confirmed by chromosomal analysis of cells obtained by chorionic villus biopsy.

– Pregnancy achieved through in vitro fertilization (IVF)

– Twin pregnancy.

PAPP-A was determined as part of first-trimester biochemical screening for aneuploidy at a LCC of 44,5 mm – 84,5 mm. Samples were processed with Brahms Kryptor immunoassay analyzer. The results were expressed as multiples of median (MoM).

The data were imported into, processed and analyzed using the SPSS version 18.0, and were statistically significant at 95% confidence level. Chi-square test was used to determine statistical significance. Frequencies and mean values for the quantitative and qualitative determinations were calculated and Pearson and Fearman tests were used to find the correlations between several variables. The difference was considered statistically significant at $p < 0.05$.

RESULTS

This study was conducted in a group of 70 pregnant women monitored at the Neamt County Hospital in the interval August 1, 2014 - September 1, 2015. Pregnancies were dated by LCC at first ultrasound, LCC ranging from 44.5mm to 84.5mm.

Physical examination and history identified the following pregnancy-associated disorders and predisposing factors of IUGR: thrombophilia in 8 cases, uterine fibroma 1 case, pre-existing thrombocytopenia 1 case, iron deficiency anemia 7 cases, preexisting hypertension 11 cases, type 1 diabetes 7

cases, and smoking in 11 cases.

The 70 pregnant women included in this study gave birth to 41 small-for-gestational age pregnancy (SGA) and 29 normal weight babies (fig. 1). Of the 41 cases with IUGR, 30 had a first trimester PAPP-A <0.5 MoM, confirming the statistically significant correlation between maternal PAPP-A level and birth weight (p=0.016).

At the baseline ultrasound performed at 12-13 weeks gestation the ultrasound appearance of placenta was also assessed (presence of deciduous hematoma, areas of angiomatous transformation, detachment of placenta), which suggests impaired first wave of trophoblast invasion and thus a possible long-term placental insufficiency and implicitly risk of IUGR. In 25 cases such changes were identified.

Thirty of the infants had low birth weight and first trimester MPV levels > 10 fl, (fig. 2). A statistically significant correlation was found between birth weight and first trimester MPV level (p = 0.001).

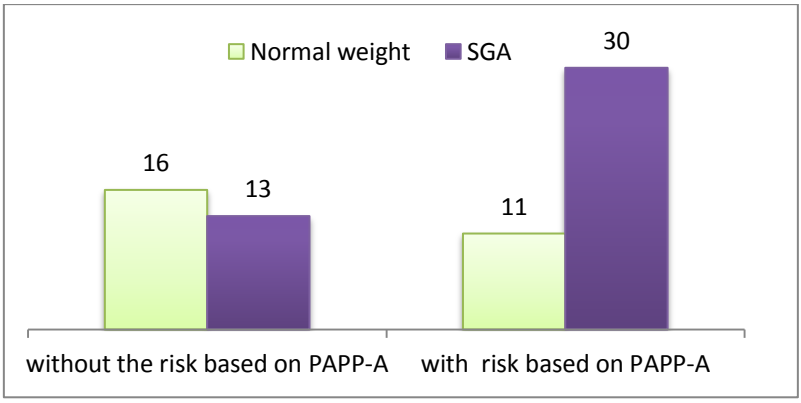

Fig. 1. Distribution of cases by birth weight and PAPP-A

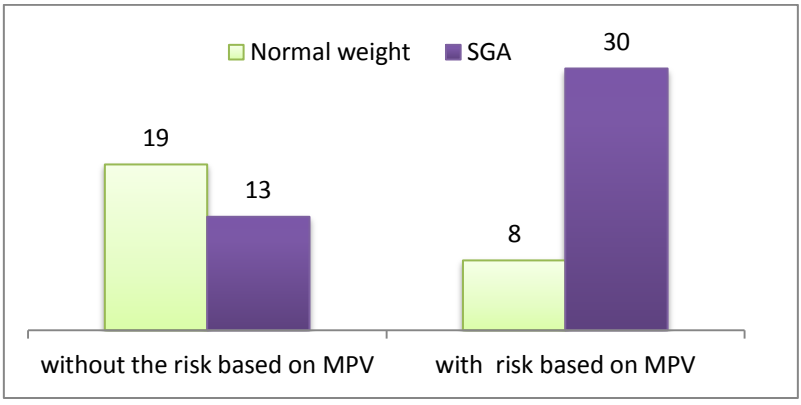

Fig. 2. Distribution of cases by birth weight and MPV

DISCUSSIONS

IUGR is a current problem and a research topic in modern obstetrics. In practice, IUGR has a statistical definition and is a fetal weight or fetal biometry below the 10th percentile or 2 SD below the mean for

gestational age (3, 4). According to the data in the literature, elevated PAPP-A levels are not correlated with increased risk of obstetric pathology, but it was found that a PAPP-A level below the 5th percentile is associated with increased risk of IUGR (17-22).

A study by M. Kwik in 894 women in the first trimester of pregnancy showed that serum PAPP-A levels <0.5 MoM have a sensitivity of 33% and a specificity of 89% in detecting fetuses with a weight below the 10th percentile. Low PAPP-A levels indirectly indicate abnormal placentation, that is a placental insufficiency which has effects on fetal growth (19).

In our study 30 of the pregnant women with IUGR had PAPP-A levels <0.5 MoM, suggesting a statistically significant correlation between IUGR and PAPP-A (p = 0.016).

Increased MPV reflects platelet activation caused by uteroplacental circulatory insufficiency, pathophysiological mechanism with repercussions on fetal growth. In this study it was found that 30 newborns had low birth weight and a first trimester MPV level > 10 fl, p = 0.001, data consistent with the literature (23-25).

A study by M. Kanakt-Pektas shows that MPV levels > 10 fl can predict IUGR with a sensitivity of 82.4% and specificity of 60% (24).

In our study, 41 pregnant women had PAPP-P levels <0.5 MoM and in 38 cases MPV level was > 10fl, which, according to the above mentioned literature data, can be used as markers for the early prediction of IUGR.

The ultrasound appearance of impaired first wave of trophoblast invasion is described as intra-and/or periplacental pathological areas which can be considered a possible cause of placental insufficiency, thus a risk of IUGR.

CONCLUSIONS

This study shows that the early detection of serum markers PAPP-A, MPV and placental changes detected at ultrasound and the detailed medical history obtained during the first trimester of pregnancy can be used as screening markers for the detection of cases at risk for IUGR. Identifying pregnancies at risk for IUGR during the first trimester requires the use of secondary preventive measures and monitoring of these cases in order to reduce neonatal morbidity and mortality.

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CEPHALOMETRIC FEATURES OF CLASS III MALOCCLUSION

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CEPHALOMETRIC FEATURES OF CLASS III MALOCCLUSION (Abstract): **Aim**: The study aimed to identify quantitative and relational characteristics of bone, dental and soft tissue structures for Class III malocclusion, according to gender and age range. **Material and methods**: 60 conventional lateral cephalograms were divided into two groups according to ANB angle: the group of cases with skeletal Class III (n=36) and a control group with skeletal Class I (n=24). There were performed 53 digital cephalometric measurements according to Steiner, Tweed and Jarabak analyzes. The Kolmogorov-Smirnov, t-student and Levene tests were used to find the characteristics of Class III, using SPSS 16.0 for Windows. **Results**: We found 14 parameters that distinguished the two classes disorders (the angles SNB, SND, FMA, IMPA, MeGoOcP, Mand 1-MeGo, NSAr, ArGoMe, NGoMe and SNPog; the distances Ao-Bo and 1u-NPog; Holdaway and AFH ratios) and 3 parameters for the Class III age ranges (NGoAr angle, Ls-NsPog' distance and S-Ar:Ar-Go ratio) ($p \leq 0.05$). There were found no significant differences between genders for skeletal Class III. **Conclusion**: Emphasizing the cephalometric characteristics of Class III malocclusion, with the overall growth together with dental and occlusion development, requires early orthodontic therapy. **Keywords**: CONVENTIONAL LATERAL CEPHALOMETRIC RADIOGRAPHY, CLASS III MALOCCLUSION, DIGITAL CEPHALOMETRIC MEASUREMENTS.

Class III malocclusion is characterized by a mesial relation of the mandible compared to the maxillary, the cause beeing skeletal and its origin is usually hereditary (1). This anomaly shows a low prevalence at the white population (1-2%) and with an increased frequency in Asian countries like Japan (4-13%) (2).

Bourdet (cit by 3) was the first researcher who described the 1737 skeletal Class III pattern in children with mandibular prognathia. In such cases, lateral cephalometric radiography is essential in establishing the skeletal, dental and facial diagnosis in order to appropriately apply orthodontic or surgical treatment (4). Sagittal discrepancies are mainly due to excessive growth of the mandible with hyperdivergence growth pattern, resulting mandibular prognathia. Most studies of skeletal Class III malocclusion were performed on Eastern Asian populations (5-9). Diversity of opinion on parameters values of cephalometric characteristics of skeletal Class III are supposed to be the expression of the ethnic composition of the groups studied (10-12).

Our research was conducted in order to investigate the cephalometric characteristics of skeletal Class III malocclusion on a sample of patients without orthodontic treatment from the northeastern region of Romania. The aim of the study was to compare the cephalometric parameters of Class III with the skeletal Class I, to identify quantitative and relational differences of bone, dental and soft tissues structures, by gender and age range.

MATERIAL AND METHODS

This retrospective and case-control study was conducted for 60 conventional lateral cephalograms, from the Orthodontics Clinic at the Ambulatory Clinic of the "St. Spiridon" University Emergency Hospital, Iasi, Romania, during January 2006 and December 2014. The cephalograms' selection criterion was their good technical quality. All radiographs were performed with the same X-ray device, the STRATO-X Orthopantomograph with magnification factor of 11.8%.

The lateral cephalograms provided from patients from northeastern Romania, (28 - 47% males and 32 - 53% females), with ages between 7–26 years (mean 14.33±5.758 years), without previous orthodontic treatment. The patients cephalograms were divided in two groups, by skeletal class (ANB angle): cases group=36 (60%) patients with skeletal class III (ANB<2°), which were 17 (42%) males and 19 (53%) females, 26 (72%) with age <14 years and 10 (28%) with age >14 years; control group=24 (40%) patients with skeletal class I (ANB≤4°), witch are 11 (46%) males and 13 (54%) females. The patients with craniofacial dysostosis, craniofacial synostosis, endocrine and genetic syndromes were excluded. The informed consent was acquired from all subjects or the patients' parents. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

The anatomical outlines of the lateral cephalograms were drafted on tracing paper with a 0.5mm diameter pencil. The tracing paper was scanned (MFD Canon Pixma MP280) in digital format (JPG File), after which it was stored on a computer (Asus Eee PC 1015BX) (13). The digital cephalometric analysis was performed with Onyx Ceph™ (Onyx CEPH 2.7.18 (174) Image Instruments GmbH, Chemnitz, Germany). The landmarks were located with the mouse pointer directly on the digital paper image on the computer display (fig. 1).

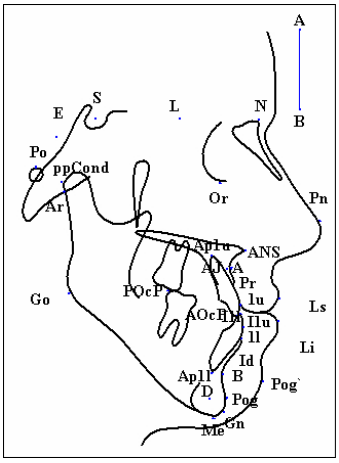

Fig. 1. Cephalometric landmarks: sella (S), nasion (N), E-point (E), L-point (L), porion (Po), condylion posterior (ppCond), articulare (Ar), orbitale (Or), gonion (Go), menton (Me), gnathion (Gn), pogonion (Pog), D-point (D), anterior nasal spine (ANS), A-point (A), A-point acc. to Jarabak (AJ), prosthion (Pr), infradentale (Id), B-point (B), upper incisor apex (Ap1u), upper incisor crown tip (1u), incision superior (I1u), incision inferior (I1l), lower incisor crown tip (1l), lower incisor apex (Ap1l), anterior point for the occlusal plane (AOcP), posterior point for the occlusal plane (POcP), pronasale (Pn), labrale superior (Ls), labrale inferior (Li) and pogonion soft tissue (Pog')

For each imagines there were measured 28 angles (°), 21 linear dimensions (mm) and 4 bone ratios (%), dental and soft tissues (n=53 measurements), using Steiner (14), Tweed (15) and Jarabak (16) analyses (fig. 2). Every single scanned paper was digitally measured under identical calibration conditions for the size of the cephalometric images. All data obtained were extracted and stored in the electronic format (Microsoft Office Excel 97-2003 Worksheet). The protocol included a quantitative evaluation of the skull basis, maxillary, mandible, and the relations between them, as well as with the dental and soft tissues.

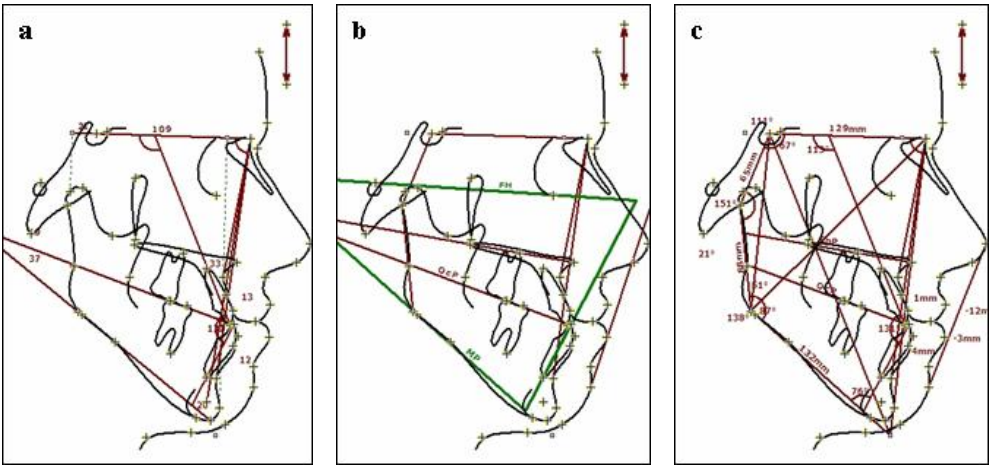

Fig. 2. Cephalometric parameters

(a) acc. Steiner: SNA, SNB, ANB, SND, II, SN-OcP, SN-GoGn, Max1-NA, Max1-SN, Mand1-NB, 1u-NA, 1l-NB, Pog-NB, Holdaway ratio, S-L and S-E. (b) acc. Tweed: FMIA, FMA, IMPA, Wits, PoOr-OcP, Z, PFH, AFH and AFH:PFH. (c) acc. Jarabak: MeGo-OcP, Mand1-MeGo, 1u-NPog, 1l-NPog, Ls-PnPog', Li-PnPog', NSAr, SArGo, ArGoMe, Sum, N-S, S-Ar, NGoAr, NGoMe, Ar-Go, S-Ar:Ar-Go, Go-Me, SN-GoMe, GoMe:NS, N-Go, S-Me, NSGn, S-Go, N-Me, SGo:NMe, SNPog and NAPog.

Statistical analysis was made with program SPSS 16.0 (SPSS Inc., Chicago, IL), for Windows. The numerical values were calculated according to the parameters of descriptive statistics (mean value, standard deviation (SD), error of standard deviation (SEM), minimum and maximum value). To compare the measured values of the two skeletal classes, sexes and age intervals, the Kolmogorov-Smirnov, t-Student and Levene tests for the equality of variances were applied, with $p \leq 0.05$ value, corresponding to the 95% confidence interval, beeing considered statistically significant.

RESULTS

There were measured the angles and distances between the bone structures, dental and soft tissues for each lateral cephalometric radiograph, using the three cephalometric analyses. The measurement mean values were compared between the two skeletal classes.

For the Steiner (14) analysis there were found statistically significant differences between skeletal classes for the sagittal position of the mandible to the anterior cranial base plane (SNB angle), sagittal position of symphysis mentalis to the ante-

rior plane of the cranial base (SND angle) and for Holdaway ratio (tab. I).

For the Tweed (15) analysis there were found statistically significant differences between skeletal classes for the mandibles' growth direction (FMA angle), sagittal position of the lower central incisor to the mandibular plane (IMPA angle), sagittal relation of maxillary-mandible (Wits or distance Ao-Bo) and for skeletal anterior height (AFH) (tab. II).

For the Jarabak (16) analysis there were found statistically significant differences between skeletal classes for inclination of the occlusal plane to mandibular basis (MeGoOcP angle), sagittal position of the lower central incisor to mandibular basis (Mand 1-MeGo angle), sagittal position of the upper central incisor to the Downs facial plane (distance 1u-NPog), temporo-mandibular joint and glenoid Fossae position (NSAr angle), type of condylar growth (ArGoMe angle), type of mandibular growth (NGoMe angle) and sagittal position of mandibular basis to the anterior cranial base plane (SNPog angle) (tab. III).

Other differences of angular, linear and ratios measured mean values were not significant.

The mean values of the digital measurements of skeletal Class III were compared between the two sexes and no statistically significant differences were found.

The mean values of the digital measurements of skeletal Class III were compared for two intervals of age <14 years and >14 years and there were found statistically significant differences for the type of mandibular growth (NGoAr), sagittal position of the upper lip (Ls-NsPog') and for the cranial base length and mandibular ramus height ratio (S-Ar:Ar-Go) (tab IV).

TABLE I

Descriptive statistics and differences between groups for cephalometric measurements of Steiner analysis

Parameters	Cases with skeletal class III (n=36)			Control with skeletal class I (n=24)			*p* value
	Mean	SD	SEM	Mean	SD	SEM	
Angular (°)							
SNA	79.7217	5.43376	0.90563	80.7038	3.09720	0.63221	0.426
SNB	80.4567	5.47972	0.91329	77.7275	3.17859	0.64883	0.032*
ANB	-0.7344	2.35933	0.39322	2.9758	0.65558	0.13382	0.000****
SND	78.1900	5.22631	0.87105	75.7771	3.05570	0.62374	0.046*
II	131.8017	11.17348	1.86225	131.9329	11.09934	2.26564	0.965
SN-OcP	15.5447	5.47329	0.91222	16.2433	5.03722	1.02822	0.619
SN-GoGn	30.3708	6.66255	1.11042	28.7871	5.03722	1.02822	0.332
Max1-NA	28.4169	7.19123	28.4169	24.6783	7.37017	1.50443	0.056
Max1-SN	108.1381	7.52379	1.25396	105.3813	7.91621	1.61589	0.179
Mand1-NB	20.5164	7.80400	1.30067	20.4154	6.85486	1.39924	0.959
Linear (mm)							
1u-NA	6.4519	2.75272	0.45879	5.5350	2.13288	0.43537	0.174
1l-NB	4.3242	2.26633	0.37772	3.6167	1.37529	0.28073	0.176
Pog-NB	2.4647	1.65978	0.27663	2.9592	1.59626	0.32584	0.256
Holdaway ratio	3.2861	2.14431	0.35738	2.0646	1.48163	0.30244	0.018*
S-L	56.6764	12.57102	2.09517	53.1608	5.57675	1.13835	0.204
S-E	19.5053	4.77858	0.79643	20.2213	2.87271	0.58639	0.513

$p>0.05$ – no statistically significant differences; *$p<0.05$ – statistically significant differences; ****$p<0.0001$ – extremely statistically significant differences.

TABLE II

Descriptive statistics and differences between groups for cephalometric measurements of Tweed analysis

Parameters	Cases with skeletal class III (n=36)			Control with skeletal class I (n=24)			*p* value
	Mean	SD	SEM	Mean	SD	SEM	
Triangle (°)							
FMIA	66.0664	8.20082	1.36680	64.9429	6.79298	1.38661	0.581
FMA	26.8550	6.57577	1.09596	22.4987	5.35326	1.09273	0.009**
IMPA	87.0789	8.14199	1.35700	92.5571	7.37926	1.50628	0.010**
Further measurements							
SNA (°)	79.7217	5.43376	0.90563	80.7038	3.09720	0.63221	0.426
SNB (°)	80.4567	5.47972	0.91329	77.7275	3.17859	0.64883	0.032*
ANB (°)	-0.7344	2.35933	0.39322	2.9758	0.65558	0.13382	0.000****
Wits (Ao-Bo) (mm)	-5.1500	4.75349	0.79225	-0.3163	3.73168	0.76173	0.000****
POr-OcP (°)	9.4183	5.07064	0.84511	8.6367	5.72637	1.16889	0.581
Z (°)	77.2153	10.15696	1.69283	75.6438	7.68934	1.56958	0.522
PFH (mm)	50.8775	9.17578	1.52930	49.7838	5.77117	1.17804	0.606
AFH (mm)	66.8661	10.86013	1.81002	61.6392	5.37680	1.09753	0.033*
AFH/PFH (%)	76.4650	9.69464	1.61577	80.9875	8.93843	1.82455	0.073

p>0.05 – no statistically significant differences; **p*<0.05 – statistically significant differences; ***p*<0.01 – highly statistically significant differences; *****p*<0.0001 – extremely statistically significant differences.

TABLE III

Descriptive statistics and differences between groups for cephalometric measurements of Jarabak analysis

Parameters	Cases with skeletal class III (n=36)			Control with skeletal class I (n=24)			*p* value
	Mean	SD	SEM	Mean	SD	SEM	
Dental analysis							
MeGoOcP (°)	17.4908	3.75773	0.62629	13.8633	3.85878	0.78767	0.001***
II (°)	131.6975	11.06530	1.84422	131.9329	11.09934	2.26564	0.936
Max1-SN (°)	108.3031	7.44283	1.24047	105.3813	7.91621	1.61589	0.152
Mand1-MeGo (°)	87.0583	8.18432	1.36405	92.5779	7.38995	1.50847	0.010**
1u-NPog (mm)	3.3825	4.08578	0.68096	6.0708	2.51376	0.51312	0.006**
1l-NPog (mm)	1.6036	3.50999	0.58500	0.4429	2.72691	0.55663	0.177
Ls-NsPog' (mm)	-5.6833	2.80010	0.46668	-4.2250	2.93086	0.59826	0.057
Li-NsPog' (mm)	-2.8067	3.19658	0.53276	-2.6613	2.94068	0.60026	0.859
Skeletal analysis							
NSAr (°)	121.4239	5.57444	0.92907	125.6046	4.79078	0.97791	0.004**
SArGo (°)	142.3531	6.31661	1.05277	141.7117	6.72933	1.37362	0.709
ArGoMe (°)	129.1639	7.70221	1.28370	122.7921	5.41522	1.10538	0.001***
Sum (°)	392.9414	7.07147	1.17858	390.1100	5.17420	1.05618	0.098
N-S (mm)	70.9967	10.02414	1.67069	71.3171	5.34401	1.09084	0.887
S-Ar (mm)	35.6836	5.92013	0.98669	34.6004	2.98528	0.60937	0.411
NGoAr (°)	53.3419	3.67529	0.61255	52.0700	4.22530	0.86249	0.221
NGoMe (°)	75.8211	6.60669	1.10111	70.7229	4.60316	0.93962	0.002**

Parameters	Cases with skeletal class III (n=36)			Control with skeletal class I (n=24)			*p* value
	Mean	SD	SEM	Mean	SD	SEM	
Ar-Go (mm)	50.8775	9.17578	1.52930	49.7838	5.77117	1.17804	0.606
S-Ar:Ar-Go (%)	71.2353	12.67297	2.11216	70.3342	9.95383	2.03182	0.771
Go-Me (mm)	68.1181	12.47722	2.07954	66.8333	7.90436	1.61347	0.656
Go-Me:N-S (%)	95.9083	10.18379	1.69730	93.9050	10.62951	2.16974	0.466
SNA (°)	79.7217	5.43376	0.90563	80.7038	3.09720	0.63221	0.426
SNB (°)	80.4567	5.47972	0.91329	77.7275	3.17859	0.64883	0.032*
ANB (°)	-0.7344	2.35933	0.39322	2.9758	0.65558	0.13382	0.000****
SNGoMe (°)	32.9414	7.07147	1.17858	30.1100	5.17420	1.05618	0.098
N-Go (mm)	114.3119	16.68898	2.78150	115.9513	8.56687	1.74871	0.659
S-Me (mm)	133.3447	19.64042	3.27340	126.0708	7.73846	1.57961	0.090
NSG (°)	66.2544	4.50370	0.75062	66.7404	3.09286	0.63133	0.647
S-Go (mm)	81.3564	12.09777	2.01629	79.3687	5.92924	1.21030	0.459
N-Me (mm)	118.7572	18.61302	3.10217	114.0229	8.46308	1.72752	0.248
S-Go:N-Me (%)	68.7256	5.66855	0.94476	69.7362	4.44223	0.90677	0.465
SNPog (°)	81.5817	5.24116	0.87353	79.2075	3.07573	0.62783	0.050*
NAPog (°)	175.2058	4.63133	0.77189	176.7813	1.97550	0.40325	0.122

p>0.05 – no statistically significant differences; **p*<0.05 – statistically significant differences; ***p*<0.01 – highly statistically significant differences; ****p*<0.001 – very highly statistically significant differences; *****p*<0.0001 – extremely statistically significant differences.

TABEL IV

Descriptive statistics and significant differences between intervals of age for cephalometric measurements of the skeletal class III

Parameters	Cases with skeletal class III (n=36)						*p* value
	Age <14 years (n=26)			Age >14 years (n=10)			
	Mean	SD	SEM	Mean	SD	SEM	
NGoAr (°)	54.3362	3.49701	0.68582	50.7570	2.87759	0.90997	0.007**
Ls-NsPog' (mm)	-4.9931	2.79605	0.54835	-7.4780	1.95437	0.61803	0.015*
S-Ar:Ar-Go (%)	73.8815	13.52723	2.65291	64.3550	6.59167	2.08447	0.008**

**p*<0.05 – statistically significant differences; ***p*<0.01 – highly statistically significant differences.

DISCUSSION

Our research has focused on the comparison of cephalometric measurements between skeletal Class III and Class I in order to identify the quantitative characteristics and bone structures relations, dental and soft tissues of Class III malocclusion, according to gender and age range. The skeletal Class was identified using the ANB angle value, beeing recognized by many authors as the skeletal discrepancies indicator (11, 13, 17, 18). We used three cephalometric analyzes used by orthodontists, in order to identify three aspects (skeletal, dental and soft tissues) characteristic to the Class III malocclusion. We chose efficient cephalometric analysis software for precision measurements.

Using statistical tests allowed us to find significant differences between skeletal Classes, which represented the defining parameters of skeletal Class III malocclusion for the studied population. Thus, we found 14 statistically significant measure-

ments (10 angular, 2 linear and 2 of percentage), that characterized the skeletal Class III: mandibular prognatia by the anterior sagittal position of the mandible to the anterior cranial base plane (increased SNB angle and decreased Ao-Bo distance); progeny by the anterior sagittal position of symphysis mentalis to the anterior cranial base plane (increased SND and SNPog angles and Holdaway ratio); mandible's hyperdivergent growth (increased FMA and NgoMe angles); lower dental retroclination (decreased IMPA and Mand 1-MeGo angles and 1u-Npog distance); occlusal plane tilting towards the mandible basis (increased Me GoOcP angle); anterior position of temporomandibular joint (decreased NSAr angle); posterior condylar growth, with the opening of the mandibular angle (increased ArGoMe angle); facial anterior height growth (increased AFH ratio).

Previous comparative studies (of Class I and Class III) reported similar parameters as prognathia, posterior mandibular rotation, forward position of the mandible and lower central incisor retroclination (3, 6-9, 19-22), as well as different parameters as cranial base shortening, underdeveloped maxillary, upper central incisor proclination and facial deficiency of the zygomatic region (11, 12, 23, 24). Results are still controversial and some authors argue that the cranial morphology in subjects with Class III does not differ from those with Class I (8, 25). Contradictions in existing trials results are due to the heterogeneity of ethnic, race and age diversity of studied groups and research methods.

In our study, the digital measurement values of skeletal Class III were compared between sexes and we did not find statistically significant differences. The literature reports significant differences between the sexes regarding the position of the upper lip (26).

In the present study, statistically significant differences between the two age ranges, prepubertal (<14 years) and pubertal-postpubertar (>14 years) of skeletal Class III, showed emphasis of several characteristics together with the growth dental-maxillofacial complex, at the studied population. Thus, we found 3 parameters that showed the emphasis of the vertical growth of the mandible (decreased NGoAr angle), mandible prognathia (decreased S-Ar:Ar-Go ratio) retrusion of the upper lip (decreased Ls-NsPog' distance). Previous studies show that specific Class III parameters are established from an early age and are maintained throughout the skeletal growth (6, 27), and puberty is of significant importance on the mandibular length growth, the maximum pubertal beeing extended in subjects with class III (10, 28).

Our study presents anthropometric importance in the population studied and has clinical-diagnostic value for a correct specialized orthodontic or surgical therapy (4).

CONCLUSIONS

The present study demonstrates that the Class III malocclusion shows a distinct pathology, characterized by a series of specific cephalometric parameters which set the diagnosis and indicate the morphological dental-maxillofacial segment that should be orthodontically corrected as early as possible.

Our study has particular importance for practitioners and suggests the necessity in their involvement in identifying at an early age these serious skeletal discrepancies. Future similar studies may extend to other types of skeletal malocclusion affecting facial esthetics and dental health of our young population.

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HISTOPATHOLOGICAL VARIANTS IN RENAL TUMORS: FROM COMMON TO UNCOMMON SUBTYPES

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HISTOPATHOLOGICAL VARIANTS IN RENAL TUMORS: FROM COMMON TO UNCOMMON SUBTYPES (Abstract): **Aim**: The assessment of renal tumors diagnosed in a department specialized in kidney pathology, following the correspondence between the identified histological subtypes and pathological background reported in the literature. **Material and Methods:** The study group included 219 patients diagnosed with renal tumors and surgically treated in the University Hospital "Dr. C.I. Parhon", Iaşi. Histopathological diagnosis was revalued reconsidering microscopic specimens consequent to analyzed cases. **Results:** In 204 (94.4%) of the 219 cases there were no differences between initial diagnosis and the diagnosis subsequent to revaluation. After microscopic examination, most cases were classified as clear cell renal cell carcinomas and urothelial carcinomas. For clear cell renal cell carcinoma and urothelial carcinomas we registered different percentages compared with data reported in literature (86.27% *vs.* 70-75%, 28.81% *vs.* 10% respectively). **Conclusions:** Our results point out the possibility of identifying epidemiological features with regional / national specificity. **Keywords**: RENAL TUMOR, HISTOLOGICAL SUBTYPE, CLEAR CELL RENAL CELL CARCINOMA.

The pathologist applies as a diagnostic operational tool the WHO histological classification of renal tumors which includes nine major classes: renal cell tumors, metanephric tumors, nephroblastic tumors, mesenchymal tumors, epithelial and mesenchymal mixed tumors, neuroendocrine tumors, hematopoietic and lymphoid tumors, germ cell tumors, and metastatic tumors (1). Additionally, should be considered the urothelial tumors category with renal pelvic origin, representing approximately 10% of renal tumors (1).

Renal cell carcinoma (RCC) represents approximately 3% of all human malignancies (2, 3) and approximately 90% of all kidney tumors. Analysis of incidence and mortality assigned to RCC shows a noticeable regional variability, which is probable the result of demographic, environment, and genetic factors influence. The efforts to identify the reversible factors that cause renal carcinoma development, have led not only to a better understanding of its etiology, but also of the genetic features associated to histological profile (4). RCC prognosis

is generally related with the tumor stage being proved that the survival of stage I is> 90% at 5 years, while of stage IV is <20-30%. Unfortunately, renal tumors often remain asymptomatic until advanced stages. Due to its origin in various tubular structures of the kidney parenchyma, RCC is characterized by a marked heterogeneity, being described many morphological subtypes (5). Clear cell RCC accounts for approximately 70-75% of cases, followed by papillary RCC (10-15%), chromophobe RCC (5%), collecting duct RCC (<1%) and unclassified form (<2%) (5-7). Sarcomatoid features can occur in all forms of RCC, as a sign of active epithelial-mesenchymal transition (5, 8).

Other rare histological types are: medullary RCC, translocation RCC, and mucinous tubular and spindle-cell RCC (5, 9). In the last years, new epithelial tumor subtypes were described: tubulocystic RCC, acquired cystic disease-associated RCC, clear cell tubulopapillary RCC, the microphthalmia (MiT) family translocation RCCs, and hereditary leiomyomatosis-RCC syndrome-associated tumors (5, 10), and 3 new entities with a provisional status were proposed: thyroid-like follicular RCC, succinate dehydrogenase B deficiency-associated RCC; and anaplastic lymphoma kinase translocation RCC (5, 10).

Our study aimed to analyze the renal tumors diagnosed in a department specialized in kidney pathology, following correspondence between identified histological subtypes and pathological background reported in the literature.

MATERIAL AND METHODS

The study group included a total of 219 patients diagnosed with renal tumors surgically treated in the Urology Department, University Hospital "Dr. C.I. Parhon", Iaşi during 2010-2013. The male patients accounted for 123 cases (55%) while female patients accounted for 101 cases (45%). The age interval ranged between 20 and 84 years (mean 62.98 years). Tissue samples from surgical procedures were processed in the Laboratory of Pathology using standard protocol in order to obtain microscopic preparations for histopathological diagnosis. A retrospective, descriptive study was initially performed, based on the existing pathology reports. Subsequent, all microscopic slides corresponding to the analyzed cases were cross-assessed by three pathologists, in order to review the diagnosis.

RESULTS

All cases were histopathologically classified according to the WHO histological classification of renal tumors (tab. I). For 204 (94.4%) of the 219 analyzed cases there were no differences between initial diagnosis and the diagnosis after re-evaluation. A total of 12 cases, 11 diagnosed as mixed renal cell carcinoma and 1 case diagnosed as renal cell carcinoma with high-grade anaplasia were assigned as clear cell renal cell carcinoma.

TABLE I

Distribution of histological subtypes

Histological subtype	Number of cases	Percentage
Cell clear renal carcinoma	132	60.27%
Urothelial carcinoma	63	28.81%
Oncocitoma	12	5.47%
Papillary renal cell carcinoma	9	4.10%
Angiomyolipoma	1	0.45%
Angiomyofibroma	1	0.45%
Metastatic digestive tumor	1	0.45%

The microscopic exam in cases classified as clear cell renal cell carcinoma (ccRCC) highlighted various aspects. Some cases

presented cystic and solid areas with infiltration limited to medulla or with cortex, capsule or perirenal adipose tissue invasion. Other cases of ccRCC showed large areas of sarcomatous transformation. We identified also ccRCC with multinodular appearance, with high index of nuclear atypia and large areas of necrosis and hemorrhage. In most cases, the large vessels hilum had no tumor emboli. In the 11 cases (5.02%) initially considered as mixed renal cell carcinoma, based on the association between clear cell renal cell carcinoma areas and large areas of tumoral cells with cytoplasm predominantly acidophile, or isolated chromophobe, with tubulo-papillary or papillary pattern, the histologic revaluation resulted in their classification as clear cell renal cell carcinoma, motivated by the certain presence of tumor cells with typical appearance of clear cell, even if in limited territories. The same diagnostic criterion was applied to 1 case initially diagnosed as renal cell carcinoma highly anaplastic.

In case of oncocytomas, *the microscopic exam* revealed the presence of round or polygonal cells arranged in nests or with alveolar or tubular pattern and rare microchystic areas, with abundant, intensely eosinophilic, and finely granular cytoplasm, and small, round, centrally located nuclei, with uniform chromatin and finely contoured nuclear membrane. The myxoid or hyalinized stroma included oncoblasts - small cell with minimum cytoplasm and small, hyperchromatic nuclei.

The microscopic structure of angiomyolipoma revealed myoide fusiform cells, areas of mature adipose tissue and dysmorphic thick walled blood vessels without elastic lamina. Areas represented by smooth muscle component showed hypercellular, atypical, pleomorphic or epithelioid appearance. We identified also necrosis and hemorrhage areas associated with stromal sclerosis. In the structure of angyomiofibroma we noted high cellular density and nuclear pleomorphism particularly in the myomatous areas.

In one case, the diagnosis was metastatic tumor. *The immunohistochemical profile* of undifferentiated tumor cells, with glandular and compact architecture, confirmed the digestive origin of the primary tumor.

The microscopic exam in urothelial carcinoma with renal pelvic origin showed papillary pattern, G2 or G3 degrees of differentiation, infiltrating adjacent renal parenchyma, with large areas of necrosis, commonly tumor emboli in lymphovascular spaces and perineural invasion in the hilum.

DISCUSSION

The classification of renal tumors registered significant changes in recent decades, due to progress in deciphering the molecular profile of this pathology (5, 9, 10). Genomic analysis demonstrated that the differences between tumor subtypes are not based only on histological criteria, but also on the specific genetic mutations (5, 11). Consequently, enhanced awareness of biological and genomic characteristics of different tumor subtypes enabled the development of targeted therapies (12). There are two aspects to be highlighted. First, although genetic alterations are important, 96% of all cases are sporadic forms of RCC and only 4% are hereditary (13, 14). Secondly, the predominant histological subtypes in the pathology of renal tumor are clear cell renal cell carcinoma and urothelial carcinoma (1, 15).

Our study complements the literature limited data on individual experience of regional centers specialized in renal tumor pathology (16-18). In this context, we draw attention to the fact that for clear cell renal carcinoma and urothelial carcinoma we

registered different percentages, higher compared to reported data in the literature (86.27% versus 70-75%, 28.81% versus 10% respectively). These figures point out the possibility of identifying epidemiological features with regional / national specificity, thus opening perspectives of deepening the study.

CONCLUSIONS

Although our study did not include uncommon or rare histological variants, their existence cannot be unnoticed - pathologist theoretical knowledge providing the basis for a diagnosis based on operational criteria of the most recent classification.

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INTRAOCULAR BIODISTRIBUTION OF INTRAVITREAL INJECTED FLUORESCENT DEXAMETHASONE-CHITOSAN NANOPARTICLES IN RABBIT EYES

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INTRAOCULAR BIODISTRIBUTION OF INTRAVITREAL INJECTED FLUORESCENT DEXAMETHASONE-CHITOSAN NANOPARTICLES IN RABBIT EYES (Abstract): **Aim:** To evaluate intraocular biodistribution of fluorescent nanoparticles composed of dexamethasone bound to chitosan after intravitreal administration in rabbit eyes. **Material and methods:** The chitosan and gelatin based nanoparticles were synthetized using a reverse emulsion-double crosslinking technique (ionic and covalent) and then dexamethasone was bound. Two units of 1% suspension of these nanoparticles in saline solution were injected intravitreally into rabbit eyes. The histologic sections obtained at 72 hours were analyzed by confocal microscopy. **Results:** The chitosan-fluorescein conjugate bound to dexamethasone was present in all ocular tissues at 72 hours. The nanoparticles were present in the retina and lens in a larger amount than in the other ocular tissues. **Conclusions:** The reverse emulsion-double crosslinking technique was efficient in synthesizing a biocompatible polymeric nanosystem. The *in vivo* study of intraocular biodistribution of fluorescein-marked nanoparticles capable of binding dexamethasone revealed their affinity for the retina and lens after intravitreal administration. **Keywords:** BIODISTRIBUTION**,** INTRAVITREAL, NANOPARTICLES, DRUG DELIVERY, CHITOSAN, DEXAMETHASONE

Nanoparticles are nanometer sized molecules that behave as a whole unit with respect to their properties and transport behavior. These molecules are beginning to be more frequently studied and used in the therapy of many diseases as safe and efficient drug carrier systems. Recent studies have demonstrated the ability of nanoparticles to overcome the eye barriers (cornea, conjunctiva, sclera and the blood-retinal barrier), thus being able to deliver therapeutic agents to the different ocular tissues. (1-5).

Current research trends in ophthalmology aim at developing intraocular drug delivery systems able of exerting their pharmacological effect over a longer period of time at a lower administration rate and with

a lower dose related toxicity rate.

The most recent therapies of choroidal and retinal neovascularization target the main pathogenic processes of the disease: inflammation and overexpression of pro-angiogenic factors. In addition to the anti-angiogenic and anti-inflammatory agents approved for use (ranibizumab, aflibercept, steroids) or widely used in "off-label" manner (bevacizumab) there are numerous ongoing researches on other potential pathogenic factors (platelet-derived growth factor, complement modulators).

The aim of this experimental study is to assess the intravitreal biodistribution of fluorescent chitosan nanoparticles loaded with dexamethasone and injected in rabbit eyes. These nanoparticle-dexamethasone systems are considered potential therapeutic agents for ocular neovascularization and its complications.

MATERIAL AND METHODS

1. Preparation of dexamethasone fluorescent nanoparticle. The polymers used for the preparation of nanoparticles were chitosan and gelatin. These polymers were chosen because they combine the benefits of proteins and those of polysaccharides, in order to obtain a good bioavailability and drug release capacity to target ocular tissues.

Chitosan is a linear polysaccharide obtained from crustacean shells. It is composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Gelatin is a polypeptide prepared by thermal, chemical and physical denaturation of collagen. In the preparation process a series of emulsifiers and surfactants were also used. A 10% fluorescein solution was used to mark the chitosan-gelatin nanoparticles.

Chitosan and gelatin nanoparticles were synthesized using the double crosslinking in the droplet method. This method was selected due to the low toxicity required by ophthalmic preparations.

The heterogeneous reaction system involved two emulsions. The aqueous phase of the first emulsion was represented by the solution of the two natural polymers, gelatin and chitosan (polymer concentration 0.5 %). The organic phase of the first emulsion, consisting of toluene and the specific amount of surfactant Span 80 was added to the aqueous solution and stirred at 15 000 rpm for 10 minutes. The thus obtained primary emulsion was dripped by syringe into a 600-ml Berzelius beaker, stirred ultra-fast for 10 minutes in order to obtain the secondary emulsion in the organic phase, represented by a volume of 260 ml toluene and the appropriate surfactant, Span 80, 2%.

After the emulsification of the two emulsions, ionic crosslinking was carried out using sodium phosphate (5%), while stirring (5000 rpm/10 min). Glutaraldehyde was dripped into the reaction vessel while stirring in order to complete the covalent crosslinking.

After the double cross linked particles were obtained, separation was done by centrifuging and purification of the particles in several stages consisting of successive cycles of washing with double-distilled water /acetone/n-hexane to remove traces of toluene and centrifugation (5000 rpm). Drying of nanoparticles was carried out in n-hexane at room temperature.

The nanoparticles were marked with fluorescein, which was covalently bound to chitosan. Fluorescence was necessary to assess the biodistribution of nanoparticles within the eye.

Chitosan–fluorescein conjugate was synthesized using a method adapted from

De Campos et al. (2004) (6). The resulting product was dialyzed using a dialysis membrane in the form of tubes with a pore size of 12,000 Da. The last step was the lyophilization of the product. Chitosan fluorescein staining was highlighted by infrared spectroscopy.

Using double crosslinking in the double emulsion process, as described above, fluorescent nanoparticles were obtained by introducing in the reaction a fluorescein-labeled chitosan.

Afterwards, dexamethasone was bound to the fluorescent nanoparticles by immersing 100 mg nanoparticles in 9 ml solution of dexamethasone for 48 hours. Then, the suspension was centrifuged and the supernatant weighed.

2. Study of in vivo release of dexamethasone loaded fluorescent nanoparticles. The study was conducted in accordance with the Declaration of Helsinki, the current modifications (Somerset West Amendment). We used a group of 5 rabbits bred in laboratory under the same conditions and fed with standard food. Each rabbit received a 0.03 ml intravitreal injection of 1% solution of dexamethasone-loaded nanoparticles in the right eye. Before performing intravitreal injections the animals were anesthetized with intraperitoneal ketamine 25 mg / kg.

The animals used in the experiment were sacrificed 72 hours after the injection of fluorescent nanoparticles after prior anesthesia with Ketamine 25 mg/kg. The right eye was harvested, transferred to ice and sectioned within 15 minutes.

The tissue samples were then treated with mouse anti-fluorescein monoclonal antibodies, which were washed after 48 hours followed by a new treatment with secondary goat anti-mouse antibodies and labeled with R- phycoerythrin.

The sections were then transferred to microscope slides and coated with the fluorescence medium. To highlight the fluorescence, a confocal microscopy setup was used, including an inverted Nikon Eclipse TE-300 microscope.Images capture was done using red fluorescence (for R-phycoerythrin) and transmitted light in order to distinguish the morphological features.

RESULTS

Analysis of the biodistribution of fluorescent dexamethasone loaded-nanoparticles within tthe eye. Following the intravitreal injection and analysis of the obtained histological sections, the fluorescent dexamethasone-nanoparticles were found to be uniformly distributed and present in all ocular tissues. Red fluorescence was present in all ocular tissues of the experimental animals at 72 hours (fig.1 A, B; fig.2 A, B; fig.3 A, B; fig.4 A, B).

The intraocular biodistribution of dexamethasone nanoparticles recorded the highest values in the lens and retina, as seen at both the microscopic examination of histological sections and RGB diagram. A fluorescence value of 17.35 ± 1.85 UA inside the lens and 13.08 ± 3.75 UA in the retina was recorded, showing a better fixation of the drug loaded nanoparticles within the tissues surrounding the injection site.

In the structure of cornea and sclera a significant amount of dexamethasone loaded nanoparticles was detected, but lower compared with the retina and lens. Recorded values were 9.75 ± 3.24 UA for the cornea and 11.78 ± 2.94 UA for sclera, thereby highlighting the migration of nanoparticles in all ocular tissues at 72 hours after injection.

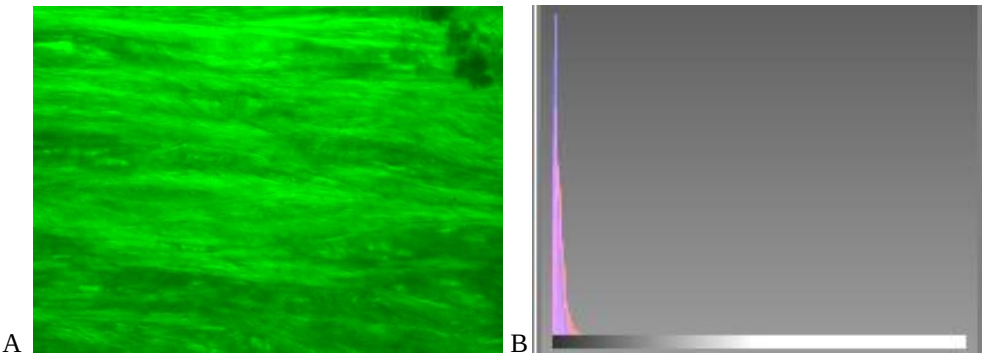


Fig. 1. A. Analysis of red fluorescence from cornea section of the right eye at 72 hours (40x) (9.75±3.24 UA, n = 5). B. Cornea RGB at 72 hours.
Red fluorescence is present at medium intensity.

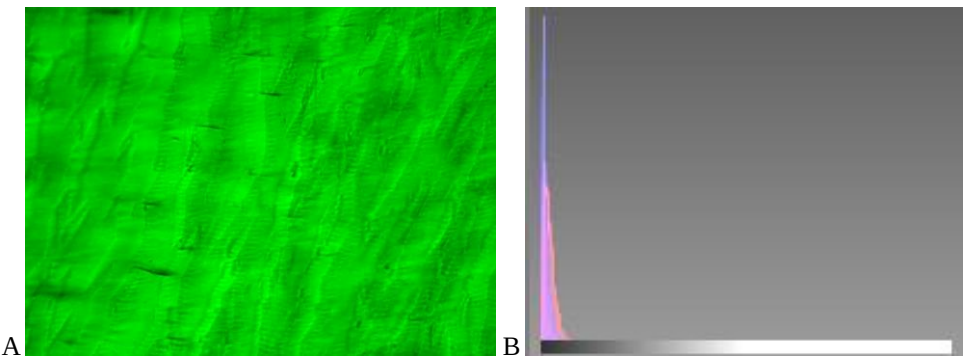


Fig. 2. A. Analysis of red fluorescence from lens section of the right eye at 72 hours (40x) (17.35 ± 1.85 UA, n = 5). B. Lens RGB at 72 hours.
Red fluorescence is present and intense.

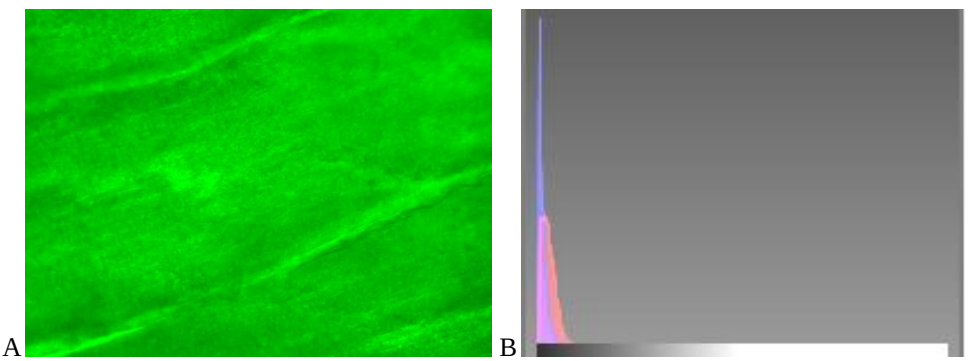


Fig. 3. A. Analysis of red fluorescence from retina section of the right eye at 72 hours (40x) (13.08± 3.75 UA, n = 5). B. Retina RGB at 72 hours. Red fluorescence is present and intense.

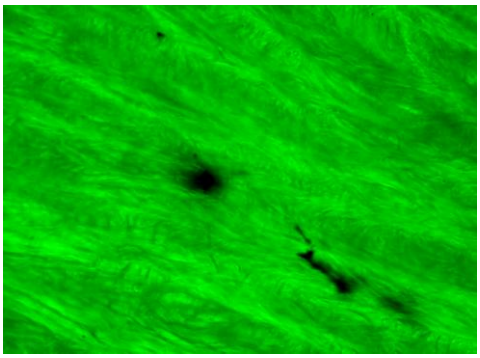
A  B

Fig. 4. A. Analysis of red fluorescence from sclera section of the right eye at 72 hours (40x) (11.78±2.94 UA, n = 5). B. Sclera RGB at 72 hours. Red fluorescence is present at medium intensity.

DISCUSSION

Nanoparticles are currently studied in terms of efficiency as carriers of many drugs with application in ophthalmology and other medical specialties. Specific controlled release properties depend on the physicochemical properties of the drug, the chemicals used in the synthesis of the nanoparticles, as well as the methods of preparing such polymer systems. In general, when using optimal drug loaded nanoparticles the ocular bioavailability of many drugs significantly increases compared to conventional formulations. Increased ability for overcoming the blood-retinal barriers and releasing biologically active principles in a safe and effective manner that opens new opportunities in the treatment of many ophthalmic diseases.

The blood-retinal barriers consist of an inner segment constituted of retinal endothelial cells, pericytes and astrocytes and an outer segment composed of the tight junctions between the retinal pigment epithelium cells. This barrier acts as a filter that restricts permeability of high molecular weight particles, including drugs, to the retina. Drug loaded nanoparticles of the appropriate size were proved to have favorable biological properties as prolonged residence time in the vitreal gel, higher availability of the drugs to the deeper layers of the retina and decreased toxicity (7-9). Also, these carriers can improve the availability of poorly water-soluble molecules to the retinal layers (10).

Nanopolimeric systems structured on chitosan and gelatin are used in the pharmaceutical composition of many hydrogels as drug carriers due to their property of being absorbed and metabolized without resulting toxic products. The blending of chitosan with gelatin increases the stability of the gel (11, 12).

Dexamethasone is a glucocorticosteroid with anti-inflammatory and immunosuppressive properties. In ophthalmology, is widely used in the topical treatment of various inflammatory diseases. The intravitreal administration of dexamethasone is used in the treatment of macular edema secondary to vascular occlusions, diabetes, uveitis. Its effectiveness in the treatment of disorders of the posterior segment of the eye is due to a reduction in vascular permeability and leukocyte accumulation, by acting simultaneously on both VEGF and cytokines path (13).

Particles stable in size and shape were obtained using the double crosslinking (covalent and ionic) in double emulsion technique. This method proved to be efficient due to covalent surface crosslinking. Also, the high swelling capacity of the nanoparticles is due to ionic crosslinking which enable a large diffusion capacity of drugs.

The histological samples were treated by indirect immunofluorescence to investigate the presence or absence of fluorescent drug- loaded nanoparticle in their structure. Tissue samples were then subjected to direct microscopy (by transmitted light) in order to highlight their morphological structure and confocal microscopy to assess the presence of fluorescently loaded nanoparticles inside ocular tissues. The presence of fluorescent dexamethasone nanoparticles was detected in the ocular tissues of experimental animals at 72 hours by marking red fluorescence.

Following intravitreal injection of fluorescently labeled polymer-drug nanosystems and analysis of the histological sections obtained from the rabbit eyeballs we found them to be uniformly distributed and present in all ocular tissues.

Comparing the results obtained at 72 hours after intravitreal administration of the fluorescent polymer-drug system with those achieved in our previous experimental study, in which only fluorescein loaded nanoparticles were injected in Wistar rat eyes, we concluded that the fluorescence values attained in the second experiment were higher (14). In both experiments the nanoparticles were predominantly fixed in the structure of retina and lens, but the fluorescence value was higher with a medium of 9 UA when dexamethasone was bound to the polymer. Although cornea and sclera presented a lower fluorescence than retina and lens in both experiments, higher values, in medium with 7 UA, were also registered when dexamethasone nanosystems were administered.

The higher values of fluorescence obtained in this experiment suggest that the dexamethasone carrier nanoparticles are stable and have the ability to persist in the tissues long enough to deliver the loaded drugs.

The results of this study are consistent with existing literature on the subject. Binding dexamethasone to polymeric nanoparticles systems has the effect of increasing the drug action period after intraocular injection. Experimentally, it was demonstrated that dexamethasone-loaded nanoparticles can inhibit the development of subretinal neovascularization, the effect being dose dependent (15). The bioavailability of the therapeutic substance is increased by its binding to the polymer carrier, dexamethasone nanoparticles are gradually released over a period of up to 50 days, with maintenance of stable therapeutic doses at least 30 days (16).

CONCLUSIONS

In our study dexamethasone carrier nanoparticles injected intravitreally were found mainly in the lens and retina at 72 hours after administration. Smaller amounts were found in the cornea and sclera at the same interval of time after administration.

We demonstrated the ability of drug-polymer systems to penetrate tissues in the immediate vicinity of administration site and migrate, especially in the retinal and lens structure, at 72 hour interval. Their stability is higher after drug binding to the polymeric structure. Also higher levels of

fluorescence were recorded in this case compared to the values obtained when only fluorescein-loaded nanoparticles were administered.

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INTRAORBITAL FOREIGN BODY

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INTRAORBITAL FOREIGN BODY (Abstract): Penetrating orbitocranial injuries caused by intraorbital foreign body are a rare cause of morbidity being most common among young people. The term intraorbital foreign body refers to a foreign body that occurs within the orbit but outside the ocular globe. We report the case of a 12-year-old male child who sustained a right cranial facial trauma due to accidental fall on a piece of wood, which penetrated intraorbitally. Native cranial magnetic resonance imaging (MRI -1.5 T) revealed the presence of an intraorbital foreign body, 6 cm long and 1.5 cm wide, near the orbital apex. In our patient, early surgical extraction of the foreign body had a decisive role on his full recovery.In this case, although a large foreign body penetrated the entire length of the orbit, it did not cause damage to any intraorbital structure. Vision and right ocular globe function had an excellent prognosis. **Keywords:** INTRAORBITAL FOREIGN BODY, ORBITAL TRAUMA, VISUAL ACUITY.

CASE REPORT

We report the case of a 12-year-old male child who sustained a right craniofacial trauma due to an accidental fall while playing with another child on a stake set firmly in the ground. During the impact, a wooden fragment 6 cm long and 1.5 cm wide penetrated intraorbitally the anterior right maxillary sinus wall and then the right orbital floor up to the orbit apex.

On local examination, the external end of the foreign body was seen just below the right lower orbital rim. The right eye was painful and showed significant palpebral edema and erythema (fig. 1).

Fig. 1. The patient photography at the first examination: a wooden foreign body, below the right lower orbital rim.

The ophthalmologic examination revealed preservation of visual acuity of the

right eye (V.A.R.E.=1) and no pathologic changes on slit-lamp biomicroscopic and ophthalmoscopic examination.

Native cranial computed tomography (CT) scan showed a right intraorbital hypodensity with a trajectory parallel to the interior wall of the orbit, medial to the right internal muscle up to the orbit apex, involving the scleral wall and a temporal aerial bulla.

Native cranial MRI (1.5 T) revealed the presence of an intraorbital foreign body which did not cause damage to the intraorbital vascular and nerve structures (fig. 2, 3, 4).

The child was admitted and the administration of antibiotics (clindamycin and metronidazole) was initiated.

Fig. 2. Sagittal T1 MRI, intraorbital foreign body (arrow).

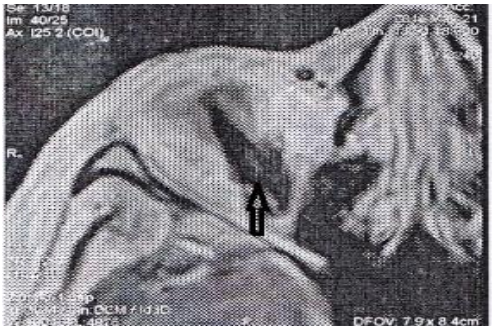

Fig. 3. Axial T1 MRI, intraorbital foreign body (arrow).

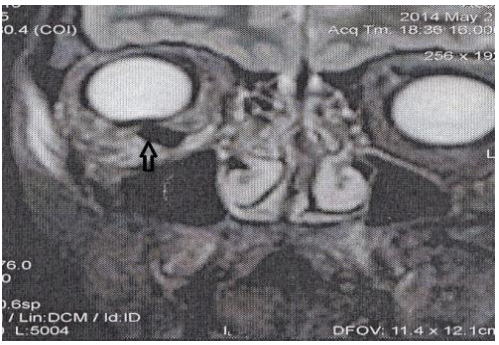

Fig. 4. Coronal T1 MRI, intraorbital foreign body (arrow).

Surgery was performed 24 h after the accident and consisted in the removal of the foreign body (wooden fragment) by enlarging the entry point (fig. 5, 6).

Fig. 5. Wooden foreign body extracted from the orbit.

Fig. 6. The post operative results.

The postoperative course was favorable, the patient being discharged 5 days after surgery.

Upon discharge, the patient showed diplopia, with left eye (L.E.) fixer and right eye (R.E.) sursumvergent +13°.

One month after discharge, diplopia remitted and visual acuity of the right eye remained 1.

DISCUSSION

Penetrating orbitocranial injuries caused by an intraorbital foreign body are a rare cause of morbidity and they occur mostly in children and adolescents.

Penetrating orbitocranial injuries are more common in people involved in military services, but they may also occur in civil life, where they usually take the form of accidents. The occurrence mechanism relies on high velocity forces like work accidents, injuries caused by weapons or even simple injuries (household accidents, play accidents). Accidents caused by intraorbital foreign bodies are more common in men than in women and young people, as compared to senior citizens.

Depending on the material, intraorbital foreign bodies may be classified into: inorganic metallic (iron, aluminum, steel, etc.), nonmetallic (plastic, glass, rock, etc.) and organic (wood, plants, thorns, etc).

The orbit is a cranial cavity where the eye and eyeball adnexa are located. Although the orbit is described as a pyramid, its walls are not exactly straight. The walls, tip and basis are curvilinear and they are pierced by foramens and fissures. An anatomical characteristic of the orbit is that structures are arranged in groups of seven: seven bones (frontal, ethmoid, lacrimal, sphenoid, zygomatic, palatine and maxillary), seven intraorbital muscles and seven nerves, respectively (1).

Foreign bodies located in the anterior 2/3 of the orbit may be approached extracranially, whereas those located in the apical area, especially in the medial section of the optic nerve, require a transcranial approach. Orbital injuries extending beyond the upper orbital fissure require a combined and complex surgical approach due to their closeness to the cavernous sinus.

The presence of the periorbita allows the classification of orbital injuries into intradural (deep in the periorbita) and extradural (when they are located between the periorbita and osseous orbit). In its turn, the muscular cone divides the orbit into two areas, the intraconal and the extraconal one. Whereas intraconal injuries are always intradural, extraconal injuries may be intra- or extradural. The intraconal area may also be divided in relation with the optic nerve into a medial, central and lateral area (2) (Figure 7).

The transcranial approach is preferred when the injuries are located at the top of the orbit and/or of the optic foramen. A direct approach through the lateral wall of the orbit requires lateral orbital margin and wall osteotomy and it is preferred when the foreign bodies are located in the upper, temporal or lower compartment and in the lateral side of the apex (3).

There are 2 basic types of surgical orbital approaches: transorbital, preferred by ophthalmologists, and extraorbital, preferred by neurosurgeons.

These are the primary transorbital approaches (3, 4):

-anterior orbitotomy without osteotomy:

-superior-incision through the eyelid, supra-orbital or sub-frontal incision,

-inferior-transconjunctival incision, subciliary incision or incision through the

lower eyelid,

-anterior orbitotomy with osteotomy of the upper orbital edge (for larger foreign bodies),

-lateral orbitotomy,

-medial orbitotomy,

-combination between lateral and medial orbitotomy.

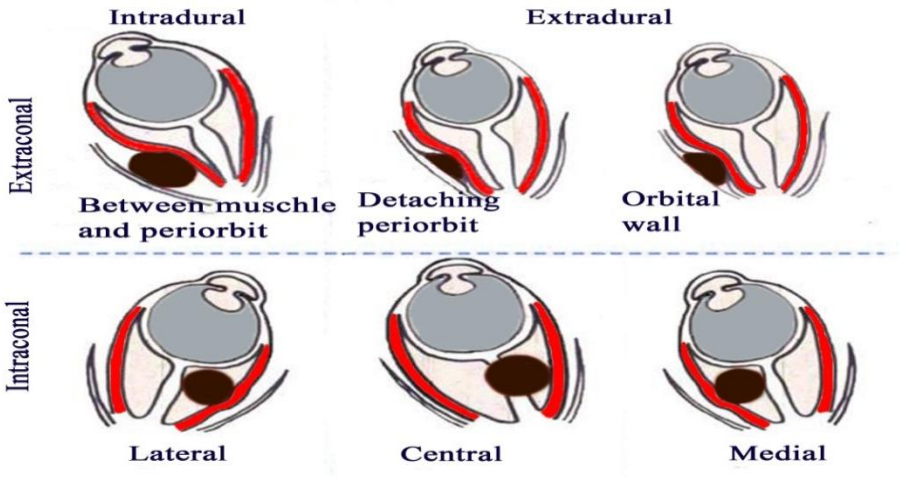

Fig. 7. Topographic classification of intraorbital lesions.

Extraorbital approaches are possible through a frontotemporal approach with or without orbital osteotomy and through a lower orbital approach. Extraorbital approaches may also be supported by endoscopic methods (4).

The approach path is set after the imaging assessment of the intraorbital injury, which reveals both the foreign body and its relations with the neighboring anatomical structures.

Eye Ultrasonography (US)

Although ophthalmic ultrasonography plays a well-determined role (5), as it provides real-time information about the eye and the orbit (especially about the intraocular structures), it may also be used during the surgical procedure. Here are some of the disadvantages of eye ultrasonography performed in case of injury caused by an intraorbital foreign body: foreign body migration (due to the probe moving around) and lack of information related to orbit fractures.

Computed Tomography (CT)

Unlike eye ultrasonography, computed tomography is available in most hospitals, does not require direct contact with the orbit, eyeball or foreign body, is superior to US in determining the size and shape of the foreign body, allows easier orbital fracture diagnosis, and is faster and cheaper than the MRI.

CT scanning should ideally observe the standard orbit examination protocol, according to which both the axial and coronal sections should be 1.0-1.5 mm. Nevertheless, foreign bodies smaller than 0.5 mm and plastic foreign bodies may be overlooked, whereas organic (wood) bodies

may have false negative results. Due to the air-containing wood cellulose matrix, the wood foreign body may be taken for pneumoorbita (6). Another disadvantage would be that orbit CT cannot be done in the operating room, the intraorbital structures are not viewed as by an eye US, and artifacts from metallic foreign bodies may occur. CT scan is recommended for: intraorbital foreign bodies, eye ball ruptures/dilacerations and orbit fractures (7).

Magnetic resonance imaging (MRI)

MRI exploration is indicated when a ferromagnetic foreign body or a foreign body of mixed organic-inorganic composition is excluded. Some of the advantages of examination by MRI are: axial, coronal and sagittal images and patient repositioning is not required (as in coronal CT sections), provides higher quality images of soft tissues compared to CT. The disadvantages of MRI exploration are: motion artifacts, long time of image acquisition and non-specificity for orbit fractures. The MRI characteristic aspect of dry or moist wood is the hypo dense signal related to intraorbital fat. T1 sections are more useful than T2 as they provide a better contrast between wood and intraorbital fat. Should there by the least suspicion of vessel injury, CT or MRI angiography are recommended to assess vascular integrity (3).

TREATMENT

The prevention of endophthalmitis is the main objective of early surgery, although recent studies found no difference between the surgery performed within the first 48 hours and surgery performed after this time interval. In all patients diagnosed with an intraorbital foreign body, antibiotic therapy must be initiated, due to the higher incidence of secondary orbital infections (5).

The surgical approach is selected based on the nature of the foreign body, its location (anterior or posterior orbit) and complications (infections, injury or compression of the optical nerve, injury to the extraocular nerves or intraorbital vessels). Asymptomatic metallic (mostly the small ones) and inert foreign bodies located within the posterior orbit may be left *in situ* and kept under control.

On the other hand, organic foreign bodies (wood or other vegetable matter) cause an intense inflammatory reaction and are not tolerated. Organic wooden foreign bodies determine a fast infection in the orbit due to its spongy texture and organic nature, which is a good environment for microbial agents (8). They may cause panophthalmia, intraorbital abscess or fistula, reason why their extraction is a surgical emergency (9).

A surgical challenge may be the transorbital penetrating intracranial injury. In this circumstance, only a multidisciplinary team may conduct a safe and successful surgery. In such cases, the upper orbital fissure and the optical channel may be gateways to the intracranial space, mostly to the cavernous sinus and the internal carotid artery (cavernous, clinoid and ophthalmic segment).

CONCLUSIONS

The patient presented to hospital immediately after the accident, which made possible the immediate initiation of antibiotic and antimycotic therapy, a possible endophthalmitis being thus prevented. The diagnosis was penetrating craniofacial trauma with a wooden intraorbital foreign body, the damage to the ocular globe supported by initial physical examination be-

ing refuted by radio-imaging investigations.

Surgical treatment is indicated in case of foreign bodies of large sizes, with cutting edges, in the presence of signs of inflammation or infection, intraorbital abscess or contrast medium uptake in the foreign body, palpable orbital mass, restricted motility, proptosis, chemosis, compression of the optical nerve, fistula, suspicion of a wooden, copper or vegetable foreign body (7).

The intraorbital foreign body as a result of a penetrating trauma is a therapeutic emergency, and the removal of an organic foreign body is a surgical emergency. The first recommended imaging method of diagnosis is computed tomography. The diagnosis and early surgical treatment influence the course and prognosis of patient's vision.

This case is peculiar because, although a large foreign body passed through the entire length of the orbit and important anatomic structures, it did not cause damage to any intraorbital structure. Vision and right ocular globe function had an excellent prognosis.

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THE ROLE OF VITAL STAINING IN THE EARLY DIAGNOSIS OF ORAL CANCER

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THE ROLE OF VITAL STAINING IN THE EARLY DIAGNOSIS OF ORAL CANCER (Abstract): The early diagnosis of oral cancer is a priority for the entire dental system. Dental specialists, who most often are the first to examine the patients with oral tumors, are responsible for their patients' future and it is not only expected but also desired that they fulfil their role with a lot of commitment, competence and professionalism. On the other hand, it is also essential to continue developing, assessing and improving the main means of primary and secondary prophylaxis and to identify and diagnose oral cancer at an early stage. Since nowadays the fight against oral and maxillofacial cancer can only be won if it is diagnosed at an incipient stage and it is followed by a radical multimodal and effective treatment, the population must be more sensitive to the oncogenic risk factors of the oral cavity and to the early manifestations of cancer. **Keywords**: VITAL STAINING, EARLY DIAGNOSIS, ORAL CANCER.

The early diagnosis refers to the diagnose made as soon as possible after a clinical manifestation when the lesion can be seen and/or palpated or when it can be noticed using laboratory tests, before reaching the maximum T_1limit, with N_0 and M_0 (1). The toluidine blue vital staining (RICHART) relies on its affinity for cell nuclei and for those nuclei undergoing mitosis (2, 3). Colour intensity is directly proportional with nuclei density, size and their division phase, that is, with their degree of malignity. The method can identify lesions of 1 mm^3 this way reducing the lower limit of detecting tumors up to the "infraclinical" stage, at an age of the tumor of almost 6 months (4).

In dentistry, toluidine blue vital staining is to be considered in the diagnostic process, completing in a useful and necessary manner the clinical examination. It requires further investigations of the suspected lesion (5, 6).

To ease the detection and the identification of risk lesions, additional methods have been introduced: toluidine blue vital staining is the most studied method that can support the early detection of premalignant lesions and squamous cell carcinoma (7, 8, 9). Toluidine blue is a cationic metachromatic dye that may selectively bind to free anionic groups such as sulfate, phosphate

and carboxylate radicals of large molecules (10). *In vivo*, toluidine blue stains nucleic and deoxyribonucleic acids which can be retained in intracellular spaces of dysplastic epithelium. Dysplastic and malignant tissues retain toluidine blue because of the loss of tumor suppressor genes that predict the progression of precancerous lesions towards carcinoma or represent the squamous cell carcinoma themselves during the diagnosis (11, 12).

MATERIAL AND METHODS

Toluidine blue is recommended for the following reasons:

- It determines premalignant oral lesions at risk of turning into squamous cell carcinoma;
- It identifies mucosal lesions with high risk molecular models with a significant potential of turning into cancer (for both high and low risk dysplasia) (13, 14);
- It evaluates the extent and the margins of carcinoma and premalignant lesions;
- It guides the biopsy site selection and accelerates the decision to biopsy (15).

The toluidine blue vital staining or the Richart test was used on 268 patients divided into three groups of physically examined patients (tab. I):

TABLE I

Groups of patients

	Patients
Group I	25
Group II	156
Group III	87
Total	*268*

From the total 268 clinical lesions, 53 had a hyperplastic vegetant appearance while 192 had an ulcerative appearance; 23 patients presented white lesions. The diagnosis made after physical examination is presented below (tab. II):

TABLE II

Diagnosis after clinical exam

Diagnosis	Patients
Traumatic ulceration on the lower lip, tongue, cheek	103
Chronic cheilitis	24
Herpetic ulceration	15
Post-burn ulceration (hard palate, tongue)	9
Aphthous stomatitis	9
Ulcerous stomatitis	23
Leukoplakia	21
Lichen planus	11
Hyperplastic benign tumors (lip, tongue, vestibule, gingivoalveolar)	53
Total	*268*

The Richart test was used for:

- completing the physical exam of benign lesions that still presented a certain degree of malignant suspicion
- identifying small, ulcerative relapse signs
- the stomatoscopic analysis of certain lesions.

The following work technique was used: we cleaned the lesion with a piece of gauze soaked in 1% acetic acid; after cleaning the area, using the water spray of the dental unit (dentior), we have isolated the lesion and dried it with warm air. The lesion was colored using a sponge saturated with toluidine blue aqueous solution. The dye was kept for 2-3 minutes after which the lesion was washed once again using the spray and then dried uniformly and without applying any pressure with a solution of 1% acetic acid.

Here are some of the cases where the Richart method was used.

1. I. I., Iasi town, came in for consultation for an ulcerative lesion, painful manifestation on the left cheek, and swelling of the surrounding mucosa, present for a few days (fig. 1). After cleaning the dye (fig. 2) we noticed a second ulcerative lesion of 2 cm in diameter which stained dark blue (positive reaction) (fig. 3). A 7 days treatment including rinses with 5% tetracycline solution led to the healing of the ulceration. The diagnosis was ulcerative stomatitis. Although the dye was retained in the central area leading to a misinterpretation of malignant lesion, in fact it was an inflammation of the oral mucosa. In this case, the toluidine blue response was false positive.

Fig. 1. The ulcerative lesion

Fig. 2. Toluidine blue aqueous solution

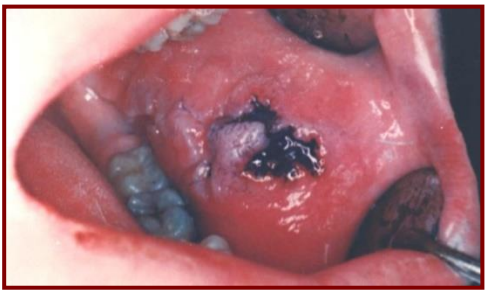


Fig. 3. Ulcerative lesion of 2 cm - stained dark blue (positive reaction)

2. S. N., Iasi county, pensioner, smoker for over 50 years and alcohol user presented with an ulcerative lesion of less than 1 cm in diameter, round, with slightly pronounced edges, without hardened base, unpainful, situated in the medial 1/3 segment of the lower lip, with the surrounding mucosa of normal appearance (fig. 3). The lesion had been present for almost 3 years. We proceeded using toluidine blue to dye the ulceration, washed the excess with acetic acid, and it resulted a dark blue stain (positive reaction) (fig. 4). Exfoliating cytology made the diagnosis of inflammatory smear with hematins, superficial and intermediary epithelial cells of normal appearance. Treatment: removal of the ulceration using a type "V" resection on the lower lip and suture of the margins.

Fig. 4. Dark blue stain (positive reaction)

The histopathological examination made the diagnosis of moderately differentiated squamous cell carcinoma with intense superficial ulceration. In this case,

the intense positive staining and the carcinoma diagnosis correlated well.

3. C. V., from Vaslui County, retired, smoker, was examined for an oval superficial ulceration of less than 1 cm in size, located on the red part of the lower lip, the surrounding mucosa having a whitish leukoplastic appearance. The lesion was noticed a few months earlier, increased in size gradually, and was slightly bleeding (fig. 5). Physical examination was completed with the toluidine blue vital staining which, after the removal of the extra amount of substance with acetic acid, remained fixed in the ulceration area. The toluidine blue vital staining was considered positive (fig. 6).

Fig. 5. Oval superficial ulceration

Fig. 6. Blue stain (positive reaction)

Treatment: excision of the ulceration from the lower lip until healthy tissue was reached. The histopathological examination led to the diagnosis of chronic ulcerative cheilitis. Although the lesion retained the dye, the reaction was *false positive* as, in the end, it turned out to be a chronic inflammation and not a malignant ulceration.

4. H. M., Iasi town, retired, non-smoker, presented with a leukoplastic area from the left edge to the ventral side of the tongue and for an ulceration of less than 1 cm in diameter on the dorsal surface of the tongue, evaluated as superficial and painful. We used the Richart test. After using acetic acid to remove the excessive dye the remaining area appeared atypical: the ulceration did not retain the dye (negative reaction) and the tip of the tongue and certain areas on the edge of the tongue as well as the leukoplastic area stained light blue (positive reaction). When analyzing the reaction 24 hours later we found that the entire area that reacted to toluidine blue regained its normal appearance (as before staining). Treatment: excision of the ulceration along with the leukoplastic area on the edge of the tongue. The histopathological examination led to the following diagnose: leucoplakia and dysplastic lesions and intraepithelial invasive carcinoma. In this case, the *atypical* Richart test was the reason why we decided to biopsy.

5. I. D., from Bacau County, smoker for 10 years, type II diabetes and third-degree obesity presented with a 1.5 cm-long ulceration situated on the ventral side of the tongue in the left half, near the tip of the tongue. The patient noticed the appearance of lesion almost a year before, but it became painful and increased in size recently. Physical examination revealed that the ulceration was highly connected to 3.5 (tooth in lingual position). The ulceration was stained with toluidine blue and then washed with 1% acetic acid solution. The ulceration did not retain the stain (negative reaction) and the surrounding mucosa

stained light blue. Exfoliative cytology demonstrated it was an inflammatory smear. Indeed it was a traumatic ulceration caused by the presence of tooth 3.5 in a vicious position and the ulceration healed in 10 days after tooth extraction.

6. C.I., Suceava County, retired, 40-year smoker and alcohol consumer on daily basis (200 g.), noticed for 3 months before the presentation the appearance of a small ulceration on the right side of the tongue in relation to 4.4 (with deep chronic marginal periodontitis), 4.3, 4.2, 4.1 (residual tooth roots). The lesion gradually increased in size developing a crater-like appearance. The Richart test was used. After removing the dye with acetic acid, the dorsal part of the tongue and the right edge along with the ulceration were stained light blue (very light) stained – the reaction being interpreted as negative. The cytological exam indicated tumor cells in the oral smear with free atypical cells and in placard. Treatment: surgical removal of the ulcer area. The histopathological examination made the diagnosis of undifferentiated squamous cell carcinoma developed deep in the chorion infiltrating strictly the muscle tissue. In this case, although the Richart test was negative, the ulceration was, in fact, a malignant lesion.

7. A. A., Iasi town, retired, smoker for 7 years, presented with a ulceration of 0.5 cm in diameter, irregular margins, the surrounding mucosa being slightly congestive, painful on palpation, situated on the edentulous alveolar crest corresponding to 4.8 (extracted 2 months earlier). During the examination the dental instrument was introduced 5 cm deep into the tooth socket. The dye was introduced and after washing it with acetic acid the ulceration appeared to have a normal (negative appearance). The cytological examination indicated an inflammatory smear. In this case, the negative result of the Richart test corresponded to the cytodiagnosis (inflammatory smear) and clinical course (the lesion healed 2 weeks later).

RESULTS

Considering the analyzed clinical lesions, the results for toluidine blue vital staining were as follows: (tables III-XI):

TABLE III

Clinical diagnosis versus true diagnosis: Ulcerations

Clinical diagnosis			***True diagnosis***		
Of 103 ULCERATIONS			87 malignant lesions and 16 inflammatory lesions		
Test	Stain	No. of patients	Lesion	No. of cases	Reaction
(+)	Dark blue	76	Malignant lesions	72	(+)
			Inflammatory lesions	4	False (+)
	Light blue	13	Malignant lesions	4	(+)
			Inflammatory lesions	9	False (-)
(-)	Uncoloured tissue	8	All cases were malignant lesions but did not retain the dye	8	False (-)
Uncertain	Atypical coloration	6	Malignant lesions	3	Atypical
			Mucosal inflammations	3	

TABLE IV

Clinical diagnosis versus true diagnosis: Cheilitis

Clinical diagnosis			***True diagnosis***
Of 24 CHEILITIS CASES			21 chronic inflammations and 3 cases that degenerated into carcinoma *"in situ"*
Test	Stain	No. of patients	
(+)	Blue	3	Carcinoma *"in situ"*
(-)	Uncoloured tissue	16	Cheilitis
Uncertain	Atypical coloration	5	Cheilitis

TABLE V

Clinical diagnosis versus true diagnosis: Leukoplakia

Clinical diagnosis			***True diagnosis***
Of 21 LEUKOPLAKIA CASES			12 leukoplakia and 9 cases that degenerated into carcinoma *"in situ"*
Test	Stain	No. of patients	
(+)	Dark blue	9	Carcinoma *"in situ"*
	Light blue	3	Leukoplakia – false reaction (+)
(-)	Uncoloured tissue	9	Leukoplakia

TABLE VI

Clinical diagnosis versus true diagnosis: Liken Planus

Clinical diagnosis			***True diagnosis***
Of 11 LICHEN PLANUS CASES			
Test	Stain	No. of patients	
(+)	Light blue	5	Lichen planus – false reaction (+)
(-)	Uncolored tissue	6	Lichen planus

TABLE VII

Clinical diagnosis versus true diagnosis: Herpetic ulcerations

Clinical diagnosis			***True diagnosis***
Of 15 herpetic ulcerations			
Test	Stain	No. of patients	
(+)	Dark blue	5	Ulcerations – false reaction (+)
(-)	Uncolored tissue	10	Ulcerations

TABLE VIII

Clinical diagnosis versus true diagnosis: Post-burn Ulcerations

Clinical diagnosis			***True diagnosis***
Of 9 post-burn ulcerations (hot food, cigars etc.)			
Test	Stain	No. of patients	
(-)	Uncoloured tissue	9	Ulcerations

TABLE IX

Clinical diagnosis versus true diagnosis: Aphthous stomatitis

Clinical diagnosis			***True diagnosis***
Of 9 APHTHOUS STOMATITIS			
Test	Stain	No. of patients	
(+)	Light blue	6	Aphthae (inflammation) – false reaction (+)
(-)	Uncolored tissue	3	Aphthae (inflammation)

TABLE X

Clinical diagnosis versus true diagnosis Ulcerative stomatitis

Clinical diagnosis			***True diagnosis***
Of 23 ULCERATIVE STOMATITIS			
Test	Stain	No. of patients	
(+)	Light blue	9	Inflammatory lesions – false reaction (+)
(-)	Uncoloured tissue	10	Inflammatory lesions
Uncertain	Atypical coloration	4	Inflammatory lesions

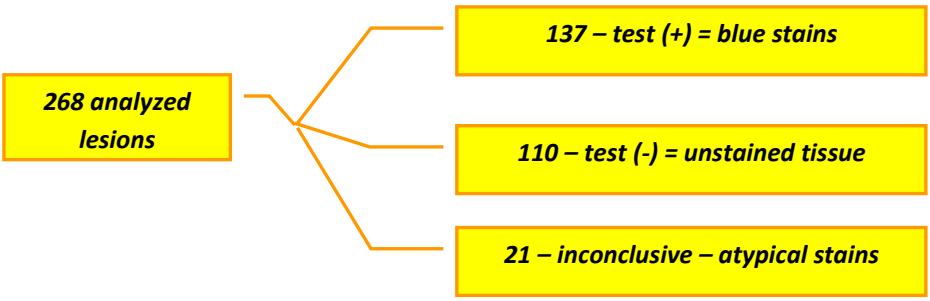
TABLE XI

Clinical diagnosis versus true diagnosis: Hyperplastic tumors

Clinical diagnosis			***True diagnosis***
Of 53 HYPERPLASTIC TUMOURS			*37 benign and 16 maligned*
Test	Stain	No. of patients	
(+)	Dark blue	6	Carcinoma *"in situ"*
	Light blue	2	Leukoplakia – false reaction (+)
(-)	Uncolored tissue	39	Benign lesions
			Malignant lesions – false reaction (-)

DISCUSSION

In conclusion, we can say that for the 268 analyzed lesions, the test results were:



From the total number of stains, some of them were *false*, as follows (tab. XII):

TABLE XII

Evaluation of the "blue stain" reaction

No of cases	Stain	No. of cases	Evaluation	%
137	stain (+) (for malignant and benign lesions)	96	Properly evaluated cases (+)	70%
		41	False cases (+)	21%
110	stain (-) (for inflammatory lesions, benign hyperplastic and malignant lesions)	94	Properly evaluated cases (-)	85%
		16	False cases (-)	12%
21	Atypical stain	3	Malignant lesions	
		18	Inflammatory lesions	

CONCLUSIONS

The dentist must diagnose and deal with precancerous lesions, and refer the patient, in useful time, to a specialized department of maxillofacial surgery (16, 17, 18, 19).

Toluidine blue staining must be considered a complementary method, useful to provide the dentist with an orientated diagnosis of pathogenic areas, that completes the physical examination.

If the false positive results do not provide the method a safety prerogative, we must then recognize its diagnostic role in locating abnormal areas on diffuse surfaces.

The simplicity of the method, the possibility of being used by any doctor, provides added value, positioning it among the techniques that should become usual in any dental office.

The main indirect form the dentist has to fight against cancer is dominated by prophylactic principles and consists in the competent practice of dental medicine.

The prophylactic principle must guide any procedure, dental or periodontal, prosthetic or surgical, for the removal of mechanical, chemical and thermal irritants from the oral cavity (20, 21).

All the above mentioned elements combined with oral septicity, on a carcinogenic background, turn into possible favoring factors for the development of oral cancer.

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NEWS

INTRALEAFLET HEMORRHAGES ARE A COMMON FINDING IN SYMPTOMATIC AORTIC AND MITRAL VALVES

Worldwide, heart valve disease is an important clinical problem and responsible for a significant mortality and morbidity. Post inflammatory valvular disease, usually rheumatic of origin, is declining in the industrialized countries but still has a high incidence in developing countries, varying from 2.5 to 3.2 cases per 1,000. Traditionally, the pathogenesis of degenerative heart valve disease was thought to be the result of time-dependent "wear-and-tear" of the leaflets, which results in thickening of valve tissue due to fibrosis, calcification, and a low-grade inflammatory response. High rates of intraleaflet hemorrhage (ILH) have recently been reported in degenerative aortic valve disease. In that study, ILH was related to the severity of stenosis and preexistent valve tricuspid or congenitally bicuspid morphology. Hemorrhages in valve tissue, as noted by Akahori et al., could relate to "wear-and-tear" processes leading to small fissures, ruptures, or friction in the highly calcified valves. Alternatively, such hemorrhages could also relate to presence of microvessels since microvessels have been described in degenerative aortic valve tissue. Because nowadays, it is not known whether hemorrhages occur in degenerative aortic valves only or whether they can also be encountered in mitral valves and in other disease states such as rheumatic heart disease, the authors investigated the prevalence, extent, and age of ILH in a consecutive series of 105 surgically resected aortic and mitral valves. In addition, they investigated the presence of microvessels and the integrity of their vessel wall as a potential source of hemorrhages. Finally, all data were related to the pathological diagnosis of valve disease. The results showed, that the prevalence of ILH is very high in resected heart valves, both aortic and mitral valves presented an association of ILH with microvessels, microvascular leakage, and calcifications. The authors concluded that repetitive microvascular-leakage-related ILH may contribute to valve dysfunction on the (very) long term (Stam OCG, van Rijswijk JW, de Mol BAJM, van der Wal AC. Intraleaflet hemorrhages are a common finding in symptomatic aortic and mitral valves. *Cardiovascular Pathology* 2017, 30: 12-18).

Doina Butcovan

FANCONI ANEMIA WITH CLEFT PALATE

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FANCONI ANEMIA WITH CLEFT PALATE (Abstract): Fanconi anemia (FA) is a rare plurimalformative syndrome (1/350,000 newborns) presenting a large phenotypic heterogeneity, chromosomal instability and autosomal recessive inheritance (OMIM 227650). **Case presentation:** Male patient followed in the genetics service since infancy, till death. The child was referred for multiple congenital anomalies: bilateral aplasia of the radius and thumb, left kidney agenesis, cardiac malformation cleft palate, bilateral cryptorchidism, ear anomalies, which were associated, in time, with facial hyperpigmentation, anomalies of dental eruption, nasal septum deviation, and lumbar scoliosis. By the age of 10 years, he develop progressive pancytopenia, aplastic anemia. The diagnosis was completed by medulogram, immunogram, and karyotype. Treatment was primarily hematologic with substitution products, immunosuppressive, marrow stimulants, antibiotic therapy. Death occurred at age of 16 by infection and pulmonary hemorrhage occurred in severe pancytopenia. **Conclusions:** It is presented a rare case of Fanconi anemia customized by the clinical association with cleft palate and instructive clinical and evolutionary complexity. **Keywords**: FANCONI ANEMIA, CLEFT PALATE, CHROMOSOMAL INSTABILITY.

Fanconi anemia (OMIM 227650) is a rare plurimalformative syndrome presenting a large phenotypic heterogeneity, chromosomal instability and autosomal recessive inheritance. Recent determination of the carrier frequency gave an estimate of more than 1/200, with an expected prevalence at birth of at least 1/160,000. In certain populations, the carrier frequency is much higher, due to founder mutations. Until now, more than 2000 cases have been reported in the literature (1).

CASE PRESENTATION

A 16 year old boy, the first child in family, born at term, birth weight 2900 g; APGAR score 8/9. The parents are young and healthy and they are non-related. From past medical history: cleft palate (DP) operated at 1 year and; at 3 years old he was operated for cryptorchidism (orchiopexy). The boy was in evidence of Genetics Department from birth with plurimalformative syndrome (cleft palate, anomalies in size and shape of upper limbs).

Physical examination at 16 years old reveals: growth retardation, pallor; on the face presents ephelides and hyperpigmentation. Craniofacial dysmorphism (fig. 1): microcephaly, long face, microphthalmia,

epicanthus, anteverted ears, sharp nose, long neck. Oral cavity: palate with keloid scar after surgery. Upper limbs: bilateral, symmetrical shortening of the forearm (shortened to half of the normal size), forearms were a single long bone with bilateral thumb agenesis; lumbar scoliosis (fig. 2).

Fig. 1. Craniofacial dysmorphism, dental anomalies, keloid scar after surgery correction of cleft palate

Fig. 2. Size and shape anomalies of the upper limbs

Laboratory investigations showed: count blood cells (CBC): severe tricyto-penia; elevated levels of urea, creatinine and urine density. Bone marrow aspirate showed global marrow hypoplasia, marrow with fat tissue. Immuno-phenotyping revealed low number of NK cells; very low levels of lymphocytes B. In absolute value: lympho-penia with a marked decrease in lymfocyte B accompanied by a moderate decrease in NK cells. CD4/CD8 ratio is at the lower limit of normal. X-Ray: bilateral agenesis of the radius; lumbar scoliosis. Abdominal ultrasound: moderate hepatomegaly, absent left kidney and right kidney is hypoplastic and misaligned. Computer tomography (CT): absence of left kidney and pelvian position of the right kidney; dextroconvex lumbar scoliosis.

Interdisciplinary checkups: cardiologic exam including EKG: minor right arterial block. ENT exam: nasal septum deviation. Psychological evaluation: IQ 90.

Genetic investigations**:** We performed karyotype from peripheral blood: it was normal: 46, XY, but does not excluded the existence of chromosome breakages. Bone marrow karyotype showed a fragile site on the long arm of chromosome 12 band q14 (fig. 3).

The differential diagnosis include: the VATER/VACTERL association, Holt-Oram syndrome and TAR syndrome (thrombocytopenia and radial agenesis) mainly due to the radial abnormalities. Other conditions to be considered include the Blackfan -Diamond anemia (especially because of the hematological features), and other chromosomal instability syndromes such as the Bloom syndrome and the atax-ia-telangiectasia syndrome (2).

Treatment was as follows: *substitution*: red cell transfusions; *bone marrow stimula-*

tion: androgens, growth factors G-MSC (Neopogen); *immunosuppressant*: Prednisone, *symptomatic*: antibiotics, vitamins, Calcium, Magnesium, Aspacardin. Marrow transplant was not performed because no compatible donors.

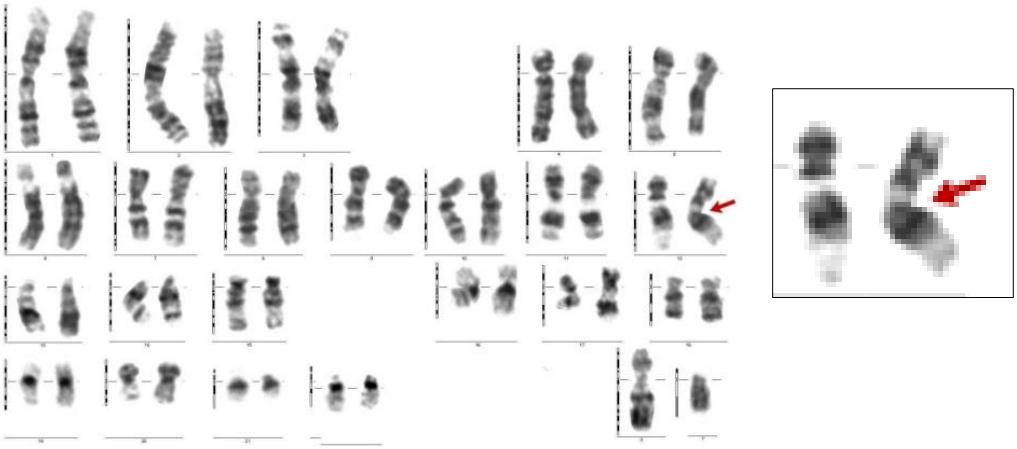

Fig. 3. Bone marrow karyotype

Evolution: at the age of 16 ½ years dies due to sepsis with multiple organ dysfunctions leading to severe pulmonary hemorrhage

DISCUSSION

Fanconi anemia, ataxia - telangiectasia and Bloom syndrome are hereditary disease resulting from mutations in genes that contribute to genome stability. Genomic instability promotes chromosomal breaks and rearrangements which is evidentiable by light microscopy examination of metaphase cells (3, 4, 5).

Today are identified 16 FA or FA-like genes: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCA, FANCF, FANCG, FANCA, FANCJ, FANCL, FANCM, FANCN, FANCP and RAD51C, XPF. Proteins codified by FA genes play a role in cellular response to oxidative stress, in cell cycle regulation, in DNA repair and chromosomal stability in tumor suppression. Majority patients have mutations in group A (60%), C (15%) or G (10%) of rest of 15% belongs to other subtypes (6, 7).

Most persons with Fanconi anemia develop malignancy, usually acute myeloid leukemia; 90% of patients develop bone marrow failure by the age of 40 years.

In 1927 Guido Fanconi described a family with three boys who had various birth defects and who subsequently developed pancytopenia (8). The syndrome is characterized by developmental defects, bone marrow failure and increased risk of cancer.

It is autosomal recessive inheritance in 99% of cases. Only FANCB gene is located on chromosome X such that its transmission will be recessive X linked (9).

The frequency in the general population is 1/350,000 births. FA occurs in all ethnic groups, but with a higher frequency (1/100 individuals) in Ashkenazi Jews and African population from South Africa (10, 11).

CONCLUSIONS

Fanconi anemia is a very heterogeneous condition clinically and patients can have a wide variety of abnormalities. Particularity of this presentation consists in associating with cleft palate, rarely described in the literature. The clinicians must recognize the considerable overlap of the FA phenotype with other malformation syndromes, and the need for applying a specific laboratory diagnostic test.

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NEWS

IN VITRO EVALUATION OF ANTIBACTERIAL EFFICACY OF PINEAPPLE EXTRACT (BROMELAIN) ON PERIODONTAL PATHOGENS

Periodontitis represents an inflammatory disease resulting in the destruction of periodontal tissues. Various treatment modalities (mechanical and surgical therapy) have been tried. Antimicrobial agents have been used as a monotherapy and as an adjunct with mechanical debridement. As antibacterial agents have been used different plant extracts. Pineapple extract (Bromelian) is one such agent. The aim of the study was to assess the antibacterial efficacy of Bromelain on aerobic and anaerobic periodontal microorganisms. Minimum inhibitory concentration (MIC) of Bromelain was tested on isolated strains of *Streptococcus mutans*, *Enterococcus fecalis*, *Aggregatibacter actinomycetemcomitans* (Aa), and *Porphyromonas gingivalis* (Pg) using serial dilution broth method. The results of the study showed that *S. mutans* showed sensitivity at the lowest concentration of 2 mg/ml as compared to *E fecalis* (31.25 mg/ml) while *P gingivalis* showed sensitivity at the lowest concentration of 4.15 mg/ml as compared to *Aggregatibacter actinomycetemcomitans* (16.6 mg/ml). In conclusion Bromelain exerts an antibacterial effect against potent periodontal pathogens; hence, it may be used as an antibacterial agent. To validate this result further trial has to be conducted. (Praveen NC, Rajesh A, Madan M, Chaurasia VR, Hiremath NV, Sharma AM. In vitro Evaluation of Antibacterial Efficacy of Pineapple Extract (Bromelain) on Periodontal Pathogens. *J Int Oral Health*, 2014; 6 (5) : 96-98).

Irina Grădinaru

SURGICAL MANAGEMENT OF PULMONARY HYDATIDOSIS IN CHILDREN

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SURGICAL MANAGEMENT OF PULMONARY HYDATIDOSIS IN CHILDREN (Abstract): Cystic echinococcosis (CE), the most common manifestation of the infestation with the larval form of the tapeworm Echinococcus granulosus, is a public health problem in endemic areas such as Moldavia. **Aim:** To retrospectively analyze pediatric patients who underwent surgery for pulmonary hydatid cyst over a 5-year period in order to assess the need for extensive lung resection. **Material and methods:** In the interval January 2007 – January 2012 30 pediatric patients with pulmonary hydatid cyst were surgically treated in the Department of Pediatric Surgery at the Iasi "St. Maria" Children's Hospital. We retrospectively reviewed the medical records of these patients **Results:** Patient age ranged from 3 to 14 years, and 21 patients (70%) came from rural areas. Respiratory symptoms (cough, chest pain, hemoptysis, fatigue, and dyspnea) were the main complaints. Chest radiography, computed tomography and serological tests made the diagnosis in all cases. The most common location of cystic lesions was the left lower lobe (40%). All patients were treated by enucleation and capitonnage of the residual cavity. Postoperative complications occurred in 5 cases (16.67%) and in one case (3.33%) a new hydatid cyst was diagnosed in the right lung after 2 years. **Conclusions:** The surgical treatment of choice was cyst enucleation with capitonnage, thus sparing the pulmonary parenchyma. Major lung resections are to be avoided in children when no severe lung destruction is detected. **Keywords*:*** PEDIATRIC PATIENTS, PULMONARY HYDATID DISEASE, ECHINOCOCCUS GRANULOSUS, ENUCLEATION, CAPITONNAGE

Cystic echinococcosis caused by *Echinococcus granulosus* is a very serious social, medical and public health problem in Romania with a yearly incidence of more than 3 cases/100.000 population in some geographical areas (1).

Echinococcosis is an anthropozoonosis with worldwide distribution caused by a small tapeworm of genus Echinococcus that can infect humans in its larval state. By passing through the intestinal wall into the portal circulation, the larva can locate itself into various organs of the intermediate host, the humans. Four types of *Echinococcus (E. Granulosus, E. Multilocularis, E. Vogeli, and E. Oligarthrus)* cause infection in humans. *Echinococcus granulosus* and *Echinococcus multilocularis* are the most common, causing cystic echinococcosis and alveolar echinococcosis, respec-

tively. Echinococcus Vogeli and E. oligarthrus cause polycystic echinococcosis and are less frequently associated with human infection (2, 3). Liver is the most commonly involved organ in more than 60% of cases, followed by lungs (10-40%), muscles, bones, peritoneum, kidneys, spleen and brain. Unlike in adults, in children the pulmonary tissue is the most commonly involved site and, unfortunately, does not have specific symptoms (4, 5). During the cystic stage the disease may remain asymptomatic for a long period of time. When symptoms occur, they are related to parasite size, location and load and caused by compression and complications (rupture, infection).

Preoperative diagnosis is based on a combination of methods such as history, physical examination, medical imaging procedures, laboratory and serological tests. Pulmonary hydatidosis is usually managed surgically or by combining surgery with administration of antiparasitic drugs. Medical treatment alone is rarely used and controversial (6, 7).

The aim of our paper was to retrospectively analyze the pediatric patients who underwent surgery for pulmonary hydatid disease over a 5-year period in order to assess the need for extensive lung resection surgery.

MATERIAL AND METHODS

The clinical records of 30 patients who underwent surgery for pulmonary hydatid disease in the interval January 2007 – January 2012 at the Department of Pediatric Surgery at St. Maria Children's Hospital, Iasi, Romania were analyzed. Hydatidosis was diagnosed based on physical findings, clinical symptoms, radiological examinations (chest X-ray and computed tomography), ultrasound, laboratory and serological test results (eosinophilia, enzyme-linked immunosorbent assay) and histopathological examination of surgical resection specimens.

Clinical, epidemiological, and laboratory data were analyzed together with surgical protocol details. IgG antibodies against *Echinococcus granulosus* were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit RIDASCREEN® Echinococcus IgG (produced by R-Biopharm AG, Landwehrstr. 54, D-64293 Darmstadt, Germany) that uses purified antigens bonded to a microwell plate. Antibodies in patient serum attach themselves to the antigens and are determined during the second phase of the test by using enzyme-labelled anti-human antibodies. The enzyme converts the colorless substrate to a blue end product, the final measurement being performed in a photometer.

Histopathological examination was performed on fragments collected during surgery, fixed by immersion in a 10% formaldehyde solution and embedded in paraffin blocks. Routine Hematoxylin and Eosin (H&E) staining was performed on sections cut from these blocks.

Surgical intervention was performed through posterolateral thoracotomy in most cases, and staged thoracotomy was the preferred surgical approach in bilateral cases. The cyst was surrounded by iodine-soaked gauze packs to prevent spillage and dissemination to surrounding structures. After neutralizing the cyst and aspirating its content, the intact germinative membrane was removed. The laminated layers were removed and then capitonnage of the residual cavity was performed. All patients received prophylaxis with Albendazole (15

mg/kg/day given orally in 2 divided doses) for 3–6 months postoperatively, in cycles consisting of 45 days of treatment alternating with 14 drug-free days). Patients were regularly followed-up postoperatively at 1 month after discharge and every 3 months thereafter for a period of 6–18 months. These visits included clinical examination, abdominal US, chest X-ray / CT if necessary, laboratory and serological tests in order to evaluate disease recurrence during the follow-up period.

RESULTS

The current study was performed in order to evaluate the clinical characteristics and surgical management of children with pulmonary hydatidosis. Of the 30 patients, 16 (53.33%) were males, 14 (46.67%) were females, and age ranged from 3 to 14 years (mean age 9.3 years). Most patients (21 cases - 70%) lived in rural areas and interacted daily with domestic animals, mainly dogs. Epidemiological investigation revealed that hydatidosis was previously diagnosed among family members of 8 (26.67%) patients. The patients presented for respiratory symptoms in 20 (66.67%) cases: cough (12 patients), chest pain (8 patients), hemoptysis (3 patients), fatigue (11 patients), and dyspnea (6 patients). A palpable abdominal mass was present in 2 (6.67%) patients having concomitant hepatic hydatid cysts. Lung hydatidosis was correctly identified on chest X-ray in 27 (90%) patients, 3 patients undergoing a computed tomography (CT) examination (fig. 1).

Laboratory test results showed eosinophilia in 21 (70%) patients, and erythrocyte sedimentation rate was elevated in 23 (76.67%) patients. Serological tests (ELISA kit RIDASCREEN® Echinococcus IgG) were performed in 12 patients and 10 (83.33%) of them were found positive, thus allowing an accurate preoperative diagnosis. The 2 patients with negative tests had uncomplicated cysts of 5.5 and 7.8 cm, respectively.

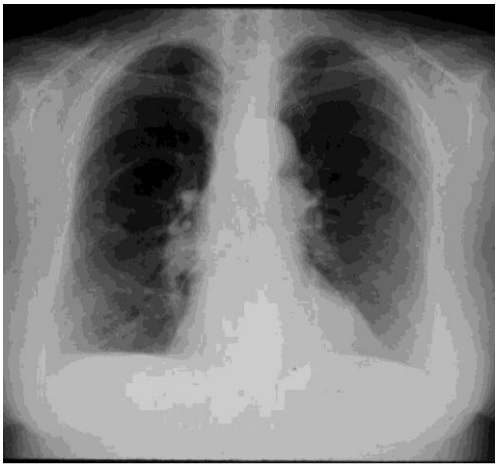

Fig. 1. Hydatid cyst located in the right lung

Cysts were located in the left lung in 14 (46.67%) patients, right lung in 12 (40%) patients, and 4 (13.33%) patients had bilateral cysts. The most frequent location was in the lower lobes, 21 (70%) patients (fig. 2).

Preoperative abdominal ultrasound was performed in all cases as part of the standard preoperative evaluation protocol, and identified concomitant liver hydatidosis in 6 (20%) patients, 2 of them with palpable masses. In 3 patients with hepato-pulmonary hydatidosis, the two locations were treated in a single operative time.

In 21 (70%) patients surgery was performed through an axillary thoracotomy; in 5 (16.67%) patients through an anterolateral thoracotomy; in 3 (10%) patients through a posterior thoracotomy; and only in one (3.33%) patient through an anterior thoracotomy. The cyst was intact in 18 (60%) patients. Bronchial fistulas were identified

in 13 (43.33%) patients and were repaired with absorbable material (Vicryl). In all cases the surgical technique consisted in enucleation and capitonnage of the residual cavity after previous inactivation with saline solution (30% NaCl) for 5-10 minutes.

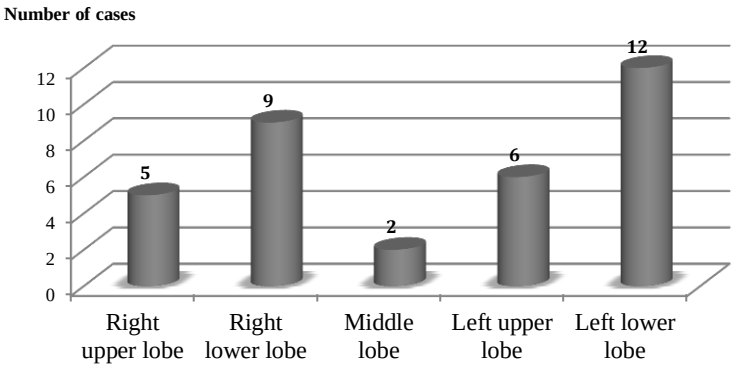

Fig. 2. Location of diagnosed hydatid cysts

Postoperative complications occurred in 5 (16.67%) patients: infection (abscess) of the residual cavity in 2 patients, treated conservatively with antibiotics; pneumothorax due to reopening of bronchial fistula in 2 (40%) patients, and atelectasis of the left lung in 1 patient. No patient required reoperation, but bronchoscopy with mucus plug aspiration was required in the patient with left lung atelectasis. Hydatidosis recurrence was recorded in one patient 2 years after the first intervention; the new hydatid cyst was located in the same lung (right), and reoperation was needed despite medical treatment with Albendazole one year after the first surgery.

Mean hospital stay was 12.7 days (range 10-25 days) with a 0% mortality rate. Mean follow-up period was 2.9 years (2 months to 5 years) by chest X-ray at a 6 months interval in the first postoperative year and then annually.

DISCUSSION

As previous studies stated, 10-20% of pulmonary hydatidosis cases are diagnosed during childhood, the statement that in children the lung is the most commonly affected organ by cystic echinococcosis being controversial (8, 9).

As in other locations, pulmonary hydatid cyst may be asymptomatic for a long period of time and diagnosed incidentally, or manifest itself only when voluminous or complicated. Pulmonary hydatidosis in children becomes symptomatic more easily compared to liver hydatidosis, due to the compressible nature of lung parenchyma, its higher vascularity and lower negative pressure (10). In endemic areas, general practitioners as well as specialist doctors (pediatrician, pediatric surgeon, general or thoracic surgeon), should be aware and suspect hydatidosis in all cases at risk (rural area, animal contact, eosinophilia, non-specific respiratory or digestive symptoms, palpable abdominal mass, acute abdomen) and rule out this disease. Chest radiography is a simple yet essential method in diagnosing lung hydatidosis as well as an ideal tool for localization and surgical planning (11). In the present study, pulmonary involve-

ment appears to be slightly more prevalent in boys (53.33%), possibly because they get into contact with infected materials more often than girls. As in order studies, pulmonary infestation is encountered more frequently in children older than 9 years (mean 9.3 years) (12).

The most frequently encountered symptoms in patients with pulmonary hydatidosis are determined by the mass effect exerted on surrounding tissues, rupture or supra-infection of the cyst causing pneumothorax, pleural effusion, or empyema. In the present study, patients were admitted with nonspecific respiratory symptoms in 2/3 of cases (20 patients – 66.67%). Serological tests like IgG ELISA can be used for diagnosing hydatidosis, but they should always be doubled by other methods as false negative results occur in uncomplicated lung cysts as it was noted in our study. In the present study, hydatid cysts were most frequently located in the lower lobes (21 patients – 70%), similar results with the ones reported in the literature (2, 5, 6).

Currently, there is no standardized treatment for pulmonary hydatidosis, the aim of any treatment being parasite eradication and recurrence prevention, while the additional aims of surgical treatment are prevention of intraoperative rupture and obliteration of the residual cavity (capitonnage). Surgical options in pulmonary hydatidosis are represented by enucleation with or without capitonnage, wedge resection, lobectomy and pneum-ectomy.

The aim of the surgical treatment is complete excision of the cyst with maximum preservation of the lung tissue. In pediatric patients there is no need to perform major lung resections like lobectomy or pneumectomy as adjacent lung parenchyma destruction is rare. In peripherally located cysts, wedge resection may be performed with minor or no sequels. In our study group, all patients were treated by enucleation with capitonnage of residual cavity. The low rate of complications, conservatively manageable, supports this type of surgical approach. Large pulmonary resections may be more deleterious to the patient and cause future morbidity.

Initially, concomitant pulmonary and hepatic hydatidosis was treated in two-stages (3 patients), enucleation of the pulmonary cyst being the first intervention due to the high risk of rupture during abdominal surgery. With the progress of anesthetic methods and better postoperative management, which allows early intensive physical therapy, hepatic and pulmonary hydatidosis have been addressed in a single surgical time (3 cases).

Axillary thoracotomy was the preferred surgical approach in our study group as it allows entering the chest only by sectioning intercostal muscles, thus producing less postoperative pain and facilitating an early recovery, crucial in pediatric surgery. At the same time, aesthetic results are better, as the scar is located in an inconspicuous area and there are no long-term sequels like scoliosis. Thoracoscopic procedures and percutaneous drainage have been tried by several authors but they are not worldwide accepted due to the high risk of dissemination and anaphylactic reaction (13).

In our study postoperative recovery was favored by an early started respiratory physiotherapy.

The use of antiparasitic drugs like Mebendazole and Albendazole has been shown in several studies to be helpful in preventing relapses (14). Their efficacy when used as a single primary treatment is debatable, as the drug has to reach the germinal layer, a process achievable only in

small cysts with thin walls. In our study, Albendazole was prescribed in 3 high-risk patients (bronchial fistulas), one of them being re-operated after two years for a new hydatid cyst in the same lung. However, due to insufficient availability of this resource and limited compliance to treatment in rural areas, a complete therapy cannot be ensured in all cases.

CONCLUSIONS

The preferred surgical treatment of pulmonary hydatidosis in our study group was cyst enucleation and capitonnage of the residual cavity, thus sparing pulmonary parenchyma. Major lung resections were avoided, as severe lung destruction is rare in children. Surgical treatment should be completed by administration of antiparasitic drugs in high-risk patients, when hydatidosis may disseminate by fistulas or secondary to perioperative complications. Pulmonary hydatidosis should be carefully considered in the differential diagnosis of respiratory or digestive symptoms in endemic areas.

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EPIDEMIOLOGY OF B/C VIRUS INFECTION HEPATITIS IN THE NORTHERN MOLDAVIAN CORRECTIONAL FACILITIES RISK FACTORS

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EPIDEMIOLOGY OF B/C VIRUS INFECTION HEPATITIS IN THE NORTHERN MOLDAVIAN CORRECTIONAL FACILITIES-RISK FACTORS (Abstract). **Aim.** To identify the specific risk factors for chronic hepatitis B/C virus infections in the correctional system in Moldova (Romania). **Material and methods.** The study included 533 subjects imprisoned in three correctional facilities located in the Northern region of Moldova. The subjects were divided into 2 groups: HBV/HBC group – 108; Control group – 425. **Results**. The risk factor for transfusion-contracted HBV/HCV was 3.73; the empirical treatment of the HBV/HBC group resulted in a relative infection risk of 2.62; syringe sharing in the HBV/HBC group accounted for a risk factor of over 4 (OR=4.33); tattooing induced a relative risk factor of 1.25, and piercing was a risk factor of approximately 2 (OR=1.97); sharing personal care items represented a risk factor of over 2 (OR=2.02). Injection drugs induced a relative risk of over 4 (OR=4.33). In the HBV/HCV group, self-aggression represented a risk factor of 1.65. **Conclusions** Prison environment, by its specific and nonspecific contamination modalities (shared with the rest of the population but more common) causes that inmates to be 2-4 times more exposed to hepatitis B/C infection. **Key words:** B/C HEPATIC VIRUS, IMPRISONED POPULATION, RISK FACTORS, EPIDEMIOLOGY

This study on inmate specific diseases and prevention possibilities reflects the epidemiological permanent concern for the needs of people deprived of their freedom. The expected impact is the reduction of morbidity and mortality among *people deprived of their freedom* (PDF) under conditions specific to the correctional system and in correlation to the trend of these indicators in the general population. The aim of this study was to identify the specific risk factors for chronic hepatitis in the correctional facilities located in North Moldavia, the seroprevalence among the studied population, and their correlation with national and international statistical data.

MATERIAL AND METHODS

The informed consent was obtained from every PDF included in the study. An epidemiological questionnaire was used to

determine the risk factors. The research was performed in the medical offices of correctional facilities in Northern Moldavia and included new inmates but also inmates sentenced to more than 5 years already in prison for some time who met the ***se****lection criteria.* The selection criteria were: age over 18; decision-making abilities; newly admitted inmates or transferred to serve their sentence; freely-expressed consent. *Exclusion criteria:* age under 18; no decision-making ability; pregnancy. 533 inmates were enrolled in this study conducted in the interval October 2010– October 2012 in three correctional facilities in Northern Moldavia. 479 (89.9%) inmates in Iaşi correctional facility and 49 (4.5%) inmates in Tg. Ocna correctional facility were subjected to epidemiological screening. Also included in the study were 30 female inmates in Bacau correctional facility. The sampling methods demonstrated that compared to the total number of inmates in Romania (n=31,720), with a sampling error of ± 4.2%, the study group was representative. Depending on the presence or absence of hepatitis B/C virus infection, the study group was divided into 2 subgroups: HBV/HCV – 108 subjects found positive; Controls – 425 subjects found negative for hepatitis B/C virus.

The study was transversal, presenting the situation at one specific point in time in study population. In order to measure the intensity of the associations between the monitored parameters we used as analytical technique the estimation of the relative risk (OR). The advantages of this type of study are: easy to perform, moderately expensive, performed in a given population easy to approach and observe, and is reproducible. This last advantage opens the perspective of using the method as a working tool for this type of population, with the aim of targeting the population to be tested and possibly diagnosed with hepatitis virus infection. To assess the specificity of the risk factors the Receiver Operating Characteristic (ROC) curve was generated by minimizing the distance between the ideal sensitivity (100%) and the 1-specificity point (0%). The data were uploaded and processed with the help of the statistical functions of *SPSS 13.0* software.

RESULTS

Medical Risk factors. 42.6% of the subjects in the HBV/HCV group and 34.4% of those in the control group had surgical interventions (p=0.139). In both groups, approximately 8% of the subjects had frequent hospital admissions, without significant differences in frequency distribution (p=0.882). The percentage of subjects who received blood transfusions was significantly higher in the HBV/HCV group (p=0.001) as compared to the control group (16.7% *vs.* 4.5%). Empirical treatment was used significantly more frequently in the HBV/HCV group (11.1%) as compared to controls (4.2%) (p=0.011). 36.1% of the HBV/HCV subjects and 27.1% of the controls had dental treatments the frequency distributions being statistically insignificant (p=0.083). Accident-related risk factors were found in 15.7% of the subjects in the HBV/HCV and 1.3% of the controls (p=0.273) (tab.I).

The ROC curve highlights that blood transfusions and use of empirical treatment were the main risk factors for hepatitis B or C virus infection (fig.1).

TABLE I

Risk factors by study subgroups

Risk factor	HBV/HCV (n=108)		Controls Lot (n=425)		Significance		OR	CI 95%
	n	%	n	%	χ^2	p		
Medical risk factors								
Surgical interventions	46	42.6	146	34.4	2.19	0.139	1.24	0.96÷1.60
Frequent hospital admissions	9	8.3	36	8.5	0.02	0.882	0.98	0.49÷1.98
Blood transfusions	18	16.7	19	4.5	17.99	**0.001**	3.73	2.03÷6.86
Empirical treatment	12	11.1	18	4.2	6.39	**0.011**	2.62	1.30÷5.27
Dental treatment	39	36.1	115	27.1	3.01	0.083	1.33	0.99÷1.79
Accident	17	15.7	48	11.3	1.20	0.273	1.39	0.84÷2.32
Non-Medical Risk Factors								
Syringe sharing	11	10.2	10	2.4	11.97	**0.001**	4.33	1.89÷9.93
Cosmetic treatment	9	8.3	30	7.1	0.06	0.804	1.18	0.58÷2.41
Tattoos	70	64.8	220	51.8	5.40	**0.020**	1.25	1.06÷1.48
Piercing	49	45.4	98	23.1	20.36	**0.001**	1.97	1.50÷2.58
Sharing personal care items	40	37.0	78	18.4	16.37	**0.001**	2.02	1.47÷2.77
Toxic substances consumption								
Daily alcohol intake	70	64.8	216	50.8	6.23	**0.013**	1.28	1.08÷1.51
Injection drug	11	10.2	10	2.4	11.97	**0.001**	4.33	1.89÷9.93
Cocaine	8	7.4	18	4,.2	1.25	0.264	1.75	0.78÷3.91
Pain killers	32	29.6	82	19.3	4.87	**0.027**	1.54	1.08÷2.18
Tuberculosis medication	11	10.2	46	10.8	0.04	0.986	0.94	0.50÷1.75
Hepatotoxic medication	19	17.6	66	15.5	0.14	0.707	1.13	0.71÷1.80
Accidental poisoning	13	12.0	14	3.3	11.93	**0.001**	3.65	1.77÷7.54
Psychiatric treatment	23	21.3	85	20.0	0.03	0.869	1.06	0.71÷1.60
Aggression								
Sexual	2	1.9	12	2.9	0.05	0.820	0.66	0.15÷2.89
Physical	20	18.5	68	16.0	0.23	0.628	1.16	0.74÷1.82
Self-aggression	42	38.9	100	23.5	9.62	0.002	1.65	1.23÷2.21

Non-medical risk factors. The proportion of HBV/HCV subjects sharing syringes was significantly greater (p=0.001) as compared to controls (10.2% *vs.* 2.4%). The frequency distribution of the subjects who had cosmetic treatment was reduced in both study groups (8.3% *vs.* 7.1%), without statistically significant percentage differences (p=0.804). The frequency of tattoos was high in both study groups, 51.8% in the control group

and 64.8% in the HBV/HCV group, but the proportion of HBV/HCV subjects was significantly higher (p=0.02). Significantly more HBV/HCV subjects had piercing (45.4% *vs.* 23.1%) (p=0.001). 37% of the HBV/HCV subjects and only 18.4% of the controls shared personal care items, statistically significant percentage differences (p=0.001). The ROC curve showed that sharing syringes and personal care items were the main risk factors for hepatitis B or C virus infection (fig. 1).

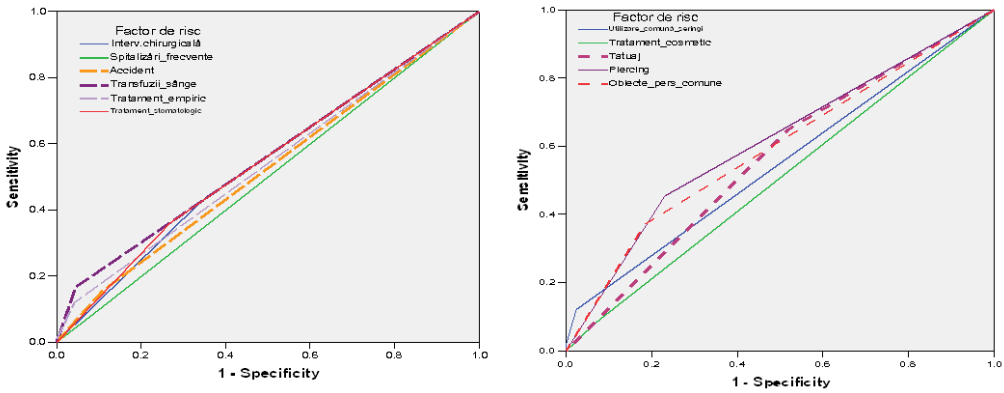

Fig. 1. The specificity of medical and non-medical risk factors

Only 28.6% of drug users had no prior criminal history, the remaining 71.4% having previously served sentences with deprivation of freedom. Of all tattooed subjects only 23.5 % had no prior criminal history, getting tattoos when free, possibly in tattoo parlors, and 76.5% got tattoos in the correctional facility, in the absence of the equipment and access to sterile tattoo instruments. Moreover, there were inmates who got tattoos while serving their twelfth sentence with deprivation of freedom, although they had not done it while serving previous sentences. Nevertheless, the majority of inmates get tattoos when serving their first sentence in a correctional facility and report current tattoos upon subsequent imprisonments (fig. 2).

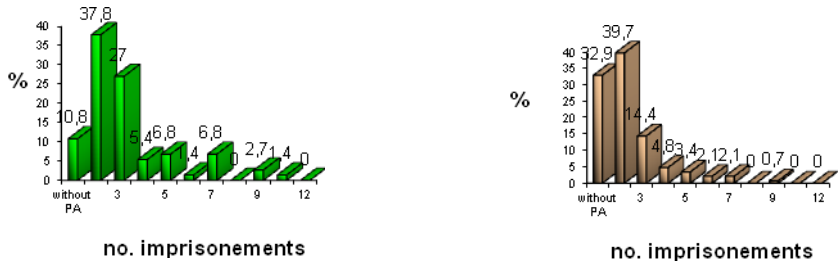

Fig. 2. Distribution of subjects with tattoos and piercings according to the number of imprisonments

As in the case of tattoos, the great majority of inmates start wearing piercings at the beginning of their „imprisonment career" (fig. 3).

Fig. 3. Distribution of subjects who commonly use personal objects, according to the number of imprisonments

Intake of toxic substances. 64.8% of the subjects in the HBV/HCV group and 50.8% of the controls reported daily alcohol intake, the percentage difference being statistically significant (p=0.013). The daily alcohol intake was of over 60 g in 62.9% of the HBV/HCV subjects and 53.7% of the controls. The frequency of subjects who use injection drugs was significantly higher in the HBV group (10.2%) as compared to the control group (2.4%) (p=0.001). Declaratively, crack cocaine sniffing was rare in both study groups (7.4% *vs.* 4.2%), the difference not being statistically significant (p=0.264). In the study series we noticed a significantly increased frequency (p=0.027) of the use of pain killers in the HBV/HCV group (29.6%) compared to the control group (19.3%). The frequency of tuberculostatic drugs use showed no significant differences between the study groups (p=0.986), being of approximately 10-11%. There were no significant differences in the intake of hepatotoxic substances between the study groups (p=0.707): 17.6% in the HBV/HCC group and 15.5% in the controls. Significantly more HBV/HCV subjects (p=0.001) experienced accidental poisoning than controls (12% *vs.* 3.3%). In both study groups approximately 20-21% of the inmates underwent psychiatric treatment. ROC curve identified daily alcohol intake of and pain killers as the main risk factors (fig. 4).

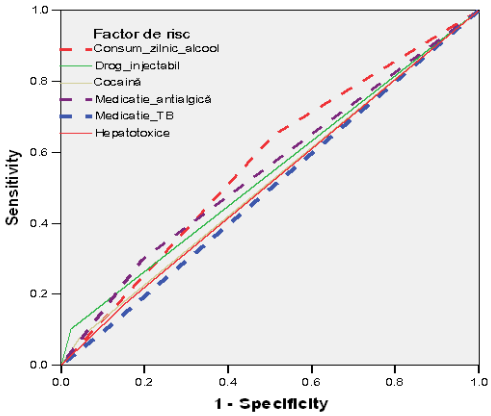

Fig. 4. Specificity of toxic substances intake as risk factors

62.8 % of inmates who drank alcohol daily had prior criminal history (fig 5).

Fig. 5. Distribution of subjects who drank alcohol daily according to the number of imprisonments

The distribution of subjects shows that 57.9 % of injection drug users and 73.1% of those who sniffed cocaine had previous criminal history.

59 % of those who chronically take pain killers, 69 % of the inmates who reported treatment with tuberculostatic drugs and 65 % of those who take psychiatric medication have been imprisoned before (fig. 7).

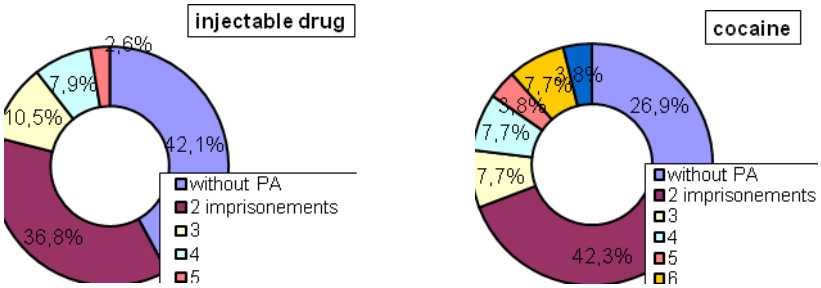

Fig. 6. Distribution of injection drug users and crack cocaine sniffers according to the number of imprisonments

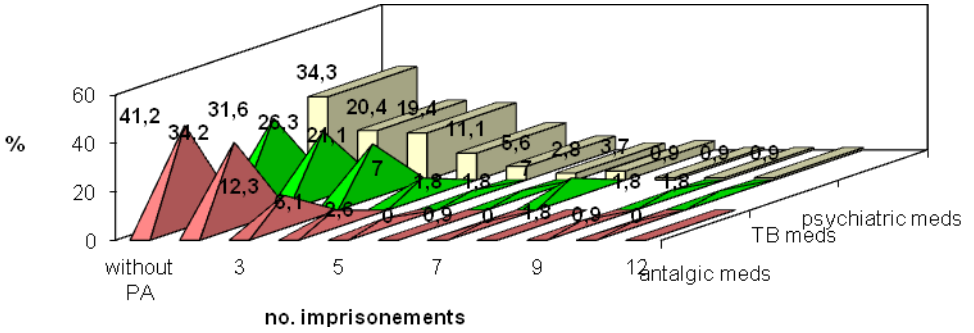

Fig. 7. Distribution of subjects by medication types according to the number of imprisonments

Other risk factors – Aggressions. 1.9% of the subjects in the HBV/HCC group and 2.8% of the controls reported they had been sexually abused, differences statistically insignificant (p=0.820). Physical aggression was reported by 18.5% of the HBV/HCV subjects and 16% of the controls, without significant difference in frequency between the study groups (p=0.628). Self-aggression was significantly more common in the HBV/HCV group (38.9%) as compared to the control group (23.5%) (p=0.002).

According to aggression type, the ROC curve confirmed that self-aggression is a main risk factor (fig. 8).

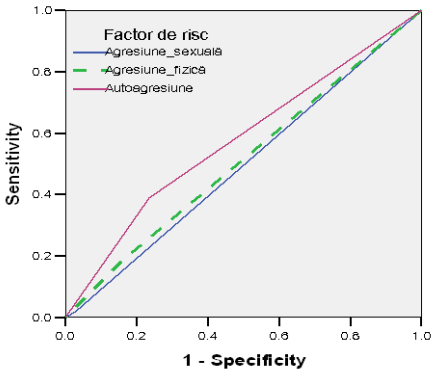

Fig. 8. Specificity of aggression as a risk factor

Physical aggression was present in 57.1% (57.1% *vs.* 42.9%) of the inmates

with criminal history, sexual aggression in 73% (73% *vs.* 27%), whereas self-aggression was more frequent in inmates who had been in prison before (58.5% *vs.* 41.5%).

DISCUSSION

Chronic liver infection is an important health problem worldwide, the strategies to prevent infections, by delivering safe blood products to injection drug users are essential and applied in most countries starting with 2000 (1,2). Many inmates are not aware that they carry the virus and are identified in the chronic stage of liver disease or in the phases of complications related to over-infection or malignant change (3). In Romania there are few nationwide evaluations of the risk of hepatitis B and C virus infection, and in the correctional system only the new acute cases reported to the Public Health Authority without identifying with certainty the infection source because of inmates' lack of trust in the health system are known. If worldwide vaccinating inmates against hepatitis B manages to keep under control this form of hepatitis, in Romania, because of the precarious situation of the public health system this prophylactic measure does not exist (4). Aggression and self-aggression are risk factors specific to correctional facilities, committed for obtaining material and non-material goods, but also a hierarchical positioning among the inmates. Many times this is a form of protest against detention conditions but also a way to emotionally touch or blackmail the staff. Self-harm is done with hand-made, improvised, and shared objects: self-made knives, needles or nails made from mattress springs, iron in the structure of furniture pieces, metal door and window parts and even glass pieces. The contamination risk is extended not only to the inmates sharing this object, but also to prisoner escort officers and security staff and even to the medical staff if aggressed by inmates or upon periodical checks of inmate personal property – accidental piercing. Minor surgical procedures as well as dental treatments represent a real epidemiological danger because they are numerous and are performed by a small number of doctors and nurses. Tattooing is another common way inmates are getting hepatitis B or C. It is a common practice among inmates, 76% of the tattooed individuals obtained it while in a correctional facility, of whom 37.8% during serving their first sentence. Tattoos are made with non-sterile, self-made and shared objects. In correctional facilities piercing is also empirically-performed, the earrings and even the piercing needle being self-made (5, 6, 7).

CONCLUSIONS

Blood transfusions account for a risk factor of 3.73; the empirical treatment induced in the HBV/HCV group a relative risk of infection of 2.62.

Sharing syringes was for the HBV/HCV group a risk factor of over 4 (OR=4.33); tattooing induced a relative risk of 1.25 in the HBV/HCV group and piercing was a risk factor of approximately 2 (OR=1.97); sharing personal care items represented a risk factor of over 2 (OR=2.02). Injection drugs induced a relative risk of over 4 (OR=4.33). In the HBV/HCV group, self-harm represented a risk factor of 1.65. Even though the frequency of crack cocaine sniffing in the HBV/HCV group was not significantly higher as compared to the

control group (7.4% *vs.* 4.2%), the calculated relative risk of infection with hepatic viruses was of 1.75 in the HBV/HCV group.

Accidental poisoning caused a relative risk of OR=3.65 in the HBV/HCV group. Psychiatric treatments had a statistically insignificant study group distribution (p=0.869).

For subjects in the HBV/HCV group self-harm was a 1.65 times stronger risk factor as compared to the control group.

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NEWS

EFLECTANCE CONFOCAL MICROSCOPY FOR MEASURING *DEMODEX* DENSITY

A study by Turgut Erdemir *et al* compared the effectiveness of standardized skin surface biopsy (SSSB) and the reflectance confocal microscopy (RCM) for measuring the *Demodex* mite density. Forty-seven healthy controls and 48 patients with pityriasis folliculorum, papulopustulary rosecea and erythema-telengiectatic rosacea were enrolled in the study. The area with the most intense erythema on the right cheek was selected for imaging with RCM (VivaScope 3000) and SSSB. RCM identified demodicosis in 48 patients (100%), while 42 patients (85.7%) showed high *Demodex* density (> 5 mites/cm2) with SSSB. The mean Demodex density measured with RCM was significantly higher than SSSB. The results demonstrate that reflectance confocal microscopy is a fast and noninvasive method, superior to SSSB, for *Demodex* mite detection (Turgut Erdemir A, Gurel MS, Koku Aksu *et al*. Reflectance confocal microscopy vs. standardized skin surface biopsy for measuring the density of *Demodex* mites. *Skin Res Technol*. 2014. doi: 10.1111/srt.12137).

Teodora Vremeră

SUBACUTE FUNGAL ENDOCARDITIS DUE TO *ACREMONIUM SPP*: A CASE STUDY AND REVIEW OF THE LITERATURE

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SUBACUTE FUNGAL ENDOCARDITIS DUE TO *ACREMONIUM SPP*: A CASE STUDY AND REVIEW OF THE LITERATURE (Abstract): A 52 years old patient is hospitalized in June 2007 in the Cardiology Clinic of Cardiovascular Diseases Medical Institute in Iasi with suspected subacute infectious endocarditis. Echocardiography shows mobile vegetation on the pulmonary valve. *Acremonium* spp is isolated from blood cultures after 2 weeks of incubation. The patient was treated with fluconazole, but died after 3 months due to renal failure.
Keywords: SUBACUTE FUNGAL ENDOCARDITIS, *ACREMONIUM SPP*, ECHOCARDIOGRAPHY.

Acremonium species are widespread in soil and are recognized as plant and insect pathogens rarely cited in humans and other mammals infections. Human infections typically occur after penetrating skin injuries, so infections are mostly in the extremities, especially mycetomas after traumatic inoculations (1).

Other sites of infection include eye, lung and gastrointestinal tract colonization, osteomyelitis, sinusitis, arthritis and peritonitis. Disseminated infections (meningitis, endocarditis, brain abscess) were rarely reported before, especially after valve replacement, dialysis, transplantation and hematologic malignancies in patients with other types of cancer (2, 3, 4, 5). In recent years the number of infections caused by *Acremonium* spp increased considerably due to predisposing conditions: invasive medical techniques, new impaired immune system conditions (6).

In what follows we will describe a case of native valve endocarditis in an immunocompetent patient.

CASE REPORT

Patient BV of 51 years without cardiopulmonary history, was admitted the Tecuci Hospital in 29.06.2007 with febrile syndrome, chest pain, cough with mucopurulent expectoration, dyspnea. The patient was diagnosed with lobar pneumonia; septic status and congestive heart failure according to NYHA class III. He received treatment with ampicillin + gentamicin, but the fever and congestive heart failure phenomena persisted. The patient was transferred to the hospital in Galati where a transthoracic echocardiography (TTE) was

performed. The echocardiography revealed:

- Increasing right heart cavities;
- Moderate pulmonary hypertension, dilatation of the aortic ring with second degree aortic insufficiency.

For furher investigation the patient was transferred to the Departament of Medical Cardiology of our institute on 2.08.2007. The patient underwent CT examination with the following results:

- On right lower lung: echodense formation, non-homogeneous, with invasive character;
- Pleural effusion in average quantity.

These elements raise the suspicion of lung cancer.

Because of persistent febrile syndrome and the congestive heart failure, the patient is still investigated for suspected endocarditis, which is why he performed a transoesophageal echocardiography (TEE) highlighting a new set of issues:

- Aortic valve: mild calcification;
- Mitral valve: normal morphology, first degree mitral insufficiency;
- Pulmonary valve: destroyed by two oscillating vegetations of 2.3 cm and 2.4 cm, third degree pulmonary valve insufficiency;
- Tricuspid valve: abnormal, modified, possible vegetation on the septal cusp, third degree tricuspid valve insufficiency.

Four sets of blood cultures were drawn (each set included one aerobic and one anaerobic bottle). Blood cultures remain negative at 9 days, but because of suspected endocarditis, we prolonged the cultures up to 3 weeks.

Early consultation with a cardiac surgeon was recommended in order to determine the best therapeutic approach. The cardiovascular surgeon decided that the surgical intervention could be postponed to allow the antibiotic treatment to take effect and avoid contamination of the prosthesis.

The patient was discharged with instructions to make fibrobronchoscopy and admission to Infectious Diseases Hospital for treatment: vancomycin 1g x 2/day + gentamicin 80mg x 2/day for 1 week and nistatin for 2 weeks.

After 14 days of incubation *Acremonium* spp was isolated from all 4 cultures and the patient received treatment with fluconazole 400mg x 2 / day intravenously for 30 days. At the beginning of treatment the patient had a second episode of pulmonary embolism.

On 10.12.2007 the patient returned for a review, in a state of afebrility but symptomatic for fatigue and dyspnea on moderate physical effort. TEE shows non oscillating vegetations on pulmonary and tricuspid valves. Blood cultures collected at that moment were negative.

In December 2007 the patient was admitted for a new episode of endocarditis, initially interpreted as acute renal failure, but blood cultures were positive for methicillin-sensitive *Staphylococcus aureus*. He received treatment with oxacillin + ciprofloxacin, but renal function (oliguria <0.5 L / day and creatinine 4 mg %) cannot be improved and the patient dies.

DISCUSSION

We found very few (only 10 studies) reporting endocarditis caused by *Acremonium* spp (7, 8, 9, 10, 11), most of them prosthetic valve endocarditis (mechanical prosthesis St. Jude no. 27, infective endocarditis on pacemaker, dura mater prosthesis). Infection in our patient affected native valve (pulmonary valve and tricuspid valve). We could not identify the gate of entry (mycetoma or trauma history). Note

that the patient was a professional driver and had a flaky lesion on the right foot, but no mycological examination was performed at the level of the injury.

Infection developed slowly over 2 months with prolonged febrile syndrome and pulmonary embolism, due to the vegetation being located in the right heart. Diagnosis of infective endocarditis was made based on TEE, blood cultures were positive after 10 days of incubation; identification of the fungus was performed after another 4 days.

Acremonium gender diagnosis is quite easy to make based on the microscopic and culture characteristics: growing moderately fast (but not more than 3 cm in 10 days), yellowish-white colonies, fasciculate (spiky), glabrous or moist. Conidiogenous cells solitary, slender (ca, 2µm wide), mostly unbranched, awl (needle-shaped) phialides. Conidia one celled, straight or curved, in slimy masses (fig.1, 2).

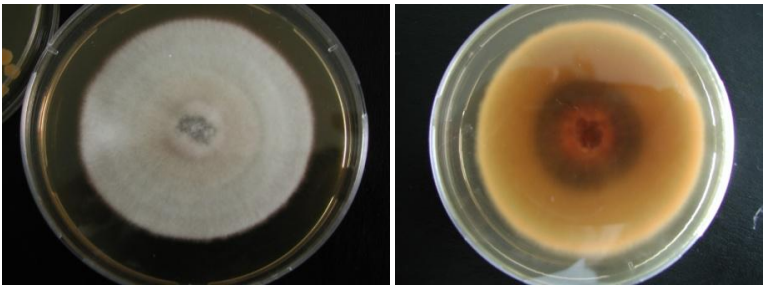

Fig 1. Appearance of culture on Sabouraud medium after 7 days of incubation (obverse and reverse)

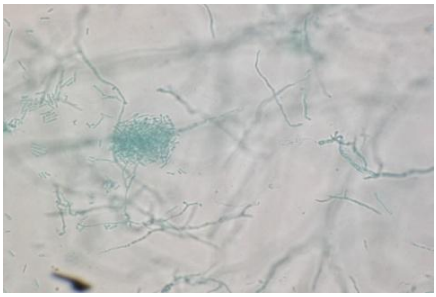

Fig. 2. Microscopic Appearance - wet preparation, 400

Acremonium species are morphologically very similar so that their identification is extremely difficult; therefore in most clinical cases etiologic agents are confused with *Acremonium* spp. This is the main reason why the actual incidence of *Acremonium* species is still unknown.

Identification of clinical isolates of *Acremonium* spp based on modern molecular methods are difficult to perform mainly due the absence of a public database of reference nucleotide sequences required for comparison for great genetic diversity of *Acremonium* spp, means that it suffers from a yearly taxonomic reshuffle. (13). We have sent our isolate to a reference laboratory, but it could not be identified to species level.

According to CLSI (M38-A2) (Clinical Laboratory Standard Institute) antifungal susceptibility should be made by the plate dilution method, a method that could not be done in our laboratory. Although, the literature data (13, 14) describes a decreased susceptibility to fluconazole (and imidazoles in general) in isolates of *Acremonium* spp. The literature generally recommends the use of amphotericin B. Because the lack of availability of this drug, the patient received i.v. fluconazole 400 mg x 2/daily for 30 days. Following this treatment, the clinical condition improved and the trans-esophageal echocardiography performed for control showed decreasing vegetation.

CONCLUSION

Acremonium spp can cause native valve endocarditis in immunocompetent persons. Fluconazole given intravenously for 30 days, two weeks more than the short-term therapy described in the literature (14), may improve the clinical condition of the patient with endocarditis.

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THE INFLUENCE OF ASOCIATING GREAT PHYSICAL EFFORT WITH NIGHT SHIFTS ON HYPERTENSIVE PATIENTS

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THE INFLUENCE OF ASOCIATING GREAT PHYSICAL EFFORT WITH NIGHT SHIFTS ON HYPERTENSIVE PATIENTS (Abstract): A great physical effort associated with work during night shifts can lead to negative effects on the worker's health. **The aim** of our study is to evaluate the risk of associating great physical effort with night shifts in hypertensive patients. **Material and methods**: We have studied 2 groups of hypertensive patients, each group being consisted of 20 male patients, age 30 to 50, that have been diagnosed with mild hypertension during the periodical medical exam, group I being consisted of subjects that performed great physical effort, and their work program implied night shifts. Group II consisted of workers that performed work that implied mild effort during day shifts. **Results and discussions**: At the end of the 2 year study period, 8 patients from group I (great physical effort associated with night shifts) developed a severe increase in blood pressure (beyond 160/100 mmHg), as well as an increase in basal cardiac frequency, whilst only one patient from group II presented such modifications. **Conclusions**: Night shift work associated with great physical effort can lead to a significant rise in blood pressure and heart rate, all the more if the subject suffers from hypertension thus predisposing the patients to coronary events. **Keywords**: WORK, NIGHT SHIFTS, HYPERTENSION, OCCUPATIONAL HEALTH

It is well known that great physical effort, performed in uncontrolled situations can lead to an increase in blood pressure and heart rate (1). Work during night shifts disrupts the circadian rhythm (2). Associating great physical effort with night shifts can have a negative impact on worker's health.

The aim of our study is to evaluate the risk of associating great physical effort with night shifts in hypertensive patients.

MATERIAL AND METHODS

We have studied 2 groups of hypertensive patients, each group being consisted of 20 male patients, age 30 to 50, that have been diagnosed with mild hypertension during the periodical medical exam.

Group I consisted of subjects that performed great physical effort and their work program implied shifts, including night shifts.

Group II consisted of workers that performed work that implied mild effort during day shifts.

The subjects of the 2 groups were monitored by Holter (blood pressure and heart

rate). All subjects had the following blood tests taken at the debut and end of our study: urea, creatinine, uric acid, glycemia, cholesterol and triglycerides. The following types of patients were excluded from our study: patients with behavioral risk factors (coffee, alcohol, smoking, excessive salt intake), as well as hypertensive patients undergoing medical treatment.

The subjects of the 2 groups were monitored for a period of 2 years, having their blood pressure, heart rate and blood tests periodically determined.

RESULTS

At the end of the 2 year study period, 8 patients from group I (great physical effort associated with night shifts) developed a severe increase in blood pressure (beyond 160/100 mmHg), as well as an increase in basal cardiac frequency. These persons necessitated immediate implementation of medical treatment, as well as modifying their program into day shifts (fig. 1).

Only one patient from group II, implying mild physical effort associated with day shifts had a severe increase in blood pressure and heart rate (fig. 2).

We noticed that night shift workers frequently experienced rises in glycemia, dyslipidemias (2) and significant rises in heart rate, which betray a sympathetic hyperactivity.

All these increased biological values represent risk factor for developing coronary events.

One of the patients from group I suffered a myocardial infarction during the study period, and a further two developed diabetes.

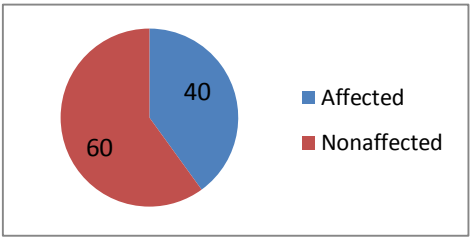

Fig. 1. Modifications found in Group I

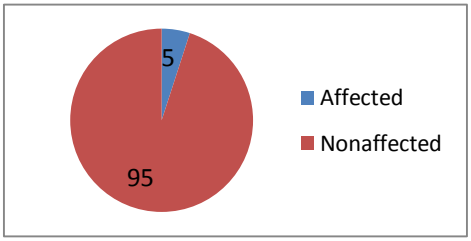

Fig. 2. Modifications found in Group II

TABLE I

Blood pressure (BP) at the end of the study

Group I				Group II			
Name	Age	Sex	BP	Name	Age	Sex	BP
GI	31	M	140/95	FG	44	M	130/70
PA	36	M	180/120	SZ	47	M	130/85
MV	45	M	150/80	LE	32	M	145/75
MS	32	M	135/95	BN	48	M	130/90
DP	44	M	155/90	LK	34	M	145/95
LA	47	M	130/90	WO	35	M	135/90
LS	34	M	175/110	TM	44	M	130/90
PF	39	M	140/95	LM	41	M	170/120
VD	41	M	165/105	CM	39	M	140/90

Group I				Group II			
CD	37	M	125/70	NP	43	M	145/85
PR	48	M	135/85	TR	32	M	140/90
TA	31	M	170/130	RD	47	M	140/90
SV	35	M	180/125	DM	39	M	130/95
MB	37	M	140/85	SA	36	M	135/80
CM	41	M	170/115	TS	32	M	140/85
PN	43	M	185/120	MO	47	M	130/75
KL	36	M	165/120	DA	42	M	140/85
SO	32	M	125/70	BS	49	M	150/95
AR	42	M	175/115	II	37	M	135/85
TI	37	M	135/90	VA	38	M	140/90

DISCUSSION

Comparing systolic and diastolic blood pressures in patients of the 2 groups at the end of the study period, we observed a significant difference in systolic blood pressure as well as diastolic blood pressure, patients that performed great physical efforts associated with night shifts having significantly increased values compared to those performing mild effort during day shifts.

Increased physical effort determines a rise in cardiac output and cardiac frequency. Physical effort determines activation of the sympathetic nervous system. Moreover, night shifts increase blood pressure and affect the variability of heart rate (2).

All these mechanisms lead to an abnormal increase in blood pressure, increase that implies the necessity of medical treatment, the modification of the work program to day shifts with mild effort.

A rise in systolic blood pressure can lead to a decrease in the individual's effort limit and an increase of myocardial oxygen consumption.

CONCLUSIONS

The results of our study have important contributions in the practice of occupational medicine, referring to active hypertensive employees who work in terms of high professional physical effort and night shifts.

We have demonstrated that the association between work night shifts and high physical efforts leads to marked increases in blood pressure and heart rate, patients in question being frequently prone to coronary events.

Our research results have to be materialized in medical and administrative measures: workplace changing, decrease of working hours or occupational reorientation, the occupational physicians taking decision in supervision of hypertensive employee.

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THE CLINICAL-EPIDEMOLOGICAL STUDY OF DRUGS USED TO TREAT ARTERIAL HYPERTENSION AND COMORBIDITIES ASSOCIATED

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THE CLINICAL-EPIDEMOLOGICAL STUDY OF MEDICINE USED TO TREAT ARTERIAL HYPERTENSION AND COMORBIDITIES ASSOCIATED (Abstract): **Aim:** The objective of this study was to investigate the drug treatment of AHT according to the grade and associated comorbidities: diabetes, chronic kidney disease, angina pectoris. M**aterial and methods:** This study is a retrospective one, comprising a total of 1064 patients admitted to the Clinical Rehabilitation Hospital Iasi, Cardiovascular Rehabilitation Clinic between 01.01.2011 - 31.12.2012. We noted the epidemiological data on the distribution of AHT according to area of origin, sex, age, and the pharmacological treatment according to the AHT grade and related diseases. **Results:** The results obtained were statistically analyzed by comparing drugs used together, their number depending on the AHT grade and associated comorbidities in order to achieve target values of blood pressure and reduce cardiovascular risk. The strategies used to control blood pressure were the gradual increase of the dose of drug or its replacement, switching from monotherapy to combination therapy, starting treatment with two drugs or administration of fixed combinations. **Conclusions:** The most used antihypertensive drugs were the angiotensin receptor blockers, ACE inhibitors, calcium blockers, diuretics and beta blockers. Some patients have even received triple therapy. **Keywords**: ARTERIAL HYPERTENSION, DIABETES, ANGINA PECTORIS, CHRONIC KIDNEY DISEASE.

Arterial hypertension (AHT) is a major risk factor for cardiovascular and renal diseases, being considered the leading cause of death. Worldwide, the disease prevalence reaches 40%, and 44.92% in Romania, prompting the need for guidelines use in order to optimize treatment (1.2). The fundamental objectives of AHT treatment are getting target values of blood pressure (BP) and reducing cardiovascular risk (3, 4). A meta-analysis that aimed at 147 clinical trials highlighted the need of lowering BP, as a reduction of 10 mm Hg of systolic blood pressure and of 5 mm Hg of diastolic one causes a decrease of 20% in the risk of coronary heart diseases and of 32% in the risk of cerebrovascular accidents in a year (5, 6).

MATERIAL AND METHODS

The aim of this study was to investigate the drug treatment of AHT according to

AHT grades and associated co morbidities: diabetes, chronic kidney disease and angina pectoris. This is a retrospective study, based on data obtained from observation sheets of a total of 1064 patients hospitalized at the Clinical Rehabilitation Hospital Iasi, Cardiovascular Rehabilitation Clinic between 01.01.2011 - 31.12.2012. This study evaluated the association of antihypertensive drugs and their effect on blood pressure value. Most hypertensive patients required more than one antihypertensive agent, so we started with the lowest dose (for patients without risk or low risk) and in patients with uncontrolled BP, doses were increased gradually until the onset of maximum effect or associated with other antihypertensive drugs.

RESULTS AND DISCUSSION

The prevalence of AHT is 38.72% men and 61.28% women aged 40-80 years (fig. 1), as follows: 21.52% under the age of 55, 40.70% between 55-65 years and 37.78% over the age of 65, being moderately prevalent in women, rural 51.03% (48.97% urban) (fig. 2, 3).

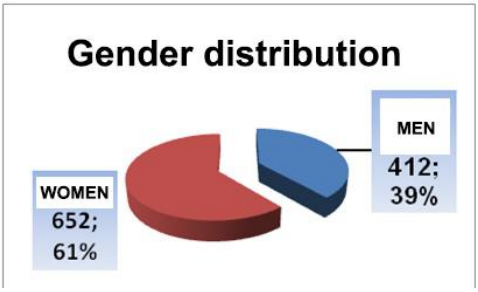

Fig. 1. Gender distribution

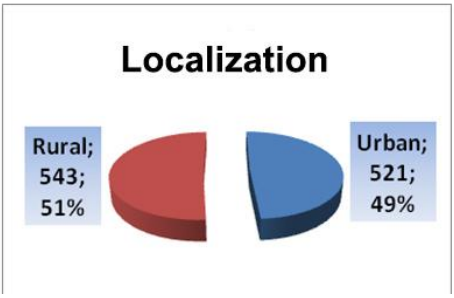

Fig. 2. Distribution on backgrounds

Consistent with literature data the age group most affected by AHT presence remains 55-65 years, predominantly females, as this age group lacks hormonal protection (fig. 4).

A a growth of AHT grade 3 of 47.84% was showed as compared with AHT grade 2 -28.29% and AHT grade 1 - 23.87% grade 1, but the difference is not statistically significant.

According to this study, associated morbidities are found in the following percentages: 26.50% diabetes mellitus (DM) 18.61% angina pectoris (AP), 14.66% peripheral artery occlusive disease (PAOD), 6.02% chronic kidney disease (CKD) (fig. 5).

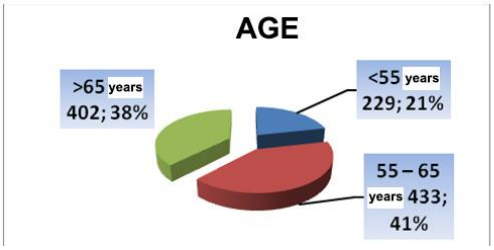

Fig. 3. AHT distribution on age

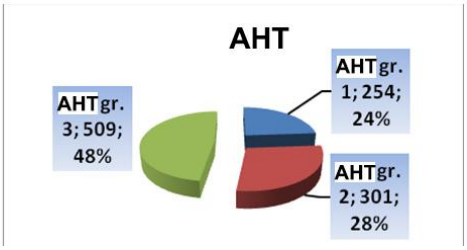

Fig. 4. Distribution on AHT grades

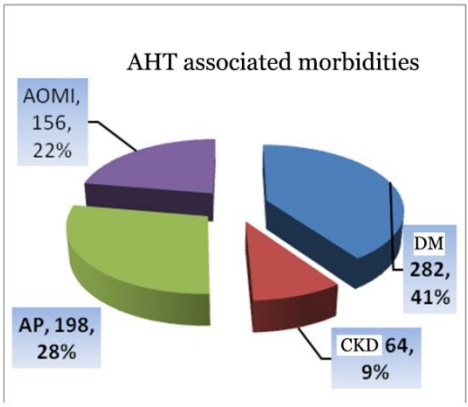

Fig. 5. Morbidities associated to AHT

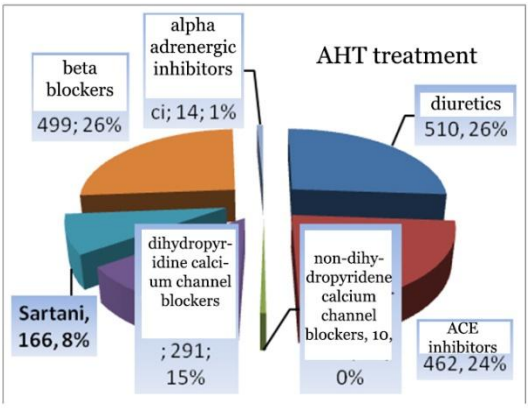

Fig. 6. AHT treatment

AHT treatment guidelines developed by the European Society of Cardiology (ESC) in 2007 and updated in 2009 support the importance of a "customized" therapy to the detriment of "staged" therapy, and recommend the use of different classes of antihypertensive and certain preparations of each class, with certain features (tab. I):

TABLE I

Treatment of HTA and co morbidities according to guidelines

HTA+comorbidities	**Recommendations therapeutic classes guidelines**
Angina pectoris	BB, CCB
Heart failure	Diuretics, BB, ACE inhibitors, ARBs, antialdosterone
Chronic kidney disease	ACE inhibitors, ARBs, loop diuretics
Diabetes mellitus	ACE inhibitors, ARBs

ACE inhibitors= Inhibitors of angiotensin converting enzyme; BB= Beta-blockers;
ARBs= angiotensin receptor blockers II /sartans; CCB= Calcium channel-blockers

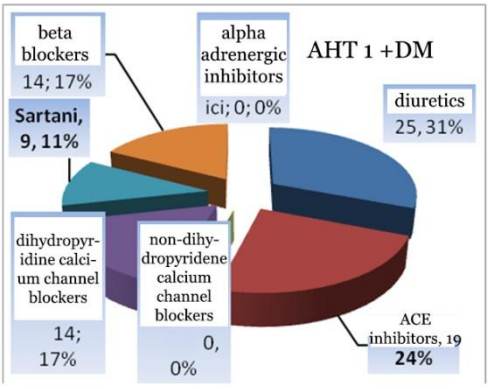

Fig. 7. Treatment of AHT grade 1 associated with DM

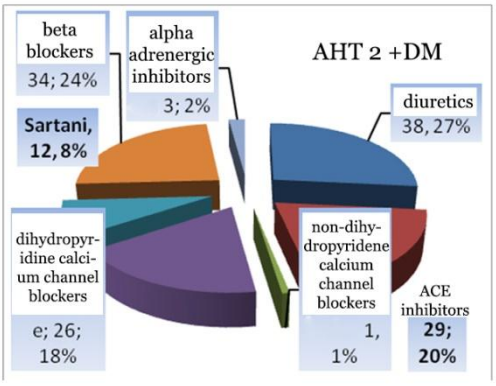

Fig. 8. Treatment of AHT grade 2 associated with DM

In our study we used different classes of antihypertensives, depending on the AHT grade and the presence of DM. along with AHT grade, the percentage of patients treated with alpha adrenergic inhibitors increases, but the increase is minimal (0 vs. 3.2% vs. 4.1%). The most significant change for all AHT grades can be found in case of beta blockers, which double at every increase in AHT grade (14.17% vs. 34.24% vs. 76.21%) ($p < 0.01$). Diuretics increase moderately from grade I to II (25.31% vs. 38.27%), and subsequently they have an exponential growth (38.27% vs. 93.26%) ($p < 0.01$), statistically significant. The same trend can be found in ARBs (9.1% vs. 12.8% vs. 42.12%), ACE inhibitors (29.2% vs.. 19.24% vs.. 83.23%), dihydropyridine calcium blockers (14.17% vs. 26.18% vs. 55.16%). In statistical terms, the greatest increase from grade I to grade III can be found in case of beta blockers (the other increases are also statistically significant) (fig. 6, 7, 8).

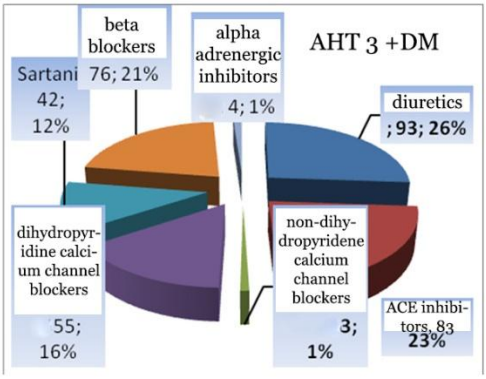

Fig. 9. Treatment of AHT grade 3 associated with DM

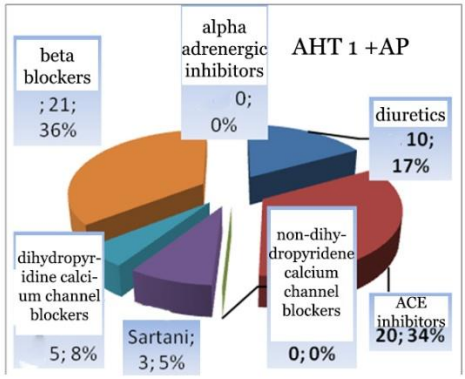

Fig. 10. Treatment of AHT grade 1 associated with AP

In our study, for the treatment of AHT associated with angina pectoris we used different medications, whose percentage ranges from one AHT grade to another. Thus, non-dihydropyridine calcium channel blockers were not used in the treatment of AHT grade 1 and 2, and a small percentage of 3.1% received these drugs in case of AHT grade 3 associated with AP. The same trend was found and the use of alpha adrenergic inhibitors (fig. 9, 10).

Diuretics were used in all grades of AHT (10.17% vs. 22.29% vs. 33.15%), and a statistically significant increase could be noted. ACE inhibitors were administered in case of AHT grade 1 in 20.34% of patients, then the percentage was dropped in case of AHT grade 2 (16.21%), and in AHT grade 3 it had a statistically significant increase (58.26%). Beta blockers were given in similar percentages in case of AHT grades 1 and 2 (21.36%, 24.32%) and in AHT grade 3 an exponential increase, statistically significant appeared (63.29%). Sartans have a similar tendency to beta blockers in all grades of hypertension (fig. 11, 12).

In case of a combination between CKD and HTA, we did not use as a treatment for AHT grades 1 and 2 alpha adrenergic inhibitors and non-dihydropyridine calcium channel blockers while in case of grade 3 percentage is minimal (2.3%, 1.1%) (fig. 13, 14).

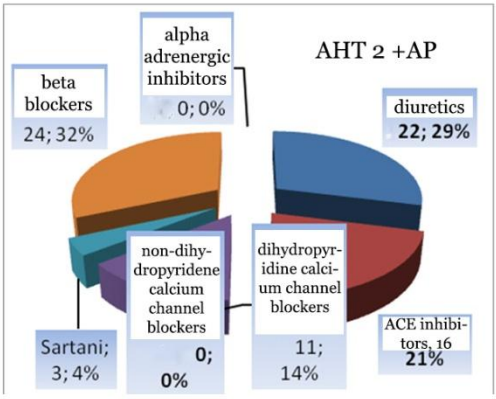

Fig. 11. Treatment of AHT grade 2 associated with AP

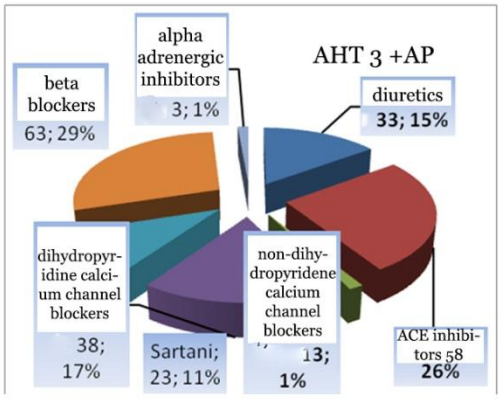

Fig. 12. Treatment of AHT grade 3 associated with AP

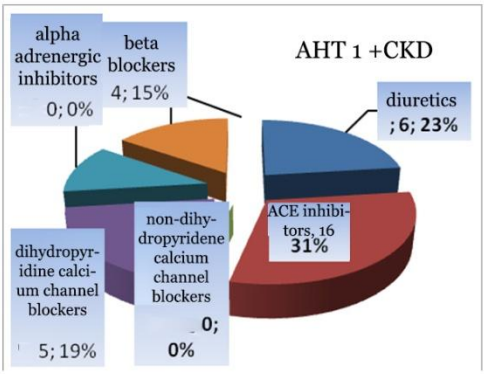

Fig. 13. Treatment of AHT grade 1 associated with CKD

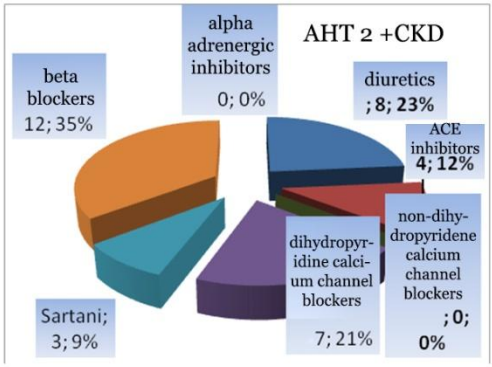

Fig. 14. Treatment of AHT grade 2 associated with CKD

Diuretics have a linear increase in HTA grades 1 and 2 (6.23% vs. 8.23%), subsequently exponential, statistically significant (21.26%). The same trend is observed in case of dihydropyridine calcium blockers and ACE inhibitors. Beta blockers significantly increase along with the AHT grade (4.15% vs.. 12.35% vs.. 19.24%) (fig. 15).

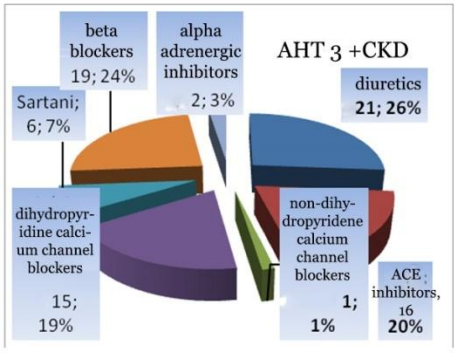

Fig. 15. Treatment of HTA grade 3 associated with CKD

CONCLUSIONS

According to current guidelines, clinical and laboratory evaluation of hypertensive patients aims to improve lifestyle through the identification of cardiovascular risk factors or clinical conditions that may influence the AHT prognosis and treatment, identification and exclusion of secondary causes of AHT and determination of the presence of target organ damage and quantification of its grade. Treatment of hypertension is also a challenge. Hypertension therapy involves both behavioral changes regarding living arrangements and customized pharmacological treatment. But hypertension can coexist with other co morbidities and thus treatment should be individualized as to not interfere with other diseases.

According to the study, AHT has a greater incidence in diabetic patients as compared to non-diabetic population of the same age. It is often associated with obesity, insulin resistance and hyperinsulinism. The AHT presence in a diabetic patient increases five times the incidence of myocardial infarction and stroke. Note that the occurrence of DM in a hypertensive patient puts the latter in a high risk group with the highest cardiovascular risk.

The goals of the pharmacological treatment of angina pectoris are to improve quality of life by reducing the severity and frequency of symptoms and thus patient's prognosis.

AHT is both a consequence and a cause of renal disease (6.02%), regardless of etiology. AHT is a major determinant of renal disease progression. Accelerated or malignant hypertension, renal artery stenosis and arterio-embolic disease are important causes of the kidney disease.

Strategies used to control blood pressure were moving from monotherapy to combinations, starting treatment with two drugs or administration of fixed combinations. Most used were angiotensin receptor blockers, ACE inhibitors, calcium blockers, diuretics and beta blockers. Some patients received even triple therapy to obtain normal values of blood pressure.

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NEWS

PHYSICAL ACTIVITY MONITORING ADVANCES AND LIFESTYLE INTERVENTIONS IN OBESITY

Obesity represents a strong risk factor for developing chronic diseases. Strategies for disease prevention often promote lifestyle changes encouraging participation in physical activity. However, determining what amount of physical activity is necessary for achieving specific health benefits has been hampered by the lack of accurate instruments for monitoring physical activity and the related physiological outcomes. Activity monitors have evolved from step counters and measuring devices of physical activity duration and intensity to more advanced systems providing quantitative and qualitative information on the individuals activity behavior. The novel techniques can monitor modes of sedentary behavior as well as the engagement in specific activity types that helps to evaluate the effectiveness of lifestyle interventions. In conclusion, advances in activity monitoring have the potential to support the design of response-dependent physical activity recommendations that are needed to generate effective and personalized lifestyle interventions for health promotion. (Advances in physical activity monitoring and lifestyle interventions in obesity. A G Bonomi, K R Westerterp. Journal: International Journal of Obesity - *Int J Obesity*, vol. 36, no. 2, pp. 167-177, 2012).

Doina Butcovan

HAND, FOOT AND MOUTH DISEASE – OUTBREAK IN ROMANIA?

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HAND, FOOT AND MOUTH DISEASE – OUTBREAK IN ROMANIA? (Abstract): Hand, foot and mouth disease (HFMD) is a viral illness usually occurring during the summer months in children younger than 5 years of age. In the North-East area of Romania the incidence is usually low, each dermatologist reporting 1-2 cases or even less per year. The diagnosis is usually based on the characteristic clinical aspect: vesicles and papules on the hands and feet and superficial oral ulcers. HFMD is typically a benign and self-limiting disease that resolves in approximately 7 days; in Asia there have been few reported severe cases that developed neurological complications and even death, while in certain areas of China this disease is a more and more serious public health problem. In the summer of 2012 in North-East Romania numerous cases of disease have been reported, some with atypical clinical manifestations and most of them with mild or moderate forms of disease. The present article is a discussion on one of these cases. The diagnosis was made based on lesions location and clinical appearance. An outbreak of HFMD should be confirmed by virology tests. **Keywords**: HAND, FOOT AND MOUTH DISEASE, EPIDEMIC

Hand, foot and mouth disease (HFMD) is a viral infection that produces vesicles and papules on the hands and feet and superficial oral ulcers (1).

Enteroviruses are RNA viruses belonging to the *Picornaviridae* family; more than 100 human serotypes are described in the literature, including 3 polioviruses, 23 coxsackie viruses A, 6 coxsackie viruses B, 31 echoviruses and 39 enteroviruses (EV68-71, EV 73-102, EV 104-107 and EV 109). From a phylogenetic point of view, there are four species of enteroviruses: A, B, C, and D (1).

The highest incidence of severe forms of disease and also the longest delay from the onset of symptoms to disease diagnosis is recorded in children. Boys are generally more susceptible to catching this disease, and a single day delay in diagnosis could increase the risk for severe forms of illness by approximately 40% and of death by

54% (2). All cases suspected of HFMD in North-East Romania in the summer of 2012 were mild and healed completely.

The disease is mainly transmitted by fecal-oral (HFMD being one of the dirty hands spread diseases) and respiratory routes. HFMD is highly contagious, especially with children 2 years old or younger, but also family members of even community. In literature cases of re-infection among brothers have been described. A person is most contagious during the first 7 days of illness, although enteroviruses persist for 1 to 4 weeks in the nose or pharynx and up to 18 weeks in stool (1).

CASE REPORT

We present a case of hand, foot and mouth disease in a 4-year-old boy.

According to his parents the boy presented a prodromal fever. At physical examination we discovered a skin eruption consisting of small papules and round-oval vesicles located periorally and on the tongue (fig. 1), palms (fig. 2), sole side of the feet (fig. 4), upper and lower limbs, and buttocks (fig. 5). Onycomadesis (fig. 3), a non-painful shedding of the nail beginning at its proximal end in one or several fingers that resolves without complications, was also present in our patient. Submandibular lymphadenopathy, common finding in this disease, was not seen in our patient.

The diagnosis was made only on clinical grounds, because we could find no laboratory able to identify the virus in our case.

a b

Fig. 1 a. Perioral vesicles and crusts b. Ulcerations on the dorsal side of the tongue

Fig. 2. Palmar lesions

Fig. 3. Onycomadesis at 4th finger

DISCUSSION

HFMD is a viral disease that affects the skin and oral mucosa.

Coxsackie virus A16 (CA16) was the first viral agent isolated from patients with HFMD; subsequently CA4, CA6, CA9 and CA10 as well as Coxsackie virus B (CB) were demonstrated as pathogens in HFMD (4). HFMD caused by EV71 has a higher incidence of neurological complications including aseptic meningitis, acute flaccid paralysis and encephalitis. Outbreaks of HFMD have occurred in Malaysia (2000-2003), Taiwan (1998-2005), Singapore (2000), Brunei (2006), Thailand (2008-2009), Korea (2008-2009) and Hong Kong (2008); therefore, the development of EV71 vaccine has become a national priority in Japan and China (3).

Human enterovirus 71 belongs to the human enterovirus A species of *Enterovirus* genus within the family of *Picornaviridae*; it is considered the major causative agent of HFMD, especially in children younger than 5 years of age, with children ages 1.0 to 2.9 years old being the most susceptible (5). EV 71 is more frequently associated with severe forms of disease or death.

Fig. 4 Plantar vesicles and lesions of lower limbs

Fig. 5. Buttock lesions

The diagnosis is clinically suspected in more than 90% of the cases, especially in the outbreak context, in our patient being made based on lesion location and clinical appearance. In Romania, the incidence of HFMD is usually low, with only 1-2 or even no cases diagnosed per year by every dermatologist. Nevertheless, in the summer of 2012 in North East Romania there was a three- or even four-fold increase in the number of reported cases. The diagnosis of certainty relies on virology lab tests: PCR for the detection of enteroviruses in blood, nasopharynx, and stool. Despite that, to our knowledge, there are no laboratory-based molecular studies on HFMD outbreak in Romania; the initially suspected outbreak has to be confirmed.

HFDM starts with a prodrome of fever, malaise, anorexia, and oral mucosal pain. These prodromal symptoms are more common in children younger than 5 years of age and precede the onset of specific lesions by 24-72 hours. In our case, the patient presented prodromal fever (5).

Skin rush manifests with small papules and especially round-oval vesicles classically located on palms and sole side of the foot; however they can be also present on the upper and lower limbs, buttocks, periorally, and even sporadically on the trunk. The typical appearance described in reference books, with lesions located solely on palms and accompanied by oral lesions, is today more rarely noticed, our case also presenting lesions located in other regions than classically described. The rash was neither pruritic nor left scars, while the vesicles were surrounded by an inflammatory halo.

Oral lesions are classically described on the anterior oral mucosa and tongue and are reported as being extremely painful, the vesicles progressing rapidly to superficial ulcers that heal typically without scars in approximately 7 days. When presenting to the dermatologist our patient had ulcerative lesions of the oral cavity and perioral vesicular lesions covered by crusts. Although in some cases cervical and submandibular lymphadenopathy may be present, our patient was lymphadenopathy-free (5).

Onycomadesis, a non-painful total separation of nail plate from the nail bed that begins at the proximal area and progresses to the free edge with nail shedding as a result of the temporary cessation of nail formation, was also present in our patient. Onycomadesis accompanying outbreaks of Coxsackievirus and enterovirus 71 has been reported in 2008 in Spain and Finland to be associated with HFMD (6). The mechanism of onycomadesis occurrence in HFMD has caused numerous debates, and it still remains a pathogenic hypothesis with several possible causes: selective tropism of certain viruses for the nail matrix; systemic effect of sepsis (nevertheless fever is not constant in HFMD), or maybe post-inflammatory consequence of periungual vesicles (7).

CONCLUSIONS

HFMD has variable expressivity, and its early diagnosis (now by modern molecular techniques) helps initiate control measures aimed at stopping virus transmission. Routine public health surveillance programs could be useful in understanding and monitoring the epidemiology of HFMD in Romania.

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NEWS

NEW THIAZOLES DERIVATIVES AS POTENT ANTIMICROBIAL AGENTS

Thiazoles are a familiar group of heterocyclic compounds possessing a wide variety of biological activities, and their usefulness as medicines is well established. Bondock et al. synthesized a series of novel 2-(3- pyridyl)-4,5-disubstituted thiazoles and characterized them by spectral and elemental analyses. The newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against ten bacterial and five fungal human pathogenic strains using the disc diffusion assay. The results clearly revealed that the substituted 2-(3-pyridyl)thiazoles exhibited good antimicrobial activity. The best antimicrobial activity was observed for 5-acetyl- 4-methyl-2-(3-pyridyl)thiazole followed by 4-methyl-2-(3-pyridyl)thiazole. Increasing the size of the substitutions either at position 4 or 5 on the thiazole nucleus decreased the antimicrobial activity. Among the synthesized compounds, 5-acetyl-4-methyl-2-(3-pyridyl)thiazole exhibited two-fold antibacterial activity of ampicillin in inhibiting the growth of *Staphylococcus epidermidis* (MIC=0.24 mg/mL) and also showed equipotent antifungal activity with amphotericin B against *Geotricum candidum* (MIC=0.48 mg/mL). In conclusion, the authors synthesized and investigated the antimicrobial activities of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles with the aim of discovering new structures that could be used as potent antimicrobial agents (Bondock S, Naser T, Ammar YA. Synthesis of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles as potent antimicrobial agents. *Eur J Med Chem* 2013; 62: 270-279).

Ana Maria Zbancioc

EXPRESSION OF BMI-1 PROTEIN IN CERVICAL, BREAST AND OVARIAN CANCER

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EXPRESSION OF BMI-1 PROTEIN IN CERVICAL, BREAST AND OVARIAN CANCER (Abstract): B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) is a member of polycomb group, which participates in axial patterning, hematopoiesis, cell cycle regulation, and senescence. Overexpression of Bmi-1 has been reported in various human cancers and proved to be associated with poor survival. **Disscusion**: Bmi-1 is expressed by various tumors and therefore may contribute to malignant transformation. Bmi-1 not only can lead mammary epithelial cells to senescence and immortalization, but also plays a key role in breast cancer. A significant correlation was observed between Bmi-1 expression and axillary lymph node metastases in lymph-ductal breast cancer. Bmi-1 is expressed in cervical cancer and correlated with a poorer prognosis, suggesting that this protein participates in the development and progress of cervical cancer. Regarding ovarian cancer, the results of several immunohistochemical studies revealed overexpression of Bmi-1, especially in poorly differentiated ovarian carcinoma. There is a strong correlation between histological grade, clinical stage and its expression. **Conclusions**: Human genes of polycomb group correlated with various hematological and epithelial cancers identify new mechanisms of malignant transformation and pave the way for developing new cancer treatments and identify new diagnostic markers. Bmi-1 and its expression in tissues taken from patients with cervical, breast and ovarian cancer could be a marker for diagnosis and prognosis, and not least a potential target of antitumor therapy. **Keywords**: BMI-1 PROTEIN, IMMUNOHISTOCHEMISTRY, ANTITUMORAL THERAPY

B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) is a member of the polycomb group, involved in hematopoiesis, cell cycle and cell aging. Recently, Bmi-1 overexpression has been reported in many cancers and was associated with a low survival rate (1). Human polycomb group genes related to various hematologic and epithelial cancers, identify new mechanisms of malignant transformation and open a path to developing new cancer treatments and identifying new diagnostic markers.

Polycomb group proteins are silent genes with key roles associated with body development by at least two multimeric complex: polycomb repressive complex 1 (PRC1) and repressive complex 2 (PRC2). Bmi-1 protein, a core member of PRC1 complex, was identified as oncogene and

together with c-myc initiates the occurrence of lymphoma. Bmi-1 gene is located on human chromosome 10p11.23 and extends over 4.9 kb, comprising 10 exons and 9 introns. It contains a RING finger domain type in its N-terminal end, and a central helix-turn-helix motif, which is required for inducing telomerase activity and immortalization of human epithelial cells (2).

It has been shown that Bmi-1 would be involved in several biological processes, such as embryonic development, organ formation, tumorigenesis, stabilization and differentiation of stem cells (3). Bmi-1 is expressed ubiquitously in almost all tissues and its expression was found to be slightly higher in the brain, spinal cord, kidneys, lungs, gonads, and placenta. However, several studies have shown that Bmi-1 expression is frequently increased in various human cancers, including lung cancer, ovarian cancer, acute myeloid leukemia, nasalpharyngeal carcinoma, breast cancer, and neuroblastoma, indicating that Bmi-1 could play important roles in the initiation and progression of cancer. The oncogenic feature of Bmi-1 was also reported to be associated with cell protection from apoptosis (4).

DISCUSSION

***Ovarian cancer*.** Prognosis of ovarian cancer has not improved substantially by standard classical treatment, in spite of new surgical techniques, chemoresponsivness of this malignancy to classical cytostatic agents, and efforts to detect it in early stages, accounting for the current global interest in ovarian cancer research. Recent molecular biology and genetic researches focus on the detection of molecular-genetic defects associated with this type of cancer, and their relation with morphopathological and clinical-imaging aspects and distinct clinical developments, which ultimately have direct therapeutic implications: molecular treatment targeting the molecular defect, customized treatment, with impact on survival (5).

Ovarian cancer has the highest mortality rate of all gynecologic malignances (6). Despite an initial response to surgery and first-line platinum- or taxane-based chemotherapy, most tumors eventually develop drug-resistant relapse. Evidence suggests that resistance to cipslatin may be the result of a defective apoptotic program. In this case, increased levels of DNA damage would be required to induce apoptosis initiation signal (7).

To investigate whether the abnormal presence of Bmi-1 is involved in the pathogenesis of ovarian carcinoma in a study conducted at the Oncology Laboratory of Sun Yat-Sen University, China in 2010, protein expression was first examined immunohistochemically in normal, benign, and borderline ovarian tissue and tissues from epithelial ovarian cancers. The results demonstrated that Bmi-1 expression in all normal ovary specimens was absent or low. In ovarian tumor specimens, a significantly increased Bmi-1 expression was observed in benign cystadenoma to borderline tumor, and carcinoma. Moreover, it was found that the frequency of Bmi-1 expression in undifferentiated ovarian carcinomas was significantly higher than in other types of carcinoma. Higher Bmi-1 expression in ovarian cancers was strongly correlated with tumor histological grade and clinical stage (pT / pN / pM). These findings suggest that Bmi-1 overexpression in ovarian carcinoma may be an acquired feature of the malignant phenotype (8).

Breast cancer is the most common malignancy in women, its mortality and morbidity continuing to grow despite the re-

markable progress in diagnosis and therapeutic approach in recent years. In the past decades, there was a continuous concern for the early detection of breast cancer and development of effective treatment modalities which to lead to a decline in breast cancer deaths and improved quality of life for women struggling with this disease. Along with conventional histopathological diagnosis of malignant breast tumors, which grades and classifies them into microscopic subtypes, significant progress in assessing the prognosis of these patients was made with the investigation of some immunohistochemical markers involved in tumor carcinogenesis.

Invasion and metastasis processes that cause great mortality are extraordinary distinctive features of breast cancer progression. Although lymph node metastases, tumor size, and histological grade of differentiation are usually considered to be prognostic markers for metastases, these occur at distant sites in 20-30% of the patients without lymph node involvement (9). So far, Human Epidermal Growth Factor Receptor (HER-2/neu), c-myc, and HOXB9 emerged as metastasis risk predictors in breast cancer. Their aberrant expression can induce the expression of growth and angiogenic factors, resulting in higher local concentrations of these factors in the tumor microenvironment, thus favoring tumor progression (10).

Bmi-1 overexpression increases motility and invasive properties of mammary immortalized epithelial cells, which is concomitant with increased expression of mesenchymal markers and decreased expression of epithelial markers. Consistent with these observations, Bmi-1 repression in metastatic breast cancer cells remarkably reduces cell motility, invasion, and transformation, as well as metastasis and tumorigenesis in mice. It has been demonstrated that Bmi-1 promotes invasion and metastasis of breast cancer and predicts poor survival (11).

Bmi-1 mRNA is significantly higher in adjacent normal breast tissue in breast cancer patients compared with normal breast tissue from patients with non-cancerous lesions. By contrast, Mel-18 mRNA level is lower in normal tissues of patients operated for breast cancer compared with breast tissue from mammoplasty. When protein expression in these two genes was evaluated, it was noticed that most epithelial cells were positive for Bmi-1 in both groups of tissue samples, although expression intensity was stronger in the normal tissue of cancer patients. Bmi-1/Mel-18 ratio can be used as a tool to stratify women at risk for developing malignancies (12).

Early detection of breast cancer is critical for improving patients' chances of survival. Techniques designed to detect recurrent or metastatic disease in the preclinical stages can contribute to this goal. The presence of Bmi-1 oncogene in plasma was assessed in a wide range of primary breast carcinomas with the aim at investigating its correlation with tumor clinicopathological parameters and survival rate. Study results suggest that Bmi-1 expression may be a marker of poor prognosis and clinically it may become a noninvasive diagnostic marker (13).

Cervical cancer. Despite significant progress in the etiology, diagnosis and treatment of cervical cancer, its incidence and epidemiology factors were not correspondingly influenced its resurgence in parallel with increased mortality both in as our country and worldwide being recognized. The reality is hard to justify and, especially, to accept, since it is the only

cancer with known etiology, viral, sexually transmitted, and cured 100%, of course in early forms, by complex, multimodal treatment. Without minimizing the undeniable progress in its etiopathogenesis, diagnostic and therapeutic means, and methods of prevention and early detection, we believe that in this field modern medicine owes, and stronger action must be taken for decreasing the incidence and improving the treatment of the disease at curable stage. It is noteworthy that this is a cancer benefiting from early detection and prevention and coded therapy, likely to cure the patients. Cervical cancer is the second most common cancer in women worldwide, with approximately 500,000 new cases diagnosed and 250,000 deaths each year. Almost 80% of cases are from developing countries (14).

Virtually all cervical cancers (99%) are related to HPV (Human Papilloma Virus) infection. In our country, every year approx. 2,800 new cases are detected, situation similar to that in developing countries from Africa, Asia and South America. According to a study by the World Health Organization, the mortality rate in Romania is 2 to 2.7 times higher than in most Central and Eastern European countries, and 6.3 times higher than EU average, the main cause responsible for this high mortality being the same factor: late diagnosis, and absence of mass screening programs, respectively. And this, in the conditions of a well organized and equipped network for the current practice of cervicovaginal exfoliative smear cytology. The natural corollary of the high incidence of cervical cancer mortality in our country is its proportional mortality, which, constantly in the last two decades, is the highest in Europe: 11.01 to 100,000 women (14).

The role of neoadjuvant chemotherapy in locally advanced cervical cancer has been studied in the past 25 years; this therapy reduces tumor size and lymph node positivity and allows minimizing the postoperative radiation therapy (15). Neoadjuvant chemotherapy, which can reduce the size and thereby increase tumor resectability, has been proposed as treatment of locally advanced cervical cancer. A study on a series of 110 patients, of which 68 received neoadjuvant chemotherapy and 42 were subjected to surgical procedure as the first therapeutic choice was conducted at the Peking University "People" Hospital. The results showed that 70.6% of patients achieved complete or partial response to neoadjuvant chemotherapy. Estimating the blood loss, duration of intervention, number of lymph nodes removed, the rate of complications during and after surgery, no significantly differences between the 2 groups were recorded. However, the rate of lymph-vascular space involvement was lower in the group who received neoadjuvant chemotherapy (16).

Neoadjuvant chemotherapy together with radical surgery increase the disease-free interval (DFS) and overall survival (OS) of patients with locally advanced cervical cancer compared with surgery as the first therapeutic act (17).

Bmi-1 is expressed in cervical cancer and correlates with a poorer prognosis, suggesting that this protein could participate in the development and progression of cervical cancer. It can be an independent prognostic of overall survival rate (18).

In a study by the Department of Gynecology and Obstetrics, University of Wurzburg, Bmi-1 expression in ovarian, breast, cervical and endometrial cancer tissues was determined by Western blot and

immunohistochemistry techniques. The results showed that Bmi-1 protein was significantly increased (19).

CONCLUSIONS

Bmi-1, polycomb group gene, regulates the proliferative activity of normal stem and progenitor cells. It is also essential for self-renewal of neural cells and hematopoietic stem cells. Bmi-1 is frequently increased in ovarian cancer and correlates with clinical stage, presence of lymph node metastasis, and poor prognosis.

Bmi-1 protein expression is more pronounced in primary breast cancer tissues compared to adjacent non-cancerous tissues. Increased Bmi-1 expression is correlated with advanced clinicopathological classifications (T, N, M) and clinical stage. In addition, high Bmi-1 levels indicate a lower overall survival and serves as a marker of increased risk for breast cancer.

Polycomb group genes related to various hematological and epithelial cancers identify new mechanisms of malignant transformation and open the door to developing new cancer treatments and identify new diagnostic markers.

Bmi-1 expression in tissues collected from patients with cervical, breast and ovarian cancer could be a diagnostic and prognostic marker, and last but not least a potential target of antitumor therapy.

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NEWS

UNIVERSAL DNA VACCINE VECTOR

Kong *et al*. describe the development of recombinant attenuated *Salmonella Typhimurium* strains that can be used as universal platform for delivering DNA vaccines to stimulate mucosal, systemic and cellular protective immunities. These *S. Typhimurium* strains have features that enable them to colonize host tissues and allow the release of bacterial contents after lysis. The purpose of the study was to transform a recombinant attenuated *Salmonella* vaccine (RASV) strain into a universal DNA vaccine-vector. The strains were genetically modified to display a hyperinvasive phenotype, in order to maximize host entry and cell internalization, to enable endosomal escape and release of the DNA vaccine into the cytosol and to decrease *Salmonella*-induced apoptosis. This will allow efficient nuclear trafficking of the DNA vaccine for synthesis of encoded protective antigens. According to this study, a DNA vaccine encoding influenza virus HA antigen, delivered by RASV strain, induced complete protection to mice against influenza virus (Kong W, Brovold M, Koeneman BA et al. Turning self-destructing Salmonella into a universal DNA vaccine delivery platform. *Proceedings of the National Academy of Sciences*, 2012; DOI: 10.1073/pnas.1217554109).

Teodora Vremeră

CORRELATIVE STUDY OF PMA SCORING IN A GROUP OF ROMANIAN PATIENTS SUBMITTED TO TOTAL HIP REPLACEMENT

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CORRELATIVE STUDY OF PMA SCORING IN A GROUP OF ROMANIAN PATIENTS SUBMITTED TO TOTAL HIP REPLACEMENT (Abstract): Elective hip and knee prosthetic surgery represent the ultimate treatment option for pain and disabilities caused by arthrosis with these particular localizations. **Material and methods**: We conducted a preliminary retrospective study on a series of 47 patients investigated and operated at the Orthopedic Clinic of the Iasi Rehabilitation Hospital in the interval March 1, 2009- March 1, 2010. **Results**: A good correlation between the general preoperative and 3 month-postoperative PMA scores (r=0.746) was found, even though the correlations between individual parameters were only moderate. **Conclusions**: The obtained results show that mean preoperative PMA scores statistically differ from the scores recorded 3 months after surgery. **Key words**: TOTAL HIP REPLACEMENT, PMA SCORE, CORRELATION, QUALITY OF LIFE.

Elective hip and knee prosthetic surgery represent the ultimate treatment option for pain and disabilities caused by the arthrosis with these particular localizations (1). The prevalence of both diseases continues to increase mainly in economically developed countries due to surgical techniques improvement and general population ageing. This trend led to increased expenses for health services, and also to longer waiting lists and implicitly waiting time for the required elective surgery.

Clear and objective criteria must be defined for the selection of patients accepted or directed to these waiting lists; also, standard diagnostic tools should be defined to determine the priorities on these lists, and to accurately assess the true emergencies and optimal benefit for the patient. Chronologic criterion should not be considered alone, but in relation with the clinical and social impact of delayed surgery effects.

In 1994, in USA, about 124,000 total hip replacements were performed as compared to 119,000 interventions in 1990. While the frequency of surgical interventions increased by 4% from 1990, the mean hospital stay decreased by 33.3%, from 10.8 to 7.2 days on average. Also, during the same interval, the number of total knee replacements increased by 62% from 129,000 to 209,000, while mean hospital stay decreased by 35%, from 10.5 to 6.8 days (2-4). Even though this information is useful to evaluate

the money spent for health services, these are enough. More specific info is required mainly regarding changes in preoperative and postoperative patient health status in order to determine how much health we can buy for the spent money.

Large joint replacement (hip, knee) is efficient in most patients, being one of the most cost-effective interventions in all surgical domains. However, some problems may occur mainly in young patients and long-term results (5, 6).

The need for hip functional assessment by a numerical score came from the declared purpose to obtain accurate data regarding operative indications and postoperative results (7). Some scores are excessively detailed and complex, thus being difficult to use. As an example, the Harris Hip Score, a hip-specific instrument, evaluates pain, function, activity, and motion on a 0–100 scale (where 100 is the best). It allows easy data collection and electronic assessment, but its main drawback consists in the fact that only 5 points are awarded for mobility; thus, a bilateral, not painful ankylosis in extension is scored 50 to 100 points, while following arthroplasty a 60 degrees flexion with 2 Canadian crutches, slight pain in sitting position will determine a score of only 32 of 100 points.

Postel Merle d'Aubigne (PMA) score, first published in 1946 by Robert Merle d'Aubigné and Michel Postel (8), is a simple scoring system, three aspects of hip function being scored 0 to 6; thus, this score was adopted world-wide. In 1990, PMA score was modified, form used by us in the present study. The main changes consist in the introduction of two new elements without changing the original principle. Practically, the incidence is determined by the level of pain, and stability is dependent on walking quality, that is separate scores for each hip. This new PMA scoring system includes 3 items scored 0 to 6: pain, mobility and stability (with unilateral load bearing, including walking) for each hip.

MATERIAL AND METHODS

We conducted a preliminary retrospective study on a series of 47 patients investigated and operated between March 1, 2009 and March 1, 2010 at the Orthopedic Clinic of the Iasi Rehabilitation Hospital.

Statistical analysis was made with the help of SPSS17 software. Statistical analysis was based on the total numerical score of each parameter and not on the clinical staging. We performed a descriptive statistical analysis together with a correlative study were carried out in order to evaluate the relation between preoperative and 3 month-postoperative PMA scores.

In this patient series we have applied the PMA scores pre- and postoperatively in order to evaluate the results and the follow-up in total hip replacement surgery. The mean patient age was of 65.49 years. Most patients were from rural areas (70.21%). All patients were retired, this not being an absolute rule but a limitation of our study group.

The mean body mass index in our study group was of 27.1, indicating that most of our patients were overweight. The body mass index may be an essential indicator in preoperative, immediate or late postoperative assessment.

Continuous variables (interval) were characterized by calculating the main statistical indicators (mean and confidence interval 95%, median, standard deviation, dispersion).

RESULTS

In our study group there was an even gender distribution of the cases (23 males –

48.94% and 24 females – 51.06%), standard deviation (SD) of 0.505. Gender distribution is important to define the allowed post-surgery activities.

Age-group distribution is also important in the evaluation using PMA scores. In our patients mean age was of 65.49 years, with a standard deviation of 7.448 for a confidence interval of 95%. The youngest patient was 49 years-old, while the oldest was 81 years-old; the true mean age varied between 63.30 and 67.68 years and median age was of 65 years. Most patients belonged to the 60-70 years age group. The follow-up of quality of life parameters depends directly on gender, activity, occupation, and social integration level of each particular patient.

Rural-urban distribution of patients is an important factor to be considered as, at least in Romania, it may directly influence the level of individual activity and also the type of the activity to be developed.

In our study group a Pearson correlation index of 0.746 was obtained, suggesting a direct powerful correlation, as this result is very close to value 1. Sig. corresponding value is equal to 0.000 - that is we have obtained a significant correlation coefficient of 0.01, meaning that there are less than 1% chances to be wrong if we claim that between the two variables there is a significant correlation. Thus we may assert that there is a powerful correlation between preoperative and 3 month-postoperative PMA scores. Therefore, the lower the preoperative PMA score, the lower the chances for postoperative PMA score to get closer to 18 (meaning excellent results). However, some debatable aspects should be considered, such as the techniques and implants available in 2009 and other subjective factors that may influence the outcome.

We have also calculated the Spearman correlation coefficient on total and partial PMA scores of the 47 study patients. Spearman correlation scores were interpreted according to the following scale (9): low correlation (rho < 0.3); moderate correlation (0.3 < rho < 0.6); good correlation (0.6 < rho < 0.8); excellent correlation (rho > 0.8)

Analysis of the obtained PMA scores showed a good correlation (r = 0,746) between preoperative and 3-month-postoperative scores. This shows that the used method can evaluate with good accuracy specific aspects of life quality in total hip replacement patient. Nevertheless, some technical aspects remain difficult to separate during scoring evaluation, according to this scoring system. Next step was to perform individual correlations between the pre and postoperative PMA scores for pain, mobility, and stability.

For the pain score, correlation analysis showed the following results: preoperative PMA scoring showed a mean of 3.45 (scale 0-6), with a standard deviation (SD) = 0.686; postoperative PMA scoring showed a mean of 5.81, with a standard deviation (SD) = 0.398; preoperative pain showed a mean score of 3.45 (min=2, max=5) with a SD=0.686; postoperative pain showed a mean score of 5.81 (min=5, max=6) with a SD=0.398

A high symmetry of Spearman score (rho = 0.541) and Pearson (0.560) index was found. A moderate correlation between the scores for preoperative and 3-month-postoperative pain was noted. Thus, pain, as a subjective but relevant factor that may influence patient's quality of life requires detailed and further attention. Individual results illustrate a main subjective component in evaluating this score, while at 3 months postoperatively most patients show good pain scores. The lack of correlation between pre and postoperative scores

shows a random evaluation of preoperative pain and a uniform positive evaluation of the postoperative pain (mild to no pain).

For the range of motion (ROM) score, correlation analysis showed that: preoperative PMA scoring showed a mean of 4.11 (range 3-6), with a standard deviation SD = 0.890; postoperative PMA scoring showed a mean of 5.60, with a standard deviation SD = 0.496; preoperative ROM scoring showed a mean of 4.11 (min=3, max=6) with a SD=0.890; postoperative ROM scoring showed a mean of 5.81 (min=5, max=6) with a SD=0.496

We may observe a high uniformity for Spearman (rho = 0.493) and Pearson coefficients (0.496). As for the pain, there was a moderate correlation between the obtained scores for pre and 3-month-postoperative ROM.

ROM should also be considered for further detailed evaluation with special references to surgical technique and/or implant type.

As to the functional score (walking/stability), the correlation analysis provided the following results: preoperative PMA scoring showed a mean of 4.00 (range 3-6), with a standard deviation SD = 0.834; postoperative PMA scoring showed a mean of 5.43, with a standard deviation SD = 0.5; preoperative functionality recorded a score of 4 (min=3, max=6) with a SD=0.834; Postoperative functionality recorded a score of 5.81 (min=5, max=6) with a SD=0.5.

We may observe a high uniformity for Spearman (rho = 0.446) and Pearson (0.496) indices. As for the pain, there is a moderate correlation between the scores for pre and 3 month-postoperative functionality.

DISCUSSION AND CONCLUSIONS

A good correlation between the general PMA scores applied pre and 3 month-postoperatively (r=0.746), even if the correlations between individual parameters were only moderate.

The results obtained in the evaluation of our patient group show that the mean preoperative PMA scores statistically differ from the scores recorded 3 months after surgery. Thus, applying PMA questionnaires may be useful for a functional evaluation, but cannot highlight the importance of a specific implant or surgical technique. We have noticed that this questionnaire cannot make the difference between different prosthesis types and various surgical techniques that may represent important impact factors on patient's quality of life, mainly long-time after surgery. The used prosthesis influences the dynamic articular behavior and the indices for different scoring systems (PMA, Harris, AAOS).

Study groups should be organized so to include prospective and retrospective studies and further detailed statistical analysis will show the flaws of classic scoring systems.

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NEWS

O ENZIMĂ ÎNDELUNG IGNORATĂ SE DOVEDEŞTE A FI CHEIA PENTRU A UCIDE BACTERIILE PATOGENE

Noi cercetări arată că o enzimă care a fost mult timp considerată relativ inutilă răspunsului imun are un rol important în determinarea celulelor sistemului imunitar să ucidă bacteriile care cauzează infecții. Cercetătorii de la Ohio State University au stabilit că această enzimă, numită caspase-11 la şoareci, permite componentelor celulare ale sistemului imunitar să fuzioneze şi să degradeze bacteriile care cauzează un tip de pneumonie numită boala legionarilor. Fără formarea fagolizosomului, aceste bacterii pot provoca boala. Nu se ştie dacă efectul este acelaşi şi contra altor bacterii. La om, enzima similară este o combinație de caspases 4 şi 5. Cercetătorii au stabilit că bacteria *Legionella pneumophila* suprimă activarea acestor două enzime din celulele umane. Dar, în cazul în care enzimele au fost reintroduse în celulele sistemului imunitar, au apărut aceleaşi evenimente de fuziune - observate şi la şoareci - care vor ucide bacteriile. Rezultatele ar putea duce la dezvoltarea de medicamente non-antibiotice concepute pentru a lupta împotriva anumitor infecții bacteriene prin activarea acestor enzime. Cei mai vulnerabili la boala legionarilor sunt fumătorii, vârstnicii, persoanele cu boli cronice sau compromise imunitar, inclusiv pacienții cu cancer şi SIDA. Majoritatea studiilor publicate până în prezent susțineau că fagocitoza era inițiată de o enzimă diferită, caspase – 1, iar caspase- 11 era necesară doar pentru activarea caspase- 1. Această concluzie se explică prin faptul că testele s-au făcut pe şoareci modificați genetic care nu aveau caspase- 1 dar nici caspase- 11 deoarece genele care codifică cele două enzime sunt situate foarte aproape una de alta. Datorită acestor studii, caspase-1 a fost considerată o țintă atractivă pentru tratament. Dar activarea caspase- 1 poate determina ca reacție adversă, o inflamație extinsă care nu apare la activarea caspase- 11, făcând din cea din urmă o ținta terapeutică mai dorită de companiile farmaceutice. Pentru noile studii, cercetătorii au utilizat culturi de celule umane şi şoareci modificați genetic, astfel încât aceştia să nu producă caspase-11. Ei au demonstrat că formarea fagolizosomului este activată de către caspase- 11 prin fosforilarea unei proteine țintă numită actin iar în celulele umane, bacteria *Legionella* a suprimat activarea caspases 4 şi 5, dar când au adăugat cele două enzime înapoi la culturile de celule imune ce conțin bacterii Legionella, bacteriile nu au mai supraviețuit. Activarea fagocitozei de către aceste enzime nu s-a produs în cazul altei bacterii patogene (*Salmonella*) ceea ce sugerează ca poate exista o diferențiere a acestor enzime funcție de tipul de bacterie (Anwari Akhter, Kyle Caution, Arwa Abu Khweek et al. Caspase-11 Promotes the Fusion of Phagosomes Harboring Pathogenic Bacteria with Lysosomes by Modulating Actin Polymerization. *Immunity*, 2012; DOI: 10.1016/j.immuni.2012.05.001Ohio State University (2012, June 11; Long-ignored enzyme turns out to be key to killing infectious bacteria. *ScienceDaily. Retrieved* June 16, 2012, from http://www.sciencedaily.com-/releases/2012/06/120611134239.htm).

Cătălina Luca

MYASTHENIA GRAVIS ASSOCIATED WITH THYMOMA AND TOXIC MULTINODULAR GOITER. A CASE REPORT

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MYASTHENIA GRAVIS ASSOCIATED WITH THYMOMA AND TOXIC MULTINODULAR GOITER. A CASE REPORT (Abstract): Adequate antithyroid drug treatment or surgery usually generates remission of myasthenia gravis (MG) in patients with thymus hyperplasia associated with Graves' hyperthyroidism. The case of a 46-year-old woman diagnosed with MG based on the clinical picture, anticholinesterase drug test and positive electromyography (EMG) is presented. The cervico-thoracic computer tomography revealed a compressive nodular goiter and normal antero-superior mediastinum and led to the diagnosis of MG secondary to the hyperthyroidism. An uneventful total thyroidectomy was performed, but postoperatively the MG symptoms worsened. Tc^{99m} tetrofosmin scintigraphy revealed an area of hyperfixation in the antero-inferior mediastinum, suggestive for thymoma, as confirmed by a repeated thoracic CT scan. Following a longitudinal sternotomy, a well incapsulated tumor of approximately 6/5 cm located in the antero-inferior mediastinum was found and an extensive thymomectomy was performed. The postoperative course was uneventful and the patient was discharged 9 days later with complete remission of myasthenia. The pathology report of the specimen revealed a mixt thymoma or AB thymoma after Muller-Hermelink and WHO classification, with invasive capsular foci corresponding to Masaoka II stadium. In conclusion, scintigraphy proved to be useful in the diagnosis and decision making of a thymoma. **Key words**: SCINTIGRAPHY, HYPERTHYROIDISM, MYASTHENIA GRAVIS, THYMOMA

The association between thyroid and thymus diseases, especially between Graves' hyperthyroidism and thymus hyperplasia with myasthenia gravis is reported in the literature but no standardized therapeutic standard is established. Both conditions can aggravate each other and a case tailored management is made by a multidisciplinary team.

The aim of this case report was to emphasize the role of a non-invasive imagistic investigation which resulted in operative decision making regarding a thymus lesion with a fatal potential if left untreated.

CASE REPORT

A 46-year-old woman, with a 2 years history of ocular, bulbar and limb muscles

weakness was admitted to the neurology department with inquiry myasthenia gravis.

Clinical examination, positive anticholinesterase drug test and positive EMG established the diagnosis of myasthenia gravis. The cervico-thoracic CT scan revealed a compressive nodular goiter (fig.1) and normal antero-superior mediastinum (fig. 2).

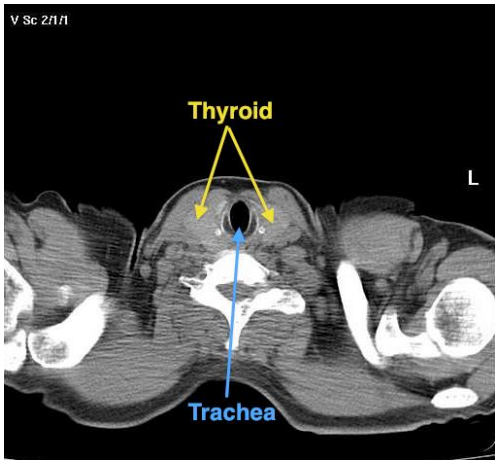

Fig. 1. The cervico-thoracic CT scan revealed a compressive nodular goiter

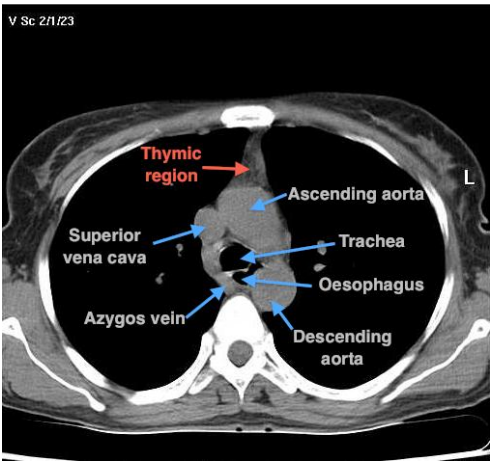

Fig. 2. The cervico-thoracic CT scan revealed a normal antero-superior mediastinum

Anticholinesterase treatment resulted in the improvement of the clinical condition and the patient was referred to the endocrinology department for further thyroid investigations.

Hyperthyroid status was revealed by hormonal tests (TSH-0.1 µUI/ml, fT4-1.2ng/dl) and ultrasound scan of the thyroid showed a multinodular goiter with degenerative areas and a total volume of 65.9 ml. Aspiration biopsy (FNAC) from the dominant nodule was benign. These findings led to the conclusion that myasthenia gravis was secondary to the hyperthyroidism and, given the large volume and nodular appearance of the goiter, the patient was referred to the surgical department for thyroidectomy. An uneventful total thyroidectomy was performed and the patient was discharged with thyroid hormone replacement and anticholinesterase treatment.

Pathology report of the goiter showed an oncocytic microfolicular adenoma with nuclear atypias and intact capsula in the left lobe and a mediofolicular adenoma with sclerohialinosis in the right lobe, developed on the multinodular goiter

Despite the expectations, five months after thyroidectomy the myasthenia gravis symptoms worsened, requiring higher anticholinesterase doses. At this stage we decided for an alternative imagistic exploration of the thymus, namely the ^{99m}Tc tetrofosmin scintigraphy. This revealed an area of hyperfixation of the radiotracer in the antero-inferior mediastinum, distal to the normal thymus location (fig. 3).

A new CT scan of the thorax showed a well delineated tumor located in the left parasternal antero-inferior region, lower than normal anatomical site of the thymus (fig. 4). Given the concordance of the two explorations, the diagnosis of thymoma with myasthenia gravis clinical stage Osserman IIB was established.

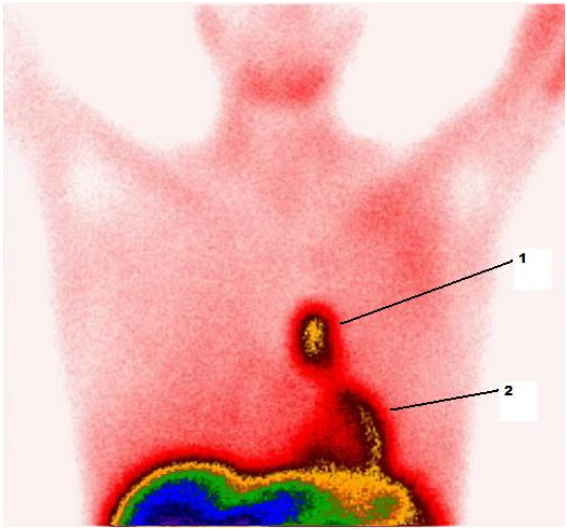

Fig.3. ^{99m}Tc tetrofosmin scintigraphy revealed an area of hyperfixation of the radiotracer in the antero-inferior mediastinum, distal to the normal thymus location

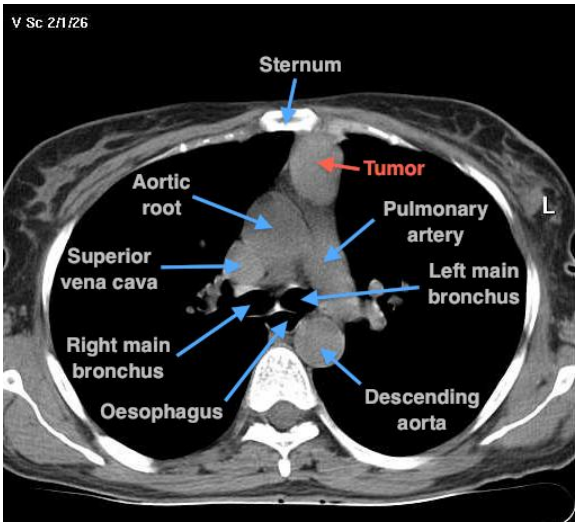

Fig. 4. A new CT scan of the thorax showed a well delineated tumor located in the left parasternal antero-inferior region

The patient was readmitted in the surgical unit and was operated on through a longitudinal sternotomy. Intraoperative exploration revealed a well encapsulated tumor of approximately 6/5 cm located in the antero-inferior mediastinum and an extensive thymomectomy was performed.

The pathology report of the specimen revealed a mixt thymoma or AB thymoma after Muller-Hermelink and WHO classification, with invasive capsular foci corresponding to Masaoka II stadium (fig. 5).

The postoperative course was uneventful and the patient was discharged 9 days later with complete remission of myasthenia.

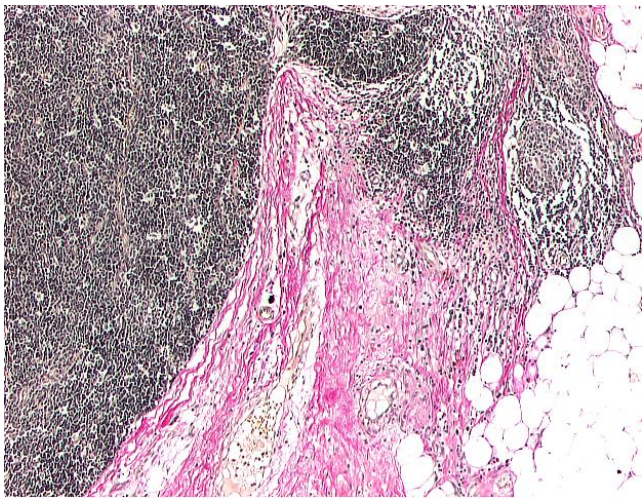

Fig. 5. AB thymoma with invasive capsular foci (Van Giesen staining)

DISCUSSION

Reports of extracardiac uptake and incidental diagnosis of mediastinal masses during ^{99m}Tc-tetrofosmin scintigraphy for the assessment of myocardial perfusion are present in the literature (1-4). Asian authors describe ^{99m}Tc-tetrofosmin scintigraphy as an useful investigation for thymus pathology, being able to detect mainly malignant tumors, tumor recurrences and metastatic lesions (5-7).

Although this investigation has not become a standard diagnostic for mediastinal tumors, it should be considered a valuable alternative when conventional investigations fail to confirm a clinical suspicion.

Thoracic CT scan has a reliable sensitivity and specificity and remains the method of choice in detecting thymus lesions. A limitation is the difficulty to differentiate thymus lymphoid hyperplasia and thymoma. On the other hand, thymus scintigraphy can distinguish after the degree of up-take of the radiotracer on the early and late images, between normal thymus, hyperplasia and thymoma.

Murakami and co-workers demonstrated the presence of thymus hyperplasia in Grave's disease patients, calculating the size and density of the thymus on untreated and treated Grave's disease patients, on CT scan images. They showed that thymus hyperplasia regresses in patients treated either with antithyroid drugs or total thyroidectomy (8).

The association of thymus hyperplasia with hyperthyroidism was reported by other authors that proven on biopsy that antithyroid treatment induces the remission of the thymus hyperplasia, avoiding such futile surgery (9).

Inoue et al reported a case of Graves' disease associated with a mediastinal mass with CT scan and MRI aspect suggestive for a thymoma. The patient underwent total thyroidectomy and thymectomy at the same stage. Pathology report showed a thymus hyperplasia (10).

Nakamura et al. demonstrated the pres-

ence of thyrotropine receptors in the hyperplasic thymus through mediastinal biopsy to a young patient with hyperthyroidism. The presence of these receptors raises the speculation that the thymus is also a target organ of the autoimmune aggression in Grave's disease (11).

Hyperthyroidism may be associated with thymus hyperplasia, in which no surgical action should be taken regarding the thymus, or with thymoma that requires surgical removal.

CONCLUSIONS

The association between thyroid and thymus lesions is challenging, both regarding diagnosis and therapy. Complementary imaging investigations such as thymus scintigraphy may prove helpful in decision making in certain cases.

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FERULIC ACID: POTENTIAL THERAPEUTIC APPLICATIONS

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FERULIC ACID: POTENTIAL THERAPEUTIC APPLICATIONS (Abstract): Ferulic acid is a phenolic compound that was first isolated in 1866 from the *Ferula foetida* resin (*Apiaceae* family) and was later obtained by synthesis. In nature, ferulic acid is found both in free and conjugated form. Due to its antioxidant effects ferulic acid can be used in the prevention and the therapy of diseases induced by oxidative stress, including Alzheimer's disease, diabetes mellitus, cancer, hypertension and atherosclerosis. The strong link between inflammation and oxidative stress suggests that ferulic acid can also be effective in the treatment of inflammatory conditions. Moreover, the specific ferulic acid structure also gives it a strong UV absorption capacity, making it a promising photoprotective agent. **Keywords:** FERULIC ACID, OXIDATIVE STRESS, ANTIOXIDANT, THERAPEUTIC EFFECTS.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) (fig. 1) was first isolated in 1866 from *Ferula foetida* (*Apiaceae* family) and synthesized in 1925 (1). The interest in ferulic acid dates to the 1970s when Japanese researchers discovered the antioxidant properties of its stearic acid esters, isolated from rice oil.

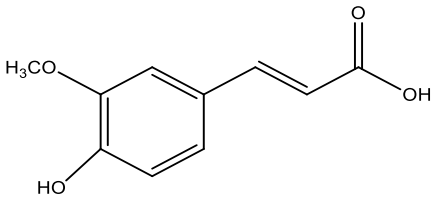

Fig. 1. Chemical structure of ferulic acid

Ferulic acid is found both in free form and as esters in vegetables, fruits, cereals and coffee. Among the richest sources of ferulic acid are wheat bran, citrus (orange juice), bananas, coffee, eggplants, cabbage, spinach, beets, bamboo shoots and broccoli (2, 3). In the western countries, extracts from various herbs, aromatics, spices represent other important sources of ferulic acid (1, 4).

It is estimated that by daily ingestion of foods of vegetable origin the intake of ferulic acid can reach 150-250 mg/day. Ferulic acid is absorbed through the gastrointestinal tract and is metabolized in the liver.

The aim of this study was to provide an outline of the different applications of ferulic acid in therapeutics, which will provide vast information to a wide range of researchers.

ANTIOXIDANT ACTIVITY

One of the most studied biological effects of ferulic acid is its antioxidant properties. Due to its phenolic nucleus and unsaturated side chain, the ferulic acid can readily form a resonance stabilized phenoxy radical which accounts for its effect of free radical removal (1). This allows ferulic acid to protect DNA and lipids from oxidation by reactive oxygen species (ROS). Ferulic acid and its derivatives exhibit antioxidant activity in response to free radicals (5, 6, 7). Given its antioxidant effects, ferulic acid may be beneficial in the prevention and treatment of disorders related to oxidative stress, including Alzheimer's disease, diabetes, cancer, hypertension and atherosclerosis (8). The strong link between inflammation and oxidative stress suggests that ferulic acid and its derivatives can also be effective in the treatment of inflammatory conditions (9, 10). Moreover, the specific ferulic acid structure also gives it a strong UV absorption capacity, making it a powerful skin protecting agent.

Based on the antioxidant effects of ferulic acid, Chinese researchers investigated the potential anti-atherosclerotic properties of ferulic acid (11). In the form of ferulate sodium salt, it is used in China for the treatment of cerebrovascular and cardiovascular diseases.

Ferulic acid at a dose of 150 mg/kg administered intraperitoneally (ip) for 4 days prevented lipid peroxidation and cellular apoptosis induced by free radicals in the Corti organ of guinea pigs exposed to noise (120 dB slp sound at a frequency of 6 kHz for 60 min). Interestingly, the administration of ferulic acid to these animals has reduced functional impairment and improved hearing function (12).

Antioxidant activity of ferulic acid appears to depend on the type of reactive species involved. In an experimental study which included rat brain microsomes, Trombino *et al.* (13) showed that ferulic acid had effectively inhibited lipid peroxidation activated by the peroxyl or peroxynitrite radical. This *in vitro* evidence agrees with the study by Kanski *et al.* (14) which demonstrated that the ferulic acid at a dose of 15-50 μM significantly attenuated cell death induced by the peroxyl radical from hippocampal neuronal cells.

Ferulic acid has been shown to influence secondary free radicals, such as those generated by carbon tetrachloride (CCl_4). Srinivasan *et al.*, showed that ferulic acid (20 mg/kg per os) prevents liver damage following CCl_4 administration in rat females. In this study, ferulic acid significantly reduced plasma liver toxicity markers as well as the protein and lipid oxidation marker levels (15).

THERAPEUTIC APPLICATIONS

Alzheimer's disease. Alzheimer's disease is a chronic neurodegenerative disorder characterized by progressive cognitive dysfunctions and memory loss (16, 17). The main role in the pathogenesis of this disease is the accumulation of beta-amyloid (βA), a peptide composed of 36-43 amino acids resulting from the amyloid precursor protein, by a proteolysis process under the action of β- and γ-secretases (18). Once produced, βA tends to aggregate in the form of oligomers or fibrils, thus forming

the core of the senile plaques. It is important to underline that βA activates specifically kinases such as glycogen synthase kinase 3 beta, cyclin-dependent kinase 5 and tyrosine-phosphorylated kinase 1A, which are responsible for hyperphosphorylation of tau protein and aggregation into neurofibrillary aggregates (19). The accumulation of oligomers, which precedes the formation of tau protein aggregates, results in an abnormal increase in ROS concentration, such as superoxide anion and hydrogen peroxide (19). There is also type 1 tumor necrosis factor alpha receptor activation, which, in the presence of apoptotic protease activator factor 1, activates the apoptosis cascade (20). It has been proven that an increased amount of βA diminishes the activity of superoxide dismutases (SOD-1 and SOD-2), leading to elevated intracellular ROS levels, which ultimately causes oxidative damage to lipids and neuronal proteins (20). At the same time, the superoxide anion (O^{2-}) excess reacts with nitrogen monoxide (NO) produced by activating microglial cells with peroxynitrite and other reactive nitrogen species (RNS) involved in protein nitration (21). The accumulation of ROS/RNS together with the activation of apoptosis and decreased SOD activity contributes to the destruction of cholinergic areas involved in cognitive performance, such as amygdala, hippocampus and cortex (22). Ferulic acid, through its antioxidant and anti-inflammatory properties, can have beneficial effects in Alzheimer's disease (23).

In adult rats, dietary supplementation with sodium ferulate (100-200 mg/kg bodyweight) for 4 weeks counteracted the increase of age-induced pro-inflammatory cytokines such as interleukin-1β (IL-1β) and helped to prevent the reduction of protein-serine/threonine kinase and protein kinase B with an important neuroprotective role in the rat hippocampus (24).

The neurotropic and neuroprotective effects of ferulic acid have also been demonstrated in the case of short-term parenteral administration. Mamiya T *et al.* (25) demonstrated that ferulic acid (5 mg/kg) administrated subcutaneously in mice for 6 days reduced brain damage due to the induction of oxidative stress by lowering the glutathione concentration and significantly increased cognitive activity.

Neoplastic Disorders. There are several factors involved in the pathogenesis of cancer, including chronic inflammation, excessive cell proliferation and/or apoptosis resistance and free radical formation. As a result, there is DNA and cellular protein damage and abnormal activation of the enzymes involved in the synthesis of proinflammatory mediators, respectively the activation of cyclooxygenase (COX) and nitric oxide synthase (NOS). From this perspective, each of these factors plays an essential role in carcinogenesis, amplifying the cascade of cell proliferation (26). The proven ability of ferulic acid to regulate cell proliferation and growth, to remove free radicals, to stimulate cytoprotective enzymes and to inhibit cytotoxic systems in both *in vitro* and *in vivo* experimental models supports the potential adjuvant role of ferulic acid in cancer therapy (27).

On colon cancer cells ferulic acid exhibited antiproliferative effects (28). The effect is due to the positive regulation of genes that encode the proteins involved in centrosome formation, of the gene for SMC1L1 protein that controls the S phase of the cell cycle as well as the negative regulation of genes involved in coding for

cyclin A2 kinase, cyclin B1 kinase, transcription factor and ornithine decarboxylase, respectively.

Nicotine plays an essential role in the pathogenesis of lung cancer by increasing the concentration of free radicals in many cell types, including leukocytes. Ferulic acid, at 10-40 mg/kg orally administered for 22 weeks, significantly reduced plasma lactate dehydrogenase and alkaline phosphatase in nicotine-treated rats. In a concentration of 100-150 μM, ferulic acid prevented nicotine-induced lipid peroxidation and DNA damage, respectively, and increased intracellular levels of SOD, catalase (CAT) and vitamin A, C and E in rat lymphocytes. It is important to point out that ferulic acid has not caused any DNA damage (29).

Another possible target of ferulic acid derivatives is cyclooxygenase (COX), which exists in the form of two major isoforms, COX-1 and COX-2. In various cancers, cyclooxygenase, the COX-2 form, is known to have superior regulation, which has led to the consideration of nonsteroidal anti-inflammatory drugs as potential anticancer drugs. Octyl-ferulic and dodecyl-ferulic esters significantly reduced the development of tumor cells in the central nervous system, colon, lungs and breasts. The half maximal inhibitory concentration (IC50) ranged from 17.05 mg/mL in breast cancer cells to 4.29 mg/mL in colon cancer cells (30). In addition, ferulic acid esters inhibited COX-2 activity, although this effect was not strictly selective and significantly decreased in esters with chain length greater than C8.

Administration of ferulic acid (40 mg/kg orally) or *Ixora javanica* extract (200 mg/kg orally), the active compound of which was identified as ferulic acid, in 7, 12-dimethylbenz[a]anthracene and methylcholanthrene-induced breast carcinogenesis demonstrated a decrease in tumor formation in rodents. The mechanisms involved in these antiproliferative effects can be associated with the antioxidant activity of ferulic acid and with the modulating effect on phase II antioxidant enzymes (31). In addition, ferulic acid reduced the development of sarcoma-180 and Ehrlich ascites carcinoma induced in mice, thus increasing the survival rate of experimental animals. Also, ferulic acid administered to rats in food at a concentration of approximately 500 mg/kg per meal for 7 weeks, significantly reduced the incidence of neoplasms, of cutaneous squamous cell papilloma and of preneoplastic lesions (hyperplasia and dysplasia) until 32 weeks (31).

Preclinical evidences have suggested that ferulic acid has potential for use as adjuvant in chemotherapy and radiotherapy. On the HeLa human cervical cancer cell line and keratinocyte cell k562, ferulic acid potentiated the cytotoxicity of platinum-based neoplastic agents (carboplatin and cisplatin) and 5-fluorouracil. Ferulic acid has been found to inhibit the growth of HeLa cells having an IC50 of 320M what is M??? (32). Furthermore, ferulic acid potentiated the effect of radiotherapy on HeLa and lung cancer cells (33).

Cardiovascular diseases. Cardiovascular diseases worldwide are associated with a high mortality rate. Hypertension and atherosclerosis are the main risk factors in the development of cardiovascular disease and, consequently, blood pressure control is particularly important in reducing the incidence of these diseases (34).

Ferulic acid (1-100 mg/kg orally) lowered blood pressure both in rat primary hypertension and in rats with genetic hy-

pertension, varying with the administered dose. It was noted that 50 mg/kg of chronic treatment with ferulic acid resulted in an antihypertensive effect comparable to that obtained with 10 mg/kg captopril. Ferulic acid reduced left ventricular diastolic rigidity, attenuated the infiltration of inflammatory cells and the collagen deposition in the left ventricle (35).

The vasodilator effect of ferulic acid has multiple valences since it involves the reduction of angiotensin II, the increase of NO synthesis and finally the reduction of NADPH-dependent peroxide anion synthesis (36).

Ferulic acid consumption (9.5 mg/kg orally) reduced triglycerides in rats and rabbits and cholesterol levels in rats. In a fat-rich diet, these lipid-lowering effects led to a significant decrease in the atherosclerotic area of the rabbit aorta and improved endothelial function (37).

Ferulic acid 0.6 mg/kg administration by intravenous bolus in rat myocardial ischemia-induced cardiac arrhythmias delayed early ventricular contractions, decreased duration of ventricular tachycardia and ventricular fibrillation, resulting in reduced mortality rate (38).

In a clinical study Wang *et al.* (39) have shown that ferulic acid as sodium salt has therapeutic potential in angina pectoris and coronary artery disease. Sodium ferulate (200-300 mg/day) administered for 2 weeks potentiated the therapeutic effects of nitrates, β-blockers, calcium channel blockers by the significant reduction in the incidence of angina attacks, reduction of nitroglycerin dose, along with an improvement in cardiac function tests. In subjects which did not respond to nitrates or β-blocker therapy, the intravenous administration of ferulic acid alone improved clinical symptoms and symptoms relief (39).

Recent studies on vascular smooth muscle cells isolated from rat thoracic aorta revealed that sodium ferulate might improve vascular functions and reduce restenosis after coronary interventions by inhibiting the neointimal hyperplasia (40).

Diabetes mellitus. Diabetes mellitus is a chronic disease characterized by the reduction of insulin synthesis and by the increased resistance of peripheral tissues to endogenous hormone in varying degrees. Increased blood glucose level affects many of the body cells, especially blood vessels and nerves. In addition, hyperglycemia is also responsible for the excess production of ROS, through the mitochondrial electron transport chain, which plays a significant role in the pathophysiology of cellular dysfunction and complications of diabetes (41).

In diabetic models on animals, ferulic acid has produced beneficial effects acting on several levels. Ferulic acid eliminates free radicals and activates antioxidant enzymes, thus reducing cellular redox imbalance. In mice with aloxan-induced or streptozotocin-induced diabetes, oral administration of 10-50 mg/kg ferulic acid demonstrated antioxidant activity, reduction of lipid peroxidation markers, stimulation of cellular response, and reduction of NF-κB immunoreactivity in pancreas, liver, kidneys and serum (42).

In streptozotocin-induced diabetic rats, ferulic acid (10 and 40 mg/kg per os) given for 3 weeks reduced blood glucose levels. This observation confirms the results obtained by Jung *et al.* (43), which demonstrated that oral administration of ferulic acid for 17 days in diabetes mellitus induced in mice increased insulin plasma concentration and reduced glucose in the blood. At the same time, the study revealed

that ferulic acid stimulates both liver glycogen synthesis and increases glucokinase activity, a key enzyme in regulating blood glucose levels. Ferulic acid also inhibits rat intestinal sucrase and maltase, acting as a natural inhibitor of alpha-glucosidase (43).

In diabetic patients, nephropathy and hypertension are some of the most common complications. The pathogenesis of these complications involves a multitude of factors including the renin-angiotensin system, activation of protein kinase C and acceleration of oxidative stress. In obese rats with induced type 2 diabetes ferulic acid (10 mg/kg/day for 20 weeks or 0.2% for 12 weeks orally) significantly reduced proteinuria level, oxidative stress marker concentration as well as expression of cytokine, diminished glomerular membrane thickness and glomerular volume compared to control group (44).

Photoprotection. On dermal application, ferulic acid has a good absorption, both at neutral and acid pH, demonstrating that the molecule can be absorbed through the skin in both dissociated and undissociated form. In healthy subjects, ferulic acid in the form of a saturated aqueous solution, pH 7.2, applied locally, has been shown to be effective in protecting skin tissue against UV light-induced erythema. The photoprotective effect of ferulic acid (0.5%) was enhanced by the combination with vitamin C (15%) and vitamin E (1%), formulation which applied locally in pigs experimentally exposed to UV radiation gave good results (45, 46).

Ferulic acid can be expected to have photoprotective effect when orally administered.

CONCLUSIONS

Our study revealed that ferulic acid and its derivatives prove to be very good antioxidants that can be used to prevent several diseases (Alzheimer's disease, neoplastic diseases, cardiovascular diseases, diabetes mellitus), and for photoprotection with a high safety profile and low financial costs. Some of the presented compounds promise wider practical applications in therapy, but clinical trials are still needed.

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EFECTELE AMINOACIZILOR CU CATENĂ RAMIFICATĂ ASUPRA HDL-COLESTEROLULUI LA ANIMALE DE EXPERIENŢĂ SUPUSE UNEI DIETE HIPERCOLESTEROLEMICE

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THE EFFECTS OF BRANCHED CHAIN AMINOACIDS ON HDL-CHOLESTEROL IN EXPERIMENTAL ANIMALS SUBJECTED TO DIETARY HYPERCHOLESTEROLEMIA (Abstract): High levels of high-density lipoprotein cholesterol (HDL) are associated with a reduced risk of cardiovascular disease, so that many studies focus on modify HDL levels for therapeutic purpose. **Aim:** The present research aims to highlight changes in plasma levels of HDL-cholesterol in experimental animals with a high-fat diet associated with some branched aminoacids - valine and leucine. **Material and methods:** The experiment was realised on 32 male Wistar rats, which were divided in four groups: group I- received a standard diet; group II – was fed with cholesterol (C), group III (C + V) - cholesterol and valine and group IV (C + L) - cholesterol and leucine. After 8 weeks animals were evaluated in relation with lipids levels in the blood. **Results:** The average values of HDL-cholesterol in group who received only cholesterol (C) were significantly lower compared with group III who received cholesterol and valine (C + V) or group IV that received cholesterol and leucine (C + L) ($p<0.001$), after one month and at the end of the experiment. **Conclusions:** Our results showed that valine and leucine increased the serum levels of HDL-cholesterol and therefore they are useful components in coronary artery disease risk reduction. The association of these aminoacids in human diets can improve clinical conditions associated with hyperlipidaemia such as atherosclerosis. **Key words**: BRANCHED AMINOACIDS, HDL-CHOLESTEROL, HYPERCHOLESTEROLEMIA, ATHEROSCLEROSIS.

Un număr considerabil de studii epidemiologice şi populaţionale au raportat că valori crescute ale HDL-colesterolului par a întârzia sau împiedica dezvoltarea aterosclerozei în timp ce niveluri reduse sunt asociate cu un risc crescut de apariţie a acestei afecţiuni (1,2). De asemenea, nivelul lipidelor sanguine, în special al colesterolului total şi al LDL-colesterolului, sunt de obicei legate de promovarea aterosclerozei, prin urmare, unele măsuri care scad nivelurile de lipide pot întârzia sau inversa progresia acestor procese.

Identificarea factorilor de risc implicaţi în apariţia aterosclerozei şi modularea factorilor cu rol protector în scopul unor acţiuni ţintite de prevenţie sau instituirii unor atitudini terapeutice care să amelioreze sau să întârzie bolile vasculare şi complicaţiile acestora constituie obiective de mare importanţă ale cercetării medicale actuale (3,4). Abordările existente sunt limitate în

capacitatea lor de a creşte HDL-colesterolul, care continuă să fie un obiectiv major în dezvoltarea de noi terapii, fie de creştere a HDL-colesterolului, fie de îmbunătăţire a funcţiei acestuia.

Studii recente abordează relaţia dintre aminoacizi şi diferite sisteme din organismul uman, subliniind rolul din ce în ce mai mare al acestor compuşi.

MATERIAL ŞI METODE

Am efectuat un studiu experimental pe un număr de 32 de şobolani Wistar masculi, cu greutatea între 250-280 g. Durata experimentului a fost de 8 săptămâni. Animalele au fost distribuite în 3 loturi a câte 8 şobolani: lot I – martor - a primit dietă standard; lotul II –a primit dietă suplimentată cu colesterol în doză de 0,4g/kg c/zi (C); lotul III –a primit dietă suplimentată cu colesterol în doză de 0,4g/kg c/zi şi cu aminoacidul valină în doză de 62,5mg/kg c/zi (C+V); lotul IV - a primit colesterol în doză de 0,4g/kg c/zi şi aminoacidul leucină în doză de 69,985mg/kg c/zi (C+L).

Pentru urmărirea nivelelor serice ale parametrilor lipidelor sanguine, la începutul experimentului, la 30 de zile şi la sfârşitul experimentului s-au recoltat probe de sânge din plexul retroorbitar de la fiecare şobolan. Anterior recoltării probelor s-a efectuat anestezia animalelor cu ketamină – 75 mg/kg intraperitoneal. Procedeele experimentale folosite în acest studiu au fost în concordanţă cu reglementările etice internaţionale. Rezultatele obţinute au fost centralizate în baze de date EXCEL şi SPSS şi prelucrate cu funcţii statistice adecvate.

Cercetarea de faţă îşi propune să evidenţieze doar efectele administrării aminoacizilor cu catenă ramificată – valină şi leucină - asupra nivelului HDL - colesterolului seric, urmând ca rezultatele cu privire la ceilalţi parametri lipidici să fie prezentate în articole viitoare.

REZULTATE

Pentru fiecare lot în parte, HDL-colesterolul a fost investigat în trei momente: la începutul experimentului - R0, după 1 lună - R1 şi la finalul experimentului - R2. Evoluţia valorilor HDL-colesterolului pe tot parcursul experimentului, precum şi varianţa seriilor de valori ale acestui parametru au fost în permanenţă raportate la lotul martor (tab.I, fig.1).

TABELUL I

Valori ale HDL-colesterolului înregistrate la loturile de studiu

Lot	R0	R1	R2
Lot I: Martor			
media	23.00	22.89	22.90
SD	1.49	1.22	1.68
CV%	6.47	**5.34**	7.35
Lot II: C			
media	22.44	19.45	15.94
SD	3.29	1.45	1.20
CV%	**14.67**	**7.47**	7.54
Lot III: C+V			
media	22.98	24.65	26.86
SD	1.48	2.79	2.95
CV%	**6.44**	11.34	11.00
Lot IV: C+L			
media	22.51	22.98	23.18
SD	2.16	1.90	1.81
CV%	9.59	8.27	**7.83**

La lotul martor, coeficientul de variație cel mai mic s-a înregistrat la 1 lună de la debutul experimentului (5,34 CV%). Varianța seriilor de valori la lotul II, III şi IV este redusă, variind de la 6,44 la 14,67 CV%, însă cele mai omogene serii de valori se observă la începutul experimentului la lotul III C+V (6,44 CV%), după 1 lună la lotul II - C (7,47 CV%), iar la sfârşitul experimentului la lotul IV - C+L (7,83 CV%). Cele mai mici valori individuale ale HDL-colesterolului se regăsesc la lotul II care primeşte numai colesterol (fig. 1).

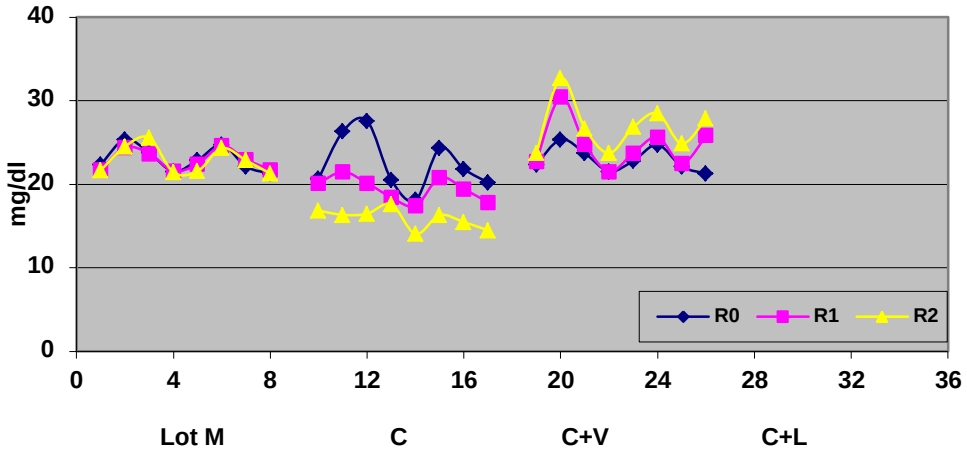

Fig. 1. Valori individuale ale HDL-colesterolului evidențiate la loturile de studiu

Valorile medii ale HDL-colesterolului la loturile II, III şi IV, în funcție de momentul investigării prezintă următoarele diferențe procentuale (fig. 2):

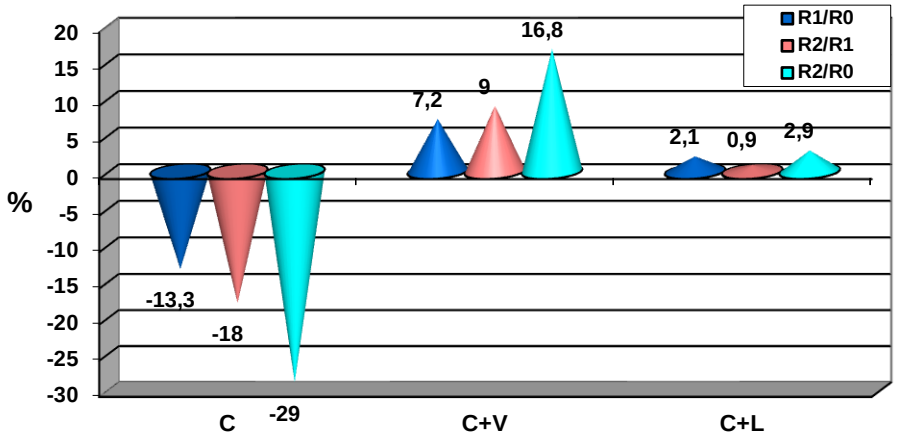

Fig. 2. Diferența procentuală a valorilor medii ale HDL-colesterolului la loturile II, III şi IV față de debutul studiului

La lotul II - C, s-a evidenţiat o scădere procentuală a valorilor medii ale HDL-colesterolului de 13,3% după 1 lună, iar la sfârşitul experimentului comparativ cu momentul iniţial, scăderea a fost de 29%; la lotul III - C+V s-a observat o creşterea procentuală a valorilor medii ale HDL-colesterolului de 7,2 % la 1 lună, iar la sfârşitul experimentului de 16,8%; la lotul IV - C+L, creşterea procentuală a valorilor medii ale HDL-colesterolului a fost de 2,1% la 1 lună, iar la sfârşitul experimentului de 2,9%.

La debutul studiului nu s-au înregistrat diferenţe semnificative între valorile medii ale HDL-colesterolului la loturile II, III şi IV şi nici între acestea şi lotul martor ($p>0,05$). Comparativ cu martorii, la 1 lună de la debutul experimentului, valorile medii ale HDL-colesterolului au fost semnificativ mai mici la lotul II- C ($p<0,001$) şi semnificativ mai mari la lotul III - C+V ($p<0,01$), la sfârşitul experimentului. Valorile medii ale HDL-colesterolului la lotul II au fost semnificativ mai mici comparativ cu lotul III sau cu lotul IV ($p < 0,001$), atât după o lună de experiment, cât şi la sfârşitul experimentului. La final, lotul IV a avut valori medii ale HDL-colesterolului semnificativ mai mici decât lotul III ($p<0,01$) (tab.II, fig. 3).

TABELUL II

Diferenţe statistice între valorile medii ale HDL-colesterolului la loturile de studiu

Moment	Lot	Lot M (n=8)	C (n=8)	C+V (n=8)
R0	C (n=8)	t = 0,44 p > 0,05	-	
	C+V (n=8)	t = 0,03 p > 0,05	t = 0,44 p > 0,05	-
	C+L (n=8)	t = 0,53 p > 0,05	t = 0,05 p > 0,05	t = 0,51 p > 0,05
R1	C (n=8)	t = 5,15 **p < 0,001**	-	
	C+V (n=8)	t = 1,63 p > 0,05	t = 4,69 **p < 0,001**	-
	C+L (n=8)	t = 0,11 p > 0,05	t = 4,19 **p < 0,001**	t = 0,28 p > 0,05
R2	C (n=8)	t = 9,54 **p < 0,001**	-	
	C+V (n=8)	t = 3,30 **p < 0,01**	t = 9,70 **p < 0,001**	-
	C+L (n=8)	t = 0,32 p > 0,05	t = 9,43 **p < 0,001**	t = 3,01 **p < 0,01**

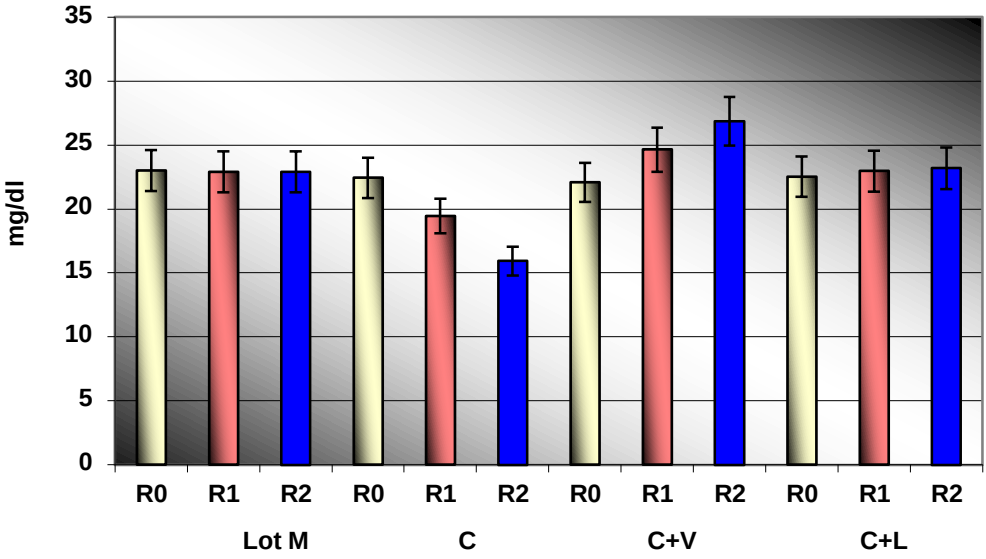

Fig. 3. Valori medii ale HDL-colesterolului la loturile de studiu

DISCUȚII

Relația dintre hipercolesterolemie şi procesul aterosclerotic a fost de-a lungul timpului în atenția a numeroase cercetări morfologice şi experimentale. Colesterolul şi trigliceridele sunt lipidele sanguine de cel mai mare interes pentru ateroscleroză şi cardiopatia ischemică (5), dar numeroase studii epidemiologice indică de asemenea o corelație indirectă puternică între concentrațiile plasmatice ale lipoproteinelor cu densitate mare (HDL) şi bolile cardiovasculare. Din colesterolul seric total, componenta majoră asociată cu un risc crescut este LDL-colesterolul, care are un rol fiziologic esențial în furnizarea colesterolului țesuturilor periferice (6,7). În contrast, HDL-colesterolul are rolul de a mobiliza colesterolul din ateroamele în formare sau din cele deja formate şi a-l transporta la ficat pentru a fi excretat în bilă. Pe lângă abilitatea de a îndepărta colesterolul de la nivel celular, HDL-colesterolul are proprietăți antiinflamatorii, antioxidante şi antitrombotice, care contribuie la îmbunătățirea funcției endoteliale şi inhibiția aterosclerozei (8). Astfel, cu cât nivelul HDL-colesterolului este mai crescut, cu atât riscul apariției aterosclerozei este mai scăzut şi, prin urmare, este de înțeles interesul special pentru dietele, medicamentele şi obiceiurile comportamentale de reducere a LDL-ului seric şi creştere a HDL-ului seric (9,10).

Tratamente care cresc HDL-ul s-au dovedit a fi eficace în reducerea incidenței bolilor cardiovasculare, atât în prevenția primară cât şi secundară, deşi nu este complet definită dovada că o creştere a HDL-colesterolului printr-un tratament farmacologic este în măsură să confere o reducere a riscurilor majore cardiovasculare independent de modificări ale LDL-ului sau de nivelurile de trigliceride (11). În diferite

modele experimentale, restricţia în carbohidraţi s-a dovedit a fi eficientă în reducerea trigliceridelor plasmatice, creşterea HDL-colesterolului şi modificarea repartiţiei subfracţiilor LDL-colesterolului (12). Atât HDL, cât şi exerciţiile fizice s-au dovedit a fi factori predictori pentru bolile cardiovasculare, în special pentru boală cardiacă coronariană. Creşterea efortului fizic este asociată cu creşterea HDL explicând în parte efectul benefic asupra HDL (13).

Dietele bogate în colesterol şi grăsimi saturate, cum ar fi cele prezente în gălbenuşul de ou, grăsimi animale şi unt, cresc nivelul colesterolului plasmatic, în timp ce dietele sărace în colesterol şi grăsimi polinesaturate îl reduc (14). Mai mult decât atât, acizii graşi omega-3, prezenţi în cantitate crescută în uleiul de peşte, sunt probabil benefici, în timp ce grăsimile nesaturate produse prin hidrogenarea artificială a grăsimilor vegetale polinesaturate şi utilizate în alimentaţie pot afecta nefavorabil profilele de colesterol, conducând la ateroscleroză. Medicamentele numite *statine* reduc colesterolul circulant indirect, prin inhibarea HMGCoA-reductazei, o enzimă cheie necesară pentru biosinteza colesterolului în ficat (15,16).

În cercetarea noastră se remarcă o scădere a valorilor HDL prin administrarea dietei bogată în colesterol faţă de animalele cu dietă standard din lotul martor ceea ce arată o diminuare a capacităţii de transport al colesterolului de la nivel vascular la nivel hepatic, conform şi altor date din literatură(8,9,10).

De-a lungul timpului au fost realizate diferite studii care au ajutat la descifrarea deprecierii metabolice care implică carbohidraţii şi acizii graşi în afecţiunile cardiace. La polul opus se află datele relativ puţine despre potenţialul impact al aminoacizilor în derularea acestor boli. Deşi majoritatea activităţilor catabolice ale aminoacizilor se desfăşoară în ficat, catabolismul aminoacizilor cu catenă ramificată (BCAA) necesită o activitate în mai multe ţesuturi nonhepatice, inclusiv muşchiul cardiac, diafragm, creier şi rinichi. O atenţie deosebită a fost acordată în ultimul timp unor modele genetice recent dezvoltate legate de defecte ale catabolismului BCAA cu impact în fiziopatologia cardiacă (17). Studii experimentale au arătat că deficitul de valină sau izoleucină are efecte similare asupra reducerii masei de ţesut adipos, într-un mod similar cu cele observate în timpul privării de leucină. Animalele hrănite cu o dietă deficitară în aceşti aminoacizi prezintă modificări semnificative ale metabolismului lipidic, fapt demonstrat de suprimarea lipogenezei în ficat şi creşterea mobilizării grăsimilor din ţesutul adipos alb (18).

CONCLUZII

Studiul biochimic a relevat, prin compararea parametrilor metabolismului lipidic în grupurile diferite conform design-ului experimentului, faptul că aminoacizii esenţiali tip valină şi leucină au un efect direct asupra hipercolesterolemiei prin creşterea HDL-colesterolului plasmatic. Consecutiv, endoteliul vascular este protejat, cu diminuarea riscului de apariţie a disfuncţiei endoteliale şi prin urmare a unor afecţiuni cardiovasculare care au ca mecanism patogenetic această perturbare. Aminoacizii cu catenă ramificată tip valină şi leucină pot fi utili astfel pentru tratamentul profilactic sau terapeutic al unor condiţii clinice asociate cu hiperlipidemie, cum ar fi ateroscleroza.

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IMPACTUL STRESULUI OXIDATIV ASUPRA REPERFUZIEI MIOCARDICE: DOVEZI ALTERNATIVE

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THE IMPACT OF OXIDATIVE STRESS ON MYOCARDIAL REPERFUSION: ALTERNATIVE EVIDENCE (Abstract): **Aim:** Our study aimed to investigate the impact of oxidative stress on myocardial reperfusion phenomenon. Since obtaining morphological evidence of oxidative stress is practically impossible in vivo, we used as "surrogate" evidence the primary percutaneous transluminal coronary angioplasty effects. **Material and methods**: The study group consisted of 22 patients hospitalized with a diagnosis of acute myocardial infarction with ST segment elevation in the Institute of Cardiovascular Diseases "Prof. Dr. George I. M. Georgescu" Iasi. Patients were evaluated by coronary angiography. To restore myocardial reperfusion we applied primary percutaneous transluminal coronary angioplasty. Evidence resume coronary flow by primary percutaneous transluminal coronary angioplasty were assessed by: (1) Thrombolysis in Myocardial Infarction (TIMI) flow, Myocardial *Blush* Grade (MBG) and complications as no-reflow, distal emboli; (2) evolution of the electrocardiographic changes. **Results**: Evaluation of myocardial blood flow resumption revealed, as final results, TIMI 3 flow with MBG 2-3 and without *no-reflow* in 21 cases. A single case has shown signs of irreversible *no-reflow*. Analysis of evolutionary electrocardiographic changes (ST segment elevation, depression) allowed assessment of the effectiveness of interventional reperfusion, dependent on the time of presentation and independent of the culprit artery patency. **Conclusions**: Assessment of myocardial reperfusion by alternative evidence provides a range of information how to restore the functional integrity of myocardium, but without allowing an analysis of biochemical and morphological substrate of the evolution of lesions, in terms of oxidative stress intervention. Studies of the dynamics of oxidative stress-induced changes at the structural level impose, under the peculiarities of myocardium, the design of an experimental model. **Key words:** OXIDATIVE STRESS, MYOCARDIAL REPERFUSION, NO-REFLOW

Stresul oxidativ reprezintă fenomenul în care există exces de radicali liberi ca urmare a excesul de sinteză a lor şi/sau prin deficitul mecanismelor de protecţie contra acestora. Acest fenomen induce anomalii biochimice, metabolice şi funcţionale (1). Are contribuţii semnificative în multe procese patologice, inclusiv în boala ischemică cardiacă şi reperfuzia miocardică. În reperfuzia miocardică este interesat şi peretele vascular, astfel încât este alterată funcţia endotelială ca urmare a modificărilor metabolice profunde

generate de formarea în exces de radicali liberi odată cu reintroducerea brutală a oxigenului molecular (2). Leziunea de reperfuzie implică astfel stres oxidativ ca urmare a excesului de radicali liberi ce apare odată cu reintroducerea bruscă a oxigenului molecular, şi deficitul mecanismelor antioxidante endogene, fie ca urmare a depăşirii acestora prin exces de radicali liberi, fie prin deficitul primar al lor ca urmare a ischemiei ce a precedat reperfuzia (3).

Tratamentul de reperfuzie miocardică aplicat în infarctul miocardic acut cu supradenivelare de segment ST reprezintă tehnica de aur în tratamentul acestei patologii, în scopul salvării miocardului la risc, şi în acest mod, de menţinere a funcţiei de pompă a inimii şi de prevenire a complicaţiilor pe termen scurt şi la distanţă (4). Obiectivul principal este reprezentat de restaurarea perfuziei tisulare, la nivel microvascular. Metodele de reperfuzie miocardică, denumite în practica clinică revascularizare, sunt reprezentate de tromboliză, angioplastie coronariană percutană transluminală (ACPT) primară, şi pontaj aorto-coronarian. ACPT primară este superioară trombolizei în ceea ce priveşte restaurarea imediată a fluxului coronarian normal, scăderea riscului de ischemie recurentă şi reinfarct, cu beneficii mai mari la grupele de pacienţi cu risc crescut, şi cu efect mai durabil (5, 6, 7).

Obţinerea unor dovezi morfologice asupra impactului stresului oxidativ in fenomenul de reperfuzie miocardică este practic imposibilă *in vivo*. În acest context, studiul nostru analizează un set de dovezi "surogat" care permit evaluarea eficacităţii fenomenului de reperfuzie miocardică, inclusiv la nivelul microcirculaţiei, prin angioplastie coronariană percutană transluminală primară.

MATERIAL ŞI METODE

Lotul de studiu a fost format din 22 de pacienţi (19 de sex masculin, 3 de sex feminin), cu vârste cuprinse între 33 şi 81 de ani, internaţi cu diagnosticul de infarct miocardic acut cu supradenivelare de segment ST (IMA) în Institutul de Boli Cardiovasculare "Prof. Dr. George I. M. Georgescu" Iaşi. Au fost incluşi pacienţii cu durere toracică persistentă şi cu supradenivelare persistentă de segment ST sau cu bloc de ram nou apărut, în primele 12 ore de la debutul infarctului, conform cu indicaţia de clasă I a liniilor ghid cele mai recente ale Societăţilor Americană şi Europeană de Cardiologie, adaptate şi aplicate de către Societatea Română de Cardiologie (4, 8, 9, 10).

La aceşti pacienţi am realizat angiografie coronariană urmărind artera responsabilă de IMA, patenţa sa, prezenţa colaterale-lor, prezenţa altor leziuni coronariene si, ulterior, am aplicat protocolul de angioplastie coronariană percutană transluminală primară (11).

Procedura invazivă a constat în aspirarea trombului şi implantare de stent metalic sau farmacologic activ. În raport cu debutul simptomatologiei, procedura a fost aplicată, pentru 14 cazuri, în primele 6 ore (aplicare precoce), iar pentru celelalte 8 cazuri, într-un interval de 6-12 ore (aplicare tardivă).

Dovezile reluării fluxului coronarian prin angioplastie coronariană percutană transluminală primară au fost apreciate prin: (1) evaluarea fluxul TIMI (Thrombolysis in Myocardial Infarction), "*blush*" miocardic-MBG (Myocardial *Blush* Grade) şi a prezenţei complicaţiilor de tip no-reflow, emboli distali (12); (2) evaluarea evoluţiei modificărilor electrocardiografice (13, 14, 15).

REZULTATE

Evaluarea circulației coronariene

Coronarografia efectuată în urgență a relevat următoarele aspecte. În 16 cazuri, ocluzie trombotică acută a arterei responsabile de IMA. În două dintre aceste 16 cazuri au fost prezente colaterale. 8 pacienți au prezentat afectare unicoronariană, iar ceilalți 8 – afectare bi- sau tricoronariană. În 6 cazuri, a fost evidențiată o arteră responsabilă de infarct patentă, cu sau fără tromb, dar cu leziune critică, 1 caz prezentând flux distal TIMI 1 pe fondul unor leziuni tricoronariene cu stenoză critică a trunchiului principal al arterei coronare stângi. Nici unul dintre cele 6 cazuri nu a prezentat colaterale, afectarea a fost unicoronariană în 3 cazuri şi bi- sau tricoronariană în celelalte 3 cazuri.

Elemente de evoluție ulterioară ACPT primare

a) Dovezi alternative ale reluării fluxului coronarian prin ACTP primară: flux TIMI, *blush* miocardic-MBG, *no-reflow*, emboli.

Evaluarea reluării circulației sanguine miocardice a evidențiat, ca rezultate finale, flux TIMI 3 cu *blush* miocardic (MBG 2-3) şi fără *no-reflow* în 21 cazuri (95,45%), cu următoarele particularități:

- 18 cazuri, la care protocolul ACPT a fost aplicat fără incidente intervenționale, au avut o evoluție clinică foarte bună, cu dispariția imediată a durerii, sau diminuarea sa importantă şi cu dispariție ulterioară, fără complicații, fiind considerate ca reperfuzie eficientă;

- 2 cazuri au prezentat embolii distale în artera descendentă anterioară (ADA) apical, dar evoluția a fost favorabilă;

- un caz a evoluat cu obținerea unui flux inițial TIMI 1 fără *blush* (*no-reflow*) şi ulterior, după administrare intracoronariană de adenozină, nitroglicerină şi ser fiziologic cald, cu flux TIMI 3 şi cu *blush*; această situație a fost încadrată ca fenomen de *no-reflow* reversibil terapeutic, evoluția pacientei fiind in final excelentă.

Un singur caz, diagnosticat cu ADA cu stenoză ostială 70-80% şi ocluzie ADA în segmentul I (bicoronarian), a prezentat flux distal TIMI 1 fără *blush*. Toate manevrele aplicate nu au condus la o îmbunătățire a fluxului, situația fiind încadrată ca fenomen de *no-reflow* ireversibil, cu evoluție nefavorabilă.

b) Dovezi alternative ale reluării fluxului coronarian prin ACTP primară: evoluția modificărilor electrocardiografice

Rezultatele analizei modificărilor electrocardiografice evolutive, cu referire la rezoluția supradenivelării de segment ST, ca indicator al reluării fluxului coronarian, sunt prezentate în tabelul I.

Caracteristicile electrocardiografice evolutive, analizate la 12 din cei 14 pacienți pentru care ACPT primară a fost practicată precoce (în primele 6 ore) au evidențiat:

- rezoluția completă a sumST (suma supradenivelărilor de segment ST din toate derivațiile) pentru supradenivelare, considerată la ≥ 70%, la 4 pacienți, iar pentru subdenivelare la 2 pacienți, dintre care doar unul se încadra la rezoluția completă a supradenivelării; dintre aceştia, doar unul a prezentat arteră patentă;

- rezoluție parțială a sumST pentru supradenivelare, considerată între 30-70%, la 8 pacienți, 3 prezentând arteră patentă;

- rezoluția slST (supradenivelarea de segment ST dintr-o singură derivație – "single lead") completă (considerată ≥ 70%) la 3 pacienți, parțială (considerată între 50-70% pentru IMA anterioare şi între 20-70% pentru cele inferioare) la 9 pacienți.

TABELUL I

Evoluția modificărilor electrocardiografice

(* indică pacienții cu arteră patentă la prezentare)

Caz	Timp (ore)	Segment ST supr_deniv (mm)	SumST supr_deniv (%)	SumST sub_deniv (%)	slST	Segment ST supr_deniv (mm) max (60 min postACTP)
1	5	6	64 %	90 %	67 %	2
2*	2,5	3	30 %	absentă	33 %	2
3	6	5	78 %	absentă	60 %	2
4*	4	absentă	-	absentă	-	Absentă
5	3	2	75 %	absentă	50 %	1
6	8	2	63 %	43 %	33 %	0,5
7*	2	5	50%	absentă	40 %	2
8	1	6	50 %	absentă	50 %	3
9*	3	2,5	60%	absentă	60 %	1,5
10	7	6	40 %	63 %	50 %	3
11*	1	2	70 %	81 %	67 %	1
12	12	5	60%	absentă	60 %	3
13	11	0,5	63 %	58 %	50 %	0,5
14	6	10	63 %	absentă	60 %	4
15	7	1	100 %	absentă	100 %	izoelectric
16	3,5	6	89 %	40 %	67 %	2
17*	12	1	100 %	absentă	100 %	izoelectric
18	3	11	60 %	absentă	73 %	3
19	2	3	33 %	54 %	75 %	1,5
20	8	5	!	absentă	!	7
21	3	0,5	echivoc	-	-	izoelectric
22	8	4	30 %	absentă	50 %	2

Supradenivelarea maximă de segmentului ST la 60 de minute postACTP, pentru IMA anterioare a permis încadrarea pacienților în grupele de risc mic – 2 cazuri (un caz cu arteră patentă), risc mediu – 3 cazuri (un caz cu arteră patentă), risc mare – 2 cazuri, iar pentru IMA inferioare ca grupe de risc mic – 2 cazuri (un caz cu arteră patentă), şi risc mediu – 3 cazuri (un caz cu arteră patentă).

Caracteristicile electrocardiografice evolutive, analizate la 8 pacienti pentru care ACPT primară a fost practicată tardiv (6-12 ore) au evidențiat:

- menținerea constantă a supradenivelării de segment ST şi aspect subacut la 9 zile pentru un caz, încadrat ca *no-reflow* ireversibil (necroza fiind deja constituită);

- reducerea supradenivelării de segment ST pentru celelalte 7 cazuri, astfel:

- rezoluția completă a sumST pentru supradenivelare a fost întâlnită la 2 pacienți (doar unul cu arteră patenta);

- rezoluția parțiala a sumST pentru supradenivelare a fost prezentă la 5 pacienți;

- rezoluția slST completă la 2 pacienți, parțială la 5 pacienți (în corespondență cu

sumST);

Supradenivelarea maximă de segment ST la 60 de minute postACTP, pentru IMA anterioare, a permis încadrarea pacienților în grupa de risc mediu – 2 cazuri (1 caz cu arteră patentă), iar pentru IMA inferioare ca grupe de risc mic – 4 cazuri (1 caz cu arteră patentă), şi risc mare – 1 caz.

DISCUȚII

Evaluarea *in vivo* a modificărilor morfologice consecutive stresului oxidativ, în corespondență cu fenomenul de reperfuzie miocardică, este extrem de dificil de realizat. Din acest motiv, am analizat posibilitatea de a utiliza o serie de metode de tip paraclinic, care pot furniza un set de dovezi funcționale – numite dovezi "surogat" - asupra fenomenului de reperfuzie eficientă, sau a leziunilor de reperfuzie.

Fluxul TIMI şi MBG (12), deşi metode subiective, sunt curent utilizate pentru definirea reperfuziei eficiente. Definiția angiografică a reperfuziei este reprezentată de asocierea TIMI 3 cu MBG 2-3; fluxul TIMI 3 este necesar, dar nu garantează reperfuzia optimă. TIMI 0-3 şi MBG 0-1 definesc fenomenul de *no-reflow*, TIMI 0-2 sugerează *no-reflow*, care însă poate fi prezent şi la TIMI 3, MBG 0-1 fiind elementul sugestiv pentru această complicație (observat în 50% din cazurile cu TIMI 3). Studiile axate pe investigarea fenomenului de reperfuzie miocardică raporteaza un prognostic foarte bun pentru MBG 2-3 şi rezoluție de segment ST > 70%, rezervat pentru MBG 0-1 şi rezoluție de segment ST < 70%, şi intermediar în rest; asocierea TIMI 3 cu MBG 0-1 aduce o mortalitate de 7 ori mai mare decât cu MBG 2-3 (6, 11, 15, 16, 17, 18). Leziunea de *no-reflow*, complicație a tratamentului de reperfuzie miocardică, este un termen utilizat în literatură pentru a descrie lipsa fluxului eficient în artera coronară responsabilă de infarct după deschiderea acesteia, fiind definit angiografic ca flux TIMI 0-3 cu MBG 0-1. În patogeneza sa multifactorială sunt implicate stresul oxidativ, prin leziunile de ischemie şi reperfuzie, emboliile distale, predispoziția individuală. Acest fenomen poate fi reversibil spontan sau terapeutic, sau susținut şi ireversibil, probabil funcție de reversibilitatea sau ireversibilitatea modificărilor microcirculației (11).

În lotul studiat, s-a practicat ACPT primară cu implantare de stent, şi s-a obținut ca rezultat final flux TIMI 3 cu *blush* miocardic şi fără *no-reflow* (MBG 2-3) în 21 cazuri (95,45%), din care 18 cazuri fără incidente intervenționale, pe care le-am apreciat ca reperfuzie eficientă (81,81%). Celelalte 4 cazuri (18,19%) au evoluat cu complicații ale tratamentului de reperfuzie, constând în fenomen de *no-reflow*. Aceste cazuri au fost definite, ca urmare a posibilităților de identificare angiografică a cauzelor de *no-reflow*, după cum urmează: 2 cazuri au prezentat embolii distale în LAD apical; un alt caz a evoluat cu obținerea unui flux inițial TIMI 1 fără *blush* (*no-reflow*) şi cu flux TIMI 3 şi cu *blush* după administrare intracoronariană de adenozină, nitroglicerină şi ser fiziologic cald - fenomen de *no-reflow* reversibil terapeutic. Un ultim caz a prezentat flux distal TIMI 1 fără *blush* în ciuda manevrelor folosite – fenomen de *no-reflow* ireversibil.

Interpretând aceste rezultate inclusiv prin rolul potențial al stresului oxidativ în leziunea de reperfuzie miocardică, putem considera că situațiile cu fenomen de *no-reflow*, reversibil şi ireversibil, au asociat un stres oxidativ important.

Analiza modificărilor electrocardiografice în dinamică constituie, prin informații-

le puternic obiective oferite, un instrument simplu dar, concomitent, extrem de adecvat pentru aprecierea eficacității reperfuziei şi implicit a prognosticului (15).

Rezoluția supradenivelării de segment ST la 60 de minute de la ACPT, măsurată la 20 de msec de punctul J, se corelează cu salvarea de miocard, permite stratificarea pacienților pe grupe de risc (mic, moderat sau intermediar, şi mare), cel mai facil pentru maxST, şi integrează perfect perfuzia miocardică şi tisulară. Acuratețea predictivă maximă este oferită de maxST, apoi de slST, şi cea minimă – de Sum ST. Astfel, dacă se interpretează SumST şi slST cu rezoluție completă şi maxST cu risc mic, există o probabilitate de > 90% ca artera responsabilă de infarct să fie patentă şi asociată cu perfuzie tisulară, iar pentru rezoluție absentă (SumST şi slST) şi maxST la risc mare, 50% au încă arteră patentă, dar fără perfuzie tisulară (11, 13, 14, 19, 20).

Rezoluția subdenivelărilor de segment ST (a modificărilor ECG reciproce, indirecte) aduce informații prognostice independente ce sunt incrementale cu cele reprezentate de supradenivelarea de segment ST (15).

Pe lotul studiat, am realizat o comparație a modificărilor electrocardiografice evolutive între pacienții pentru care ACPT primară a fost practicată precoce şi, respectiv, tardiv. În baza datelor furnizate prin evaluarea diferențelor de supradenivelare a segmentului ST, respectiv subdenivelare, putem aprecia că reperfuzia intervențională poate fi tradusă prin informații electrocardiografice. Aceste informații susțin eficacitatea ACPT primare pentru salvarea de miocard şi stratificarea riscului. Rezultatele favorabile sunt dependente de timpul de prezentare, şi independente de patența arterei afectate. Consecutiv, putem aprecia că modificările electrocardiografice evolutive pot fi considerate ca dovezi indirecte pentru leziunile produse prin stres oxidativ indus de reperfuzia miocardică, în condițiile în care intensitatea şi severitatea lor este dependentă şi de factorul timp.

CONCLUZII

Evaluarea prin dovezi alternative a reperfuziei miocardice oferă o serie de informații asupra modului de restabilire a integrității funcționale a miocardului, fără însă a permite o analiză a substratului biochimic şi morfologic al evoluției leziunilor, sub raportul intervenției stresului oxidativ. Studiul aprofundat al dinamicii modificărilor induse de stresul oxidativ, la nivel structural, impune, în condițiile particularității miocardului, un model experimental.

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IMPLANTUL COHLEAR BINAURAL – OPŢIUNE AVANTAJOASĂ ÎN TRATAMENTUL SURDITĂŢII PROFUNDE NEURO-SENZORIALE

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BINAURAL COCHLEAR IMPLANT – GOOD CHOICE IN THE TREATMENT OF DEEP SENSORINURAL HEARING LOSS (Abstract). Nowadays, cochelar implant is the only choice of treatment in deep bilateral deafness. **Aim:** To evaluate the benefits of binaural implantation of a single receiver-stimulator device by using audiometric tests at 3 and 6 months intervals. **Material and methods:** The study included 3 patients with bilateral sensorineural deafness of cochlear origin. Following implantation they were assessed by pure tone audiometry (PTA), speech audiometry (SA), and sound localization tests (LT). **Results:** PTA showed a significant decrease in hearing thresholds level during the 6-month interval following implantation. As to the degree of speech discrimination in both quiet and noisy environment, SA showed evident progress at 6 months compared to the results at 3 month. The location tests revealed improved sound localization in both ears at 6 months compared to the results at 3 months. **Conclusions:** Compared to unilateral implant, the binaural cochlear implant has the advantage of stereophonic hearing, facilitating the communication through a better understanding of speech in both quiet and noisy environment, and improving the quality of life by the location of sounds similar to natural hearing. **Key words:** BINAURAL COCHLEAR IMPLANT, SENSORINEURAL HEARING LOSS, AUDIOMETRIC TESTS

Implantul cochlear reprezintă metoda de tratament a surdităţii profunde bilaterale de câteva decenii. Conceptul implantării bilaterale a apărut odată cu rezultatele studiilor comparative efectuate la pacienţii cu auz normal sau cu proteze auditive convenţionale versus implant unilateral (monoaural), când au fost evidenţiate dezavantajele audiţiei monoaurale în viaţa de zi cu zi (1).

Totodată, mai multe colective de cercetare au dovedit şi au descris fenomenele de maturare neurologică şi de plasticitate a sistemului neural auditiv, fenomene posibile numai sub stimulare sonoră caracteristică (2, 3, 4). Astfel a apărut conceptul implantării bilaterale cu efectul său tehnologic, dezvoltarea unui sistem de implant cohlear binaural.

Implantarea bilaterală este posibilă, însă puţin probabilă în condiţiile din România datorită costului ridicat. Implantarea binaurală, adică folosirea unui singur dispozitiv pentru ambele urechi reprezintă o soluţie posibilă în cazul pacienţilor adulţi cu surditate bilaterală neuro-senzorială profundă, cu costuri mai mici. Singurul produs de

acest tip de pe piaţă este Implantul Cochlear Binaural Digisonic SP.

Implantarea cochleară binaurală, reprezintă o premieră în România, fiind realizată în Clinica ORL din Spitalul Clinic de Recuperare Iaşi, motiv pentru care prezentăm avantajele acestui tip de implant faţă de implantarea cochleară monoaurală.

CONSIDERAŢII ASUPRA IMPLANTULUI BINAURAL

1. Audiţia binaurală normală

La subiecţii cu auz binaural normal, spre deosebire de auzul monoaural, există un efect de creştere a senzaţiei de tărie sonoră cu 3 dB la nivelul pragului şi cu 10 dB la peste 30 dB, creştere esenţială pentru localizarea sursei sunetelor în spaţiu, pentru supresia ecourilor şi pentru auzul în mediul cu zgomot. Avantajele audiţiei binaurale se transformă în impedimente când este necesară testarea fiecării urechi în parte: fenomenul de transcraniere (capacitatea sunetului prezentat urechii testate de a se propaga prin craniu până la urechea opusă), poate conduce la erori audiometrice, în special în audiometria subiectivă. Datorita dezvoltării metodelor de mascare a urechii contralaterale celei testate, acest impediment a fost minimizat.

2. Audiţia binaurală prin implantul cochlear

Implantarea binaurală reprezintă o soluţie posibilă în cazul pacienţilor adulţi cu surditate bilaterală neuro-senzorială profundă, cu costuri mai mici. Implantarea cochleară binaurală prezintă faţă de implantarea cochleară unilaterală avantajele audiţiei stereofonice facilitând întelegerea şi localizarea sunetului mai bine şi mai natural (5, 6).

Considerăm acest tip de implant cochlear o alternativă terapeutică de interes major cu impact important în viaţa socială a pacientului reprezentând un factor de prognostic favorabil în evoluţia audiţiei pacientului. Singurul produs de acest tip de pe piaţă este implantul cochlear Digisonic SP Binaural (fig. 1).

Fig. 1. Implant cochlear *Digisonic SP Binaural*

Studiul îşi propune evaluarea avantajelor implantării binaurale şi monitorizarea, înregistrarea şi analizarea evoluţiei în timp a audiţiei tonale şi a perceperii vorbirii în mediul liniştit şi în zgomot, în corelaţie cu fenomenele de plasticitate cerebrală şi de adaptabilitate a pacienţilor la mediul sonor. În consecinţă, aceste avantaje conduc inerent la îmbunătătirea calităţii vieţii pacienţilor implantaţi.

MATERIAL ŞI METODĂ

Am inclus în studiu 3 pacienţi cu hipoacuzie neuro-senzorială bilaterală de origine cochleară şi cu inteligibilitate mai mică de 50% la 60 dB SPL cu proteză auditivă şi fără labiolectură.

Criteriile de excludere au fost următoarele: vârsta mai mică de 18 ani şi mai mare de 70 de ani, pacienţi pe care nu avem posibilitatea de a-i urmări în timp, pacienţi cu stare generală alterată, cu deficite neurologice anterioare, probleme ale scalpului,

alexie, infecţii ale urechii medii, durata surdităţii profunde a urechii celei mai afectate mai mare de 20 de ani şi diferenţa de durată a hipoacuziei profunde între cele două urechi mai mică de 20 de ani.

Pacienţii au fost implantaţi cu implantul cochlear tip Digisonic SP Binaural. Acesta este constituit din două părţi: partea externă şi partea implantabilă sau receptor-stimulatorul. Partea externă constă dintr-un singur procesor de sunet dotat cu microfoane ipsilaterale, la care se conectează microfonul auxiliar contralateral. Informaţia este transmisă părţii interne printr-o antenă sub formă de unde radio. Receptor stimulatorul este conectat la un port-electrod scurt pentru cochleea homolaterală şi un port-electrod lung pentru cochleea contralaterală (realizat special pentru a ajunge pe sub scalp de la o mastoidă la cealaltă) (fig. 2).

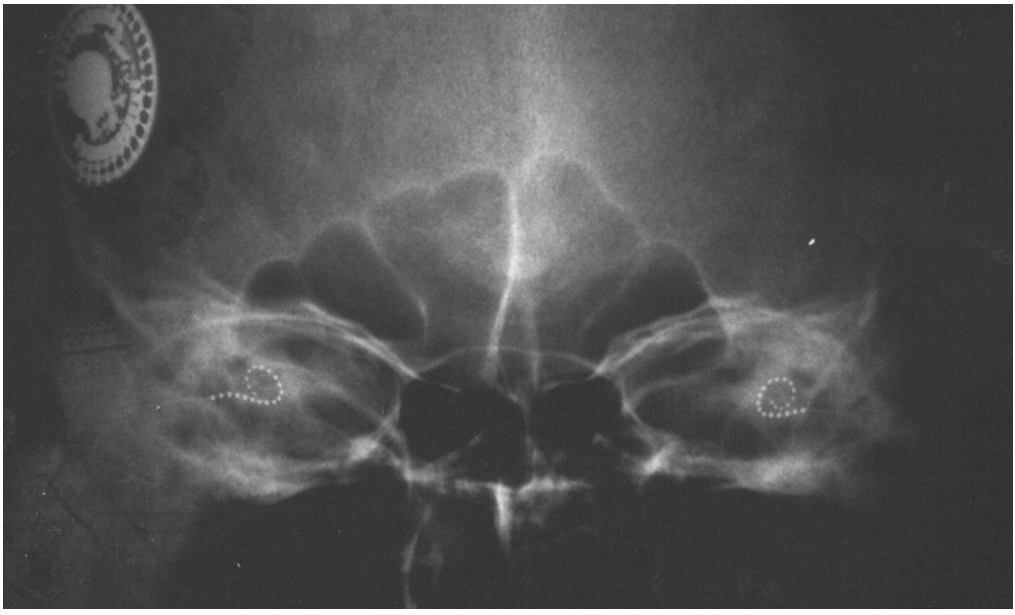

Fig. 2. Aspect imagistic al poziţionării implantului binaural (receptor-stimulator şi cei doi portelectrozi intracochleari).

Acest implant are câte 12 canale pe fiecare port-electrod. La fiecare port-electrod este conectat un electrod de masă. Procesorul este în totalitate digital şi permite flexibilitate în ajustarea parametrilor de stimulare prin intermediul softului de reglaj.

Receptor-stimulatorul unic este controlat printr-un procesor retroauricular care primeşte informaţii din ambele hemicâmpuri auditive graţie microfoanelor ipsilateral şi contralateral. Procesorul de sunet este capabil sa prelucreze 2 semnale stereo concomitent, procesarea stânga - dreapta putându-se realiza independent.

Decalajul temporal dintre cele două urechi este asemănătoare cu cel natural şi corespunde distanţei necesare sunetului de a ajunge la microfonul ipsilateral şi respectiv controlateral. Cel mai mare avantaj faţă de implantarea bilaterală îl reprezintă stimularea cu un singur procesor şi astfel se obţine o mai bună precizie temporală între

cele două urechi.

În decursul studiului s-au efectuat mai multe teste audiologice conform protocolului stabilit: pre-operator, la data activării, la 3 luni şi ultimele la 6 luni de la activare. Testele de evaluare au durat în medie o oră şi jumătate pe şedinţă şi au fost aplicate pentru testarea audiţiei monoaurale pe fiecare ureche, iar apoi pentru testarea audiţiei binaurale. Acestea s-au desfăşurat în incintă cu atenuare fonică, folosind echipament calibrat (audiometru Interacoustics, preamplificator, sistem de 5 boxe amplasate în semicerc la fiecare 45°, sistem de reglaj al implantului). Testele folosite sunt descrise mai jos.

Ca prim test s-a realizat audiometria tonală liminară pentru urechea dreaptă, urechea stângă şi ambele urechi. Se determină pragurile auditive la stimularea în câmp liber pe frecvenţele de 250, 500, 1000, 2000 şi 4000 Hz. Rezultatul ATL în câmp liber permite stabilirea performanţei auditive a tonurilor pure prin implantul cohlear.

Un al doilea test folosit a fost audiometria vocală. Prin prezentarea unor liste adaptate de câte 10 cuvinte bisilabice standardizate la intensităţile de 70 şi 80 dB SPL, s-a evaluat performanţa pacientului în recunoaşterea limbajului atât în mediul liniştit, cât şi în mediul cu zgomot. S-a înregistrat curba de inteligibilitate vocală, identificându-se pragul de inteligibilitate (intensitatea la care subiectul a recunoscut 50% dintre cuvintele prezentate) (9).

Deoarece este vorba despre pacienţi la care, prin implantul binaural, s-a restaurat audiţia stereofonică, după activarea implantului am urmărit evoluţia performanţelor pacienţilor privind identificarea localizării susei sonore. Testele au fost efectuate în cabina izolată fonic, utilizând sistemul de 5 boxe amplasate conform figurii 3. Distribuţia spaţială a boxelor are ca scop identificarea de către pacient a direcţiei de unde sunt emise sunetele. Boxele sunt calibrate, iar semnalul este emis prin intermediul unui amplificator de semnal etalonat, conectat la audiometru. Stimulul este un zgomot alb prezentat la intensitatea de 65dB SPL. Pentru fiecare pacient a fost folosit un algoritm de lucru care a cuprins câte 10 teste pentru fiecare boxă, emise aleatoriu, deci în total 50 de stimuli pentru care pacientul a trebuit să identifice direcţia din care a fost prezentat.

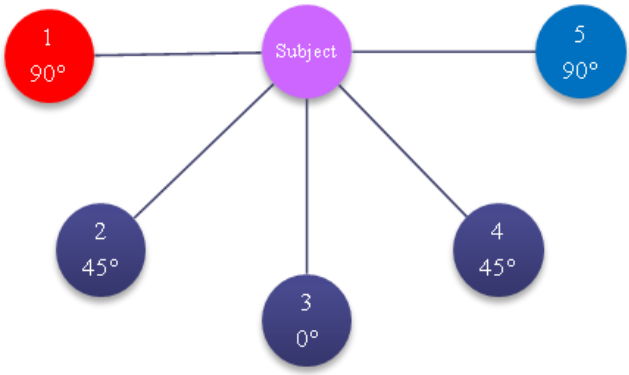

Fig. 3. Distribuţia spaţială a boxelor în câmp liber

REZULTATE ŞI DISCUŢII

În cazul ATL efectuate la cei 3 pacienţi incluşi în studiu se poate observa o scădere semnificativă a valorii pragurilor auditive înregistrate de la activare până la 6 luni după aceasta, deci o îmbunătăţire a percepţiei tonale atât pentru fiecare ureche în parte (dreapta-roşu, stânga – albastru), dar mai ales pentru auzul binaural (verde) (fig. 4).

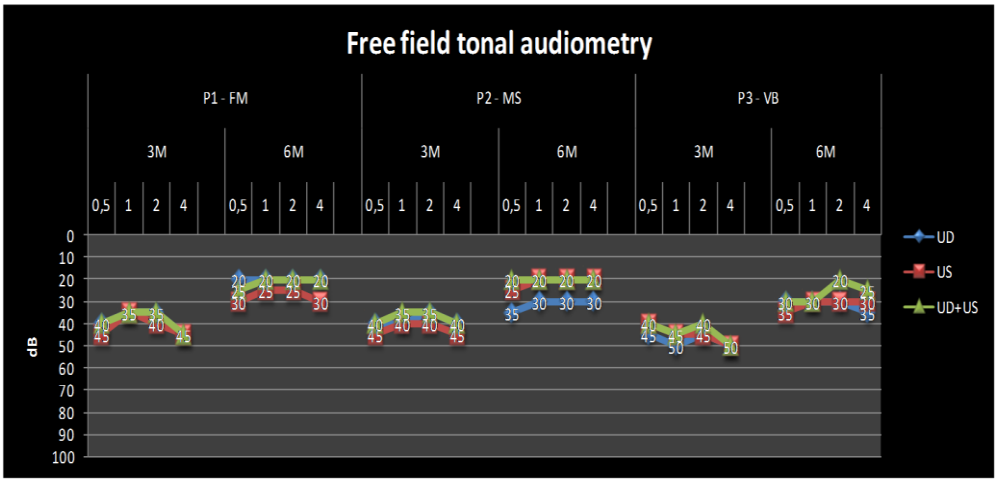

Fig. 4. Audiogramă tonală liminară

Putem observa că nivelul de audiţie în momentul testării ambelor urechi este pentru primele 2 cazuri testate (P_1 şi P_2) la nivelul maxim de audiţie pe care l-a înregistrat pacientul cu oricare ureche testată individual, iar în cazul pacinetului P_3 pragurile auditive pentru cele 2 urechi testate concomitent (la 6 luni) sunt chiar mai scăzute (pentru frecventele 2.000Hz şi 4.000Hz) decât pragurile înregistrate pe oricare ureche testată individual, deci auzul s-a îmbunătăţit considerabil de la sesiunea de reglaj şi control la 3 luni de la implantare şi apoi la 6 luni la toţi pacienţii.

În cazul discriminării vorbirii la 60 şi 70 dB SPL în linişte, rezultatele înregistrate la 6 luni de la activare comparativ cu cele înregistrate la 3 luni de la activare sunt similare pentru primul pacient (P_1). Putem observa o recunoaştere mai bună a cuvintelor la 60 dB SPL la testarea auzului binaural decât testarea pentru fiecare ureche în parte. În schimb pacientul P_2 a prezentat o îmbunătăţire semnificativă la 6 luni a recunoaşterii cuvintelor atât pentru fiecare ureche în parte, dar mai ales în testarea binaurală. De asemenea, gradul de recunoaştere a cuvintelor la testarea ambelor urechi concomitent a fost mai mare decat în cazul testării fiecărei urechi în parte. Acest tipar de evoluţie este asemănător cu majoritatea cazurilor implantate binaural pe care le-am analizat în comunicările din literatura de specialitate (10, 11). La ultimul pacient testat s-a înregistrat o îmbunătăţire a audiţiei pentru urechea dreapta de la 3 luni la 6 luni însă fară un progres evident pentru urechea stângă. Performanţa testării binaurale la 3 luni a fost la nivelul audiţiei înregistrate cu urechea dreaptă la acelaşi moment. La testarea ambelor urechi la 6 luni la 60 dBSPL

s-a înregistrat o ameliorare a gradului de recunoaştere a cuvintelor, dar nu până la nivelul atins de urechea dreaptă, iar pentru intesitatea de 70 dBSPL s-a înregistrat chiar o scădere a gradului de recunoaştere a cuvintelor, ajungâdu-se la nivelul audiţiei urechii stângi (fig. 5).

Putem observa că la testarea de la 6 luni după momentul activării, în cazul tuturor pacienţilor, se înregistrează apariţia fenomenului de recruitment prin scăderea gradului de recunoaştere a cuvintelor la intensitatea de 70 dB faţă de 60 dB. În cazul testării la 3 luni putem observa fenomenul de recruitment pentru pacientul P_1 pentru fiecare testare în parte iar pentru pacientul P_2, fenomenul a apărut doar în momentul testării separate a fiecărei urechi.

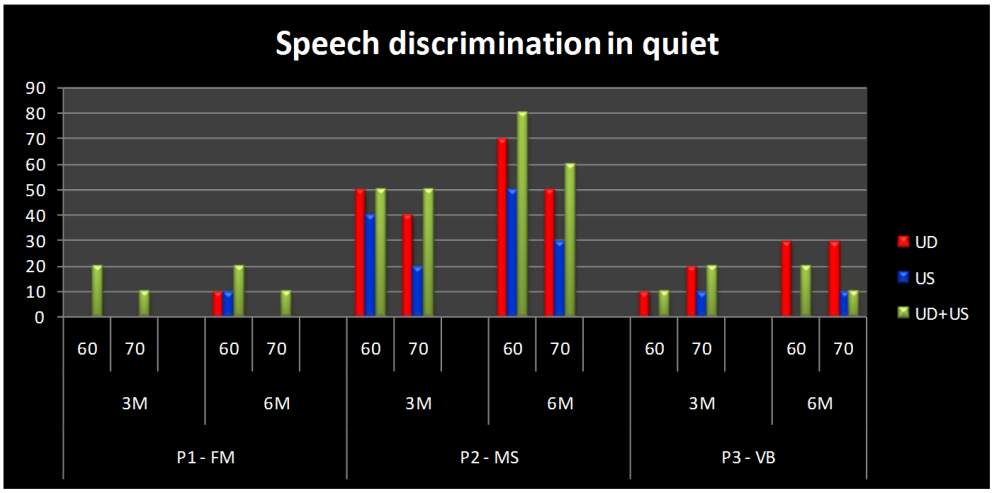

Fig. 5. Audiogramă vocală în linişte

Pentru audiograma vocală în mediul cu zgomot (zgomot alb cu intensitatea de 60 dB) pentru discriminarea vorbirii la 60 şi 70 dB SPL putem observa lipsa răspunsului şi la 3 luni şi la 6 luni din partea pacientului P_1. Pentru pacientul P_2 se poate observa o audiţie binaurală la fel de bună ca şi pentru cea mai bună ureche testată (urechea dreaptă) la 3 luni după activare şi o îmbunătăţire a gradului de recunoaştere la 6 luni, comparativ cu testarea la 3 luni, pe fiecare ureche individuală testată cât şi pentru ambele urechi testate concomitent. În schimb audiţia binaurală la 6 luni a înregistrat o îmbunătăţire a gradului de recunoaştere faţă de urechea cea mai bună testată (urechea dreaptă) atât pentru intesitatea de 60 dBSPL cât şi pentru 70 dBSPL. În cazul acestui pacient am observat apariţia fenomenului de recruitment la testarea de la 3 luni după activarea implantului cochlear binaural pentru urechea dreaptă.

Pentru pacientul P_3 s-a înregistrat o îmbunătăţire a gradului de recunoaştere la 6 luni comparativ cu testarea la 3 luni pentru audiţia cu ambele urechi concomitent şi o staţionare pentru urechea stângă pentru ambele intesităţi testate. Pentru urechea dreaptă nu s-a obţinut răspuns la 60 dBSPL pentru ambele momente de testare însă s-a înregistrat o îmbunătăţire la 6 luni pentru intesitatea de 70dB SPL, gradul de recunoaştere fiind chiar mai mare decât în cazul audiţiei binaurale (fig. 6).

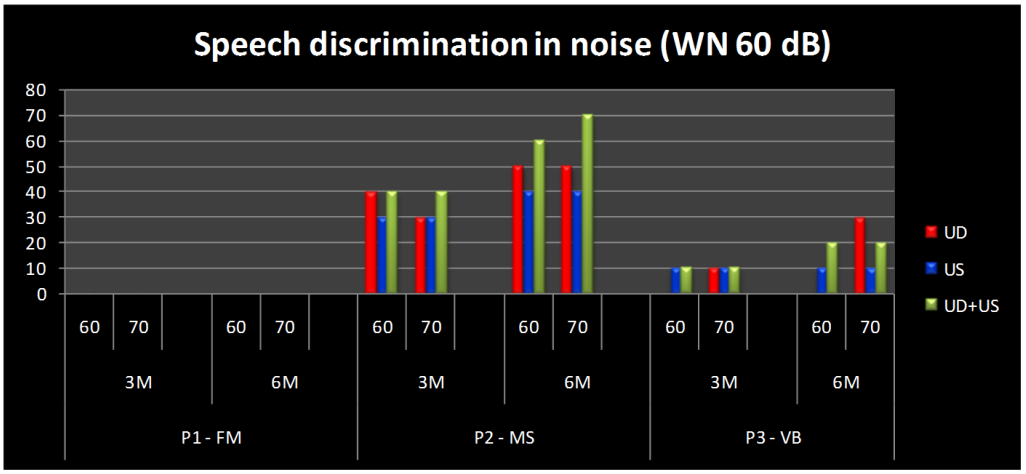

Fig. 6. Audiogramă vocală în mediu cu zgomot

În cazul testelor de localizare cu 5 boxe şi zgomot alb de 65 dBSPL s-a observat o bună localizare a originii sunetului spre dreapta în momentul stimulării urechii drepte şi o bună localizare a originii sunetului spre stânga în momentul stimulării urechii stângi (fig. 7).

Pentru stimularea binaurală localizarea apare cu precizie mai mică decât în cazul urechilor stimulate individual pe partea corespunzătoare fiecăreia, însă cu rezultate bune pentru localizarea laterală de fiecare parte. Pentru sunetele prezentate din boxa frontală localizarea a fost slabă, dar mai precisă decât în cazul stimulării individuale pe fiecare ureche. S-a observat o îmbunătăţire în ceea ce priveşte localizarea sursei zgomotului în audiţia binaurală de la testarea iniţială după 3 luni de la activare şi testarea la 6 luni de la activare în cazul tuturor pacienţilor testaţi. Imbunătăţirile s-au înregistrat atât pentru localizarea boxelor laterale dar mai ales pentru localizarea boxelor din regiunea centrală.

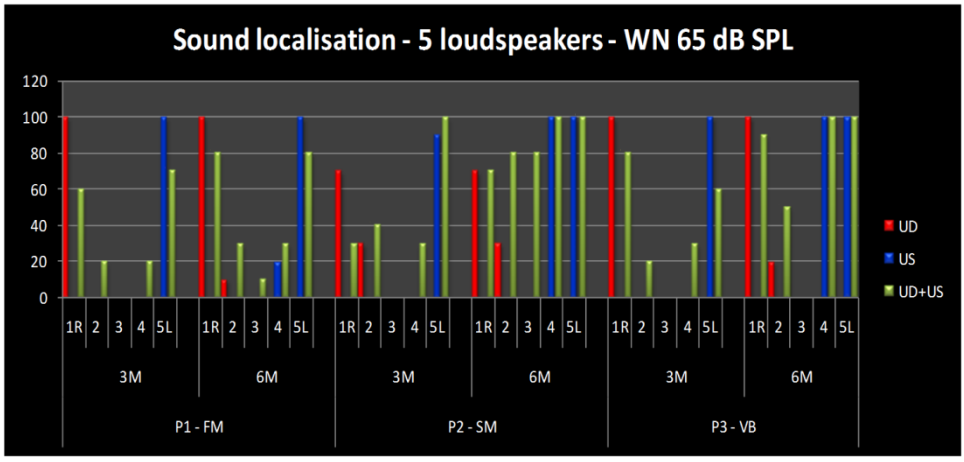

Fig. 7. Localizarea sursei sunetului

Din datele înregistrate în cursul studiului la audiograma tonală liminară am observat că auditia binaurală permite detecţia unor praguri mai scăzute sau cel puţin egale cu pragurile obţinute în cazul audiţiei monoaurale şi am notat o evoluţie în timp spre scăderea pragurilor auditive de la 3 luni la 6 luni. De asemenea am notat un progres la testarea de la 6 luni faţă de testarea de la 3 luni după activare în ceea ce priveşte gradul de discriminare a vorbirii atât în linişte cât şi în mediul cu zgomot în cazul testării audiţiei binaurale pentru pacienţii P_2 şi P_3.

Pentru testele de localizare s-a înregistrat o îmbunătăţire a localizării originii zgomotului (atât pentru boxele laterale dar mai ales pentru boxele centrale) în audiţia binaurală la testarea de la 6 luni faţă de testarea de la 3 luni după activare în cazul pacienţilor testaţi. Acest fapt indică o evoluţie în timp spre audiţia cât mai apropiată de cea naturală stereofonică şi se poate explica prin fenomenele de plasticitate neuronală şi de adaptabilitate a fiinţei umane la o nouă formă de percepere a mediului sonor.

Implantul cohlear binaural fiind constituit dintr-un singur procesor de sunet, un singur receptor-stimulator şi de 2 port-electrozi, reprezintă o alternativă mai puţin costisitoare, cu o mai bună precizie temporală între cele 2 urechi şi cu un timp chirurgical mai scurt decât implantarea bilaterală. În schimb, în cazul în care s-ar produce o defecţiune a receptor-stimulatorului, pacientul şi-ar putea pierde complet capacitatea de a auzi.

Implantarea cochleară binaurală prezintă avantajele audiţiei stereofonice facilitând întelegerea şi localizarea sunetului mai bine şi mai natural.

CONCLUZII

În tratamentul hipoacuziilor neuro-senzoriale profunde şi unele hipoacuzii severe, metodele de protezare convenţională nu pot fi utilizate. În aceste cazuri soluţia terapeutică o reprezintă implantul cochlear. Implantarea binaurală prezintă faţă de implantarea unilaterală avantajele audiţiei stereofonice, facilitând comunicarea printr-o mai bună înţelegere a vorbirii în linişte şi în mediul cu zgomot şi îmbunătăţind calitatea vieţii oferind posibilitatea localizării surselor sonore asemănător auzului natural.

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REZOLUȚIA INFLAMAȚIEI - IMPLICAȚII FARMACOLOGICE

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RESOLUTION OF INFLAMMATION - PHARMACOLOGICAL ASPECTS (Abstract): Inflammation is a fundamental biologic process evolutionally preserved by a germ line code. The interplay of the epigenetic with the environment directs the code to temporally distinct inflammatory responses, which can be acute or chronic. The aim of this study is to present new aspects regarding the resolution of inflammation**.** Acute inflammation normally resolves by mechanisms still somewhat elusive. Current evidence suggests that an active coordinated program initiated the first few hours after the inflammatory response begins and its failure lead to chronic inflammation. This process is essential for appropriate host responses, tissue protection and the return to homeostasis. Prostaglandins and leukotrienes are lipid mediators that play important roles in host defense and acute inflammation. Granulocytes promote the switch of arachidonic acid–derived prostaglandins and leukotrienes to lipoxins, active anti-inflammatory and pro-resolution mediators. The apoptosis of the neutrophils coincides with the biosynthesis of resolvins and protectins from omega-3 polyunsaturated fatty acids and releases anti-inflammatory and reparative cytokines. This information could lead to new treatments for inflammatory diseases**. Key words:** INFLAMMATION, RESOLUTION, MEDIATOR, ARACHIDONIC ACID

Termenul de ***inflamație*** defineşte un proces protectiv complex de răspuns tisular inițiat secundar acțiunii unor factori mecanici, chimici, fizici, bacterieni, ce au drept rezultat lezarea sau chiar apoptoza celulară (1). Lezarea celulară determină eliberarea de mediatori de tip inflamator, în categoria cărora sunt incluse citokinele, TNF-alfa, interleukinele, leucocitele, monocitele sau macrofagele (2). Actualmente se consideră că citokinele intervin în stimularea chemokinelor, imunoglobulinelor, altor citokine pro-inflamatorii şi în stimularea expresiei moleculelor de adeziune (3). **Procesul de rezoluție a inflamației** este reprezentat de mecanismele endogene antiinflamatorii care protejează organismul împotriva unor leziuni inflamatorii exagerate şi stimulează procesele de reconstrucție tisulară postinflamatorie (4, 5).

1. INFLAMAȚIA ACUTĂ

Inflamația acută, caracterizată prin recrutarea leucocitelor din circulație, se defineşte clasic printr-un mecanism de inițiere a unui trafic de PMN (polimorfonucleare neutro-

file), urmat de monocite, care se diferențiază ulterior pe plan local în macrofage. Invariabil, acest răspuns este declanşat de mastocitele tisulare şi macrofagele rezidente, a căror degranulare şi activare eliberează secvențial mediatori inflamatori, inclusiv amine bioactive (histamina şi 5-HT), citokine, chemokine precum şi mediatori lipidici care recrutează şi activează celulele inflamatorii, determinând formarea edemului (6).

Răspunsul inflamator se caracterizează prin activarea coordonată a diferitelor căi de semnalizare care reglementează expresia mediatorilor pro- şi anti-inflamatorii din celulele rezidente tisulare şi leucocitele recrutate. În prezent, argumentele privind semnalizarea inflamației derivă din studiile asupra membrilor familiei receptorilor interleukinei (IL) precum IL-1R, TNF şi TLR, care aparțin familiei IL-1R (7). IL-1 şi TNF alfa reprezintă citokine arhetipale pro-inflamatorii care sunt rapid eliberate la nivelul leziunii sau infecției tisulare (8) Căile de semnalizare proinflamatorie induc expresia paralelă de mediatori antiinflamatori (de exemplu, IL-10 (tab. I).

TABELUL I

Citokinele şi rolul lor în inflamație (5)

Citokine	Celula sursă	Celula țintă	Acțiune
Citokine proinflamatorii			
IL-1	Macrofagele Celulele dendritice	Limfocitele Celulele endoteliale Celulele din SNC Celulele hepatice	Stimularea răspunsului Activare celulară Febră, stare de rău Sinteza şi eliberarea proteinelor de fază acută
IL-6	Macrofagele Celulele dendritice Endoteliu Celule Th2	Celulele hepatice Celula B	Sinteza şi eliberarea proteinelor de fază acută Proliferare
TNF-alfa	Macrofagele Celulele dendritice Celule Th1	Celulele endoteliale Neutrofilelor Hipotalamusului Celulelor hepatice	Activarea endoteliului vascular prin creşterea permeabilității si stimularea moleculelor de adeziune Activare celulară Febră Sinteza şi eliberarea proteinelor de fază acută
Citokine antiinflamatorii			
IL-10	Macrofagelor Celulelor Th2	Macrofagelor Celulelor dendritice	Inhibă producerea de IL-12 Inhibă sinteza de citokine proinflamatorii
IL-12	Macrofage Celule dendritice	CD4 + celule T helper Celule NK	Diferențierea Th1 Sinteza IFN-gamma
Citokine implicate în apariția răspunsului imun			
IL-2	Celule T	Celule T Celule NK Celule B	Proliferare Activare şi proliferare proliferare
IL-4	Celule Th2 Celule mastocitare	Celule T Celule B Macrofage	Dezvoltarea celulelor Th2 Inhibă activarea IFN-gamma
IFN-gamma	Celule Th1 Celule T citotoxice Celule NK	Celule T Celule B Macrofage	Dezvoltarea celulelor Th2 Activare

Dispariția stimulului determinant al procesului inflamator acut inițiază inhibarea sintezei de mediatori proinflamatori (eicosanoizi, chemokine, citokine, molecule de adeziune, etc), stimularea catabolismului lor şi blocarea căilor de semnalizare proinflamatorie (tab. I). Astfel se opresc procesele de recrutarea de leucocite şi formare a edemului, etape care, asociate cu eliberarea factorilor care previn traficul PMN-urilor şi eozinofilelor, reprezintă "începutul sfârşitului" şi anume ***rezoluția răspunsului acut inflamator*** şi reîntoarcerea la homeostazie (9)

Eşecul rezoluției răspunsului acut inflamator determină apariția inflamației cronice.

2. REZOLUȚIA INFLAMAȚIEI

O serie de semnale endogene antiinflamatorii, de tipul corticosteronului, moderează gravitatea şi limitează durata fazei de debut. Pe parcursul progresiei procesului inflamator, acțiunea anumitor "semnale stop" pe o serie de "puncte de control" previne traficul leucocitar tisular suplimentar. Semnalele stop includ ***lipoxine, resolvine şi prostaglandine (PG) din seria D*** care deschid calea pentru migrarea şi diferențierea monocitelor în macrofage fagocitante cu rol în eliminarea celulelor moarte, şi care părăsesc ulterior *situs*-ul inflamator (10).

Lipoxinele

Literatura de specialitate (9) descrie trei căi majore de generare a lipoxinelor (LXA4, LXB4, epi-LXA4 şi epi-LXB4) pornind de la acidul arahidonic: calea 5 lipooxigenazei (eng. *5*-lipoxygenase - 5LO), calea 15 lipooxigenazei (15LO) şi calea epi-lipoxinelor declanşate de acidul acetil salicilic (ASA), care implică ciclooxigenaza 2 (eng. *cyclooxygenase-2* - COX2) şi 5-LO.

Expunerea la citokine induce expresia COX-2 în celulele endoteliale vasculare. Această moleculă este acetilată de ASA, iar activarea celulară prin receptorii membranari generează 15R-HETE, transformat de 5-lipooxigenaza (5-LO) neutrofilelor în 15-epi-lipoxine (fig. 1).

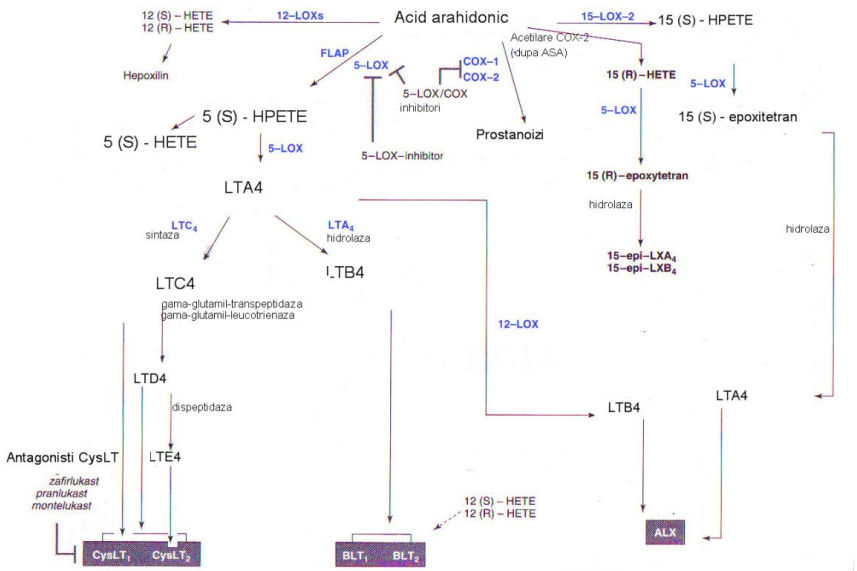

Fig. 1. Metabolismul acidului arahidonic (adaptat după Goodman **&** Gilman's)

ASA acetilează COX2 şi declanşează activitatea catalitică pentru transformarea acidului arahidonic în 15R-HETE (eng. *15-Hydroxyeicosatetraenoic acid* (**15-HETE**) *receptors*). 15R-HETE este eliberat din celulele endoteliale şi epiteliale şi transformat de 5-LO în lipoxine declanşate de ASA (ATL).

Lipoxinele, în special LXA4, constituie semnale puternice de contrareglare a unor mediatori endogeni proinflamatori, de tipul lipidelor (leucotriene, PAF) şi citokinelor (TNFα, IL-6), ce determină inhibiția inflamației dependentă de leucocite (11).

Rezolvinele şi protectinele

Studii recente au identificat noi mediatori generați din acizii graşi polinesaturați (eng. *polyunsaturated fatty acids* - **PUFA**) din dietă, cum ar fi **acidul eicosapentenoic (**eng. *eicosapentaenoic acid* - **EPA) şi acidul docosahexenoic (**eng. *docosahexaenoic acid* - **DHA)** (12). Aceşti compuşi, numiți rezolvine, docosatriene şi neuroprotectine, îndeplinesc rol antiinflamator şi au acțiuni prorezoluție, constituindu-se în inhibitori puternici ai migrării endoteliale a PMN-urilor şi ai expresiei citokinelor (13).

Compuşii derivați din EPA, care prezintă acțiuni biologice importante, sunt desemnați ca seria E, având în vedere precursorul lor EPA, notate şi ca **resolvine** din seria E (resolvine E1 sau RvE1), iar cele cu precursor DHA sunt rezolvine din seria D (resolvine D1 sau RvD1). Atât clasa de rezolvine D cât şi E sunt modulatori ai infiltrării PMN-urilor. Derivații bioactivi ai DHA, conjugați cu structuri de tip triene, sunt docosatrienele (eng. *docosatriene* – **DTS**), imunomodulatoarele şi neuroprotectoarele denumite **protectine/neuroprotectine.**

Având în vedere că aceşti mediatori sunt generați endogen în timpul unor intervale definite în timpul răspunsului inflamator, se poate concluziona că nu determină doar blocarea sau inhibarea inflamației, ci intervin în activarea rezoluției în exudatul inflamator, promovând întoarcerea la homeostazie.

Faza terminală a rezoluției

Faza terminală a rezoluției, constitutivă (mediată de PMN şi eozinofile) sau adaptativă (mediată de limfocitele), prezintă o serie de etape comune. La finalizarea inflamației acute, PMN-urile şi eozinofilele sunt eliminate într-o manieră controlată şi eficientă, proces denumit *moarte celulară programată sau apoptoză.*

Etapele parcurse de leucocitele inflamatorii includ recircularea sistemică sau moartea locală a PMN, eozinofilelor sau limfocitelor infiltrate, urmată de fagocitarea lor de monocitele derivate din macrofage. Odată fagocitoza finalizată, macrofagele pot părăsi țesutul inflamat pe cale limfatică, deşi o mică parte a acestora sunt distruse apoptotic la locul de fagocitoză (14).

Celulele stromale de tipul fibroblaştilor, contribuie la rezoluția inflamației prin retragerea semnalelor de supraviețuire şi normalizarea gradientului chemokinelor, permițând leucocitelor infiltrante să urmeze căile apoptotice sau să părăsească țesutul prin drenare limfatică (15, 16). Această secvență conduce la *rezoluție tisulară completă* şi, important, la restaurarea țesutului inflamat la starea de echilibru fiziologic.

3. IMPLICAȚII FARMACOTERAPEUTICE ALE FENOMENULUI DE REZOLUȚIE A INFLAMAȚIEI

Rezoluția inflamației acute cuprinde mecanisme endogene anti-inflamatoare care limitează prejudiciul excesiv tisular şi pro-

movează refacerea structurii şi funcţiei ţesutului. Rezoluţia este un proces activ, iar eşecul său conduce la iniţierea proceselor caracteristice inflamaţiei cronice. Descoperirea naturii active a procesului de rezoluţie a deschis modalităţi pentru o abordare nouă în terapia antiinflamatorie - iniţierea sau accelerarea rezoluţiei inflamaţiei acute, ca mecanism pentru tratamentul inflamaţiei cronice.

1. În timpul rezoluţiei inflamaţiei, neutrofilele activate trebuie eliminate în siguranţă prin apoptoză. Datorită implicării s**uperoxid-dismutaza (**eng. *superoxide dismutases* - **SOD)** în reglarea apoptozei neutrofilelor, s-a sugerat că ar putea fi utilizată ca agent terapeutic în tulburările inflamatorii. Tratamentul cu analogi SOD reduce producţia de citokine proinflamatorii, desfiinţează expresia moleculelor de adeziune celulară la suprafaţa celulelor endoteliale şi inhibă infiltrarea neutrofilelor. Analogii SOD sunt cunoscuţi în inhibarea producţiei de citokine, inclusiv a factorului de necroză tumorală alfa (TNF-a), interleukina (IL)-1b, şi IL-6, după cum se arată în modelele inflamatorii studiate (17).

Neutrofilele activate secretă specii reactive de oxigen (eng. *reactive oxygen species* - **ROS**) şi constituenţi primari granulari, iar acestea conduc uneori la prejudiciu tisular. Raportul oxidanţi/antioxidanţi este parţial implicat în patogenia inflamaţiei. În condiţii de stres oxidativ **superoxid dismutaza** acţionează ca un sistem de apărare celulară endogen, degradând superoxidul (O2) în oxigen şi peroxid de hidrogen, ca sunt ulterior detoxifiate de glutation peroxidază sau catalază. De aici derivă potenţialul terapeutic al SOD în tratamentul bolilor inflamatorii (18, 19).

2. Un alt pas important în procesul de rezoluţie îl reprezintă producerea de **mediatori lipidici pro-rezoluţie**, molecule mici care derivă din acizii graşi esenţiali omega-6 (acid arahidonic) şi omega-3 (acidul eicosapentaenoic şi acidul docosahexanoic), acizi graşi polinesaturaţi în alimentaţie. Primul agent dovedit a modula rezoluţia a fost **lipoxina**, o moleculă derivată din acidul arahidonic, apărută ca membru prototipic al lipidelor endogene/mediatori chimici activate în faza de rezoluţie a unui răspuns inflamator, şi care acţionează ca "semnalele stop" în inflamaţie (20, 21).

Alte două noi familii de mediatori lipidici recent identificate sunt **resolvinele** (produşi de interacţiune în faza de rezoluţie) şi **protectinele,** derivaţi din acizi graşi polinesaturaţi omega-3. Între aceşti controlori endogeni, căile lipooxigenazei şi ciclooxigenazei au funcţii protective şi proinflamatorii, cu rol precis pentru fiecare enzimă, exercitat în funcţie de stimuli, faza răspunsului inflamator acut (când aceste enzime sunt exprimate) şi momentul rezoluţiei (22).

În concluzie, rezolvinele şi DTS sunt familii noi de mediatori lipidici, cu structură unică şi proprietăţi antinflamatorii complementare ce pot înlătura inflamaţia şi prejudiciul mediat de PMN în multe boli umane (23, 24).

3. Cercetările recente au evidenţiat că **glucocorticoizii,** a căror proprietăţi antiinflamatorii erau bine cunoscute, influenţează mecanismele implicate în determinismul rezoluţiei, mai precis apoptoza (moartea celulară programată) la majoritatea leucocitelor şi stimulează *clearance*-ul celulelor apoptotice de către fagocite. *Clearance*-ul leucocitelor apoptotice poate fi un mecanism suplimentar prin care glucocorticoizii exercită acţiuni antiinflamatorii (25).

CONCLUZII

Rezoluţia inflamaţiei poate fi considera-

tă parte integrantă a procesului inflamator acut. Procesele implicate în rezoluție sunt influențate de interacțiunile dintre mediatorii chimici, de apoptoza leucocitelor sau de fagocitoză (26, 27). Factorii exprimați în timpul rezoluției ar putea fi folosiți direcționarea inflamației în curs de desfăşurare spre o cale prorezolutivă. O astfel de abordare ar însemna molecule în curs de dezvoltare care exercită efecte multiple la diferite faze ale răspunsului inflamator-limitarea traficului de leucocite, accelerarea *clearance*-ului celular, şi ajută la restabilirea țesutului stromal inflamat la starea sa anterioară (28, 29).

Medicamentele anti-inflamatorii curente blochează sau antagonizează căi ale mediatorilor pro-inflamatori implicate în inițierea unui răspuns inflamator acut (30). Prin urmare, cea mai mare parte a AINS blochează căile biochimice şi/sau mecanismele cheie de semnalizare pro-inflamatorie. Acest lucru ar putea fi definit ca **terapie de depleție (31).**

Abordarea terapeutică bazată pe *fenomenul rezoluției* ar necesita o schimbare a accentului de pe **terapia de depleție** pe **terapia de substituție**. Medicamentele rezoluției, precum cele revizuite în acest material, ar acționa antagonic, servind ca activatori, de exemplu, agonişti ai receptorilor, care activează mecanisme de protecție ce conduc la rezoluția inflamației acute şi reîntoarcerea la homeostazie.

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THE ANCIENT INDIAN FLAP: A TECHNIQUE STILL UP TO DATE

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THE ANCIENT INDIAN FLAP: A TECHNIQUE STILL UP TO DATE (Abstract): **Aim**: To reevaluate an old surgical technique that is still used and even up to date. **Material and methods:** A series of 24 patients was reviewed: 10 women and 14 men aged 55 to 84 years who underwent surgery between 2011 and 2017. All patients had various facial lesions removed from the nasal pyramid, nose and cheek region, nose and upper lip, and from the internal angle of the orbit. Most of the removed lesions were malignant- basal cell and squamous cell carcinomas, with only one benign lesion consisting of a hemangioma. For covering of the post-excisional defect, the Indian flap was used and mobilized into the defect by rotation, transposition or tunneling of an island flap. **Results:** Favorable results were achieved from a cosmetic and functional point of view, without any flap loss. Illustrative cases for each type of lesion and performed flap are presented and the postoperative outcomes are exemplified. **Conclusions**: The Indian flap remains the gold standard to cover defects in the nasal and neighboring regions. **Keywords**: INDIAN FLAP, BASAL CELL CARCINOMA, SPINOCELULAR CARCINOMA, PEDICLED FLAP.

The term flap has various connotations in anatomy and medicine. In plastic and reconstructive surgery, a flap is the tissue transferred from the place of origin (donor area) to another site (receiving area), to cover a post-traumatic or post-excisional defect. When a flap contains skin and subcutaneous cellular tissue it is called a cutaneous (skin) flap. The flaps may also contain other tissues (fascia, muscles and even bones) and are thus called composite flaps. Often, for the reconstructive surgery of the face, skin flaps are used. Flaps can be further classified into free flaps and pedicled flaps.

Free flaps are usually composite flaps that do not have a connection with the donor area. After transfer the blood supply is provided by the vessels of the receiving area that are anastomosed to the flap vessels by microsurgical techniques. The transfer of free flaps was possible thanks to the development of microsurgery technology and techniques and it represents an important breakthrough in plastic and reconstructive surgery (1, 2).

Pedicled flaps are vascularized from the region where they come from. They remain a priority indication for covering small and medium sized defects, especially on the face (3). The theoretical and technical principles governing the mobilization of pedicled skin flaps have originally been devel-

oped empirically. The use of frontal skin flaps for nasal reconstruction dates from ancient India. Around the year 700 BC, nasal amputation was a common punishment for a variety of crimes. The treatment of this infirmity was described in Sushruta Samhita (4). The technique today called "Indian flap" was brought to Europe in the fifteenth century and finally reached the United States in the 1830s. Subsequently, the name "Indian flap" was extended by some authors to other types of pedicled flaps on the face. The theoretical and technical principles of these types of flaps have been corrected, completed and well established over time, based on experimental and clinical studies (5, 6). Today it is known that for skin flaps, the vascularization from the donor area can be random and un-systematized (a random flap or joint flap). In order to have reliable viability, the length of this flap must be equal to the width. The blood supply of the donor area can also be provided by the subcutaneous vascular paths in the flap axis: the axial flap. The length of the axial flap may well exceed the width. The Indian flap falls into this last category (7).

The mobilization of the flaps, whether common or axial, is performed by various techniques (advancement, rotation, transposition, tunneling of island flaps). When the donor area is not adjacent to the receiving area, sutures inserted at the defect edges hold the pedicle of the flap as a bridge between the two areas. About 21 days (that can be prolonged in some situations) are needed for establishing the vascular connections in the new site. After this time, the vascularization (linking) with the donor area can be cut. The surgical technique is called flap autonomization and it involves cutting the pedicle and then performing the reconfiguration of the flap. For island flaps, autonomization is not necessary (8).

MATERIAL AND METHODS

Our study is a retrospective analysis of 24 patients with post-excisional defects of the face, who underwent surgery between 2011 and 2017, of which 10 (41.7%) females and 14 (58.3%) men, aged 55 to 84 years. The primary lesion was classified as basal cell carcinoma (BCC) in 19 (79.2%) cases (of which four were tumor recurrences), squamous cell carcinoma (SCC) in 4 (16.7%) patients and hemangioma in one (4.1%) patient (tab.I). The lesions were located as follows: seven (29.2%), on the nasal pyramid, eight (33.3%) on the nose and cheek regions, four (16.7%) on the nose and upper lip, and five (20.8%) at the internal angle of the orbit. For covering the surgical defect, the Indian flap (the axial paramedian frontal flap) was used. The mobilization of the flap was performed using the rotation technique in four (16.7%) cases, by flap transposition (the "classic flap") in 19 (79.2%) cases and in one (4.1%) case an insular flap was performed.

TABLE I

Distribution of the study patients

Sex				Tumor histology						Initial tumor localization							
M		F		BCC		SCC		Hemangioma		Internal orbital angle		Nose		Nose and genian region		Nose and upper lip	
No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
14	58,3	10	41,7	19	79,2	4	16.7	1	4.17	5	20.8	7	29.2	8	33.3	4	16.7

TABLE II
Type of flap

Type of Indian flap					
Rotated		Trans positioned		Insular flap	
No	%	No	%	No	%
4	16.7	19	79.2	1	4.17

RESULTS

The functional and esthetic outcomes were good in all patients with accurate restoration of the defect area and absence of functional disturbances related to palpebral position and closure. There were no cases of complete flap loss or partial flap necrosis. The only encountered complication was edema and flap congestion in one patient, solved by superficial pricking using a syringe needle.

The reconstruction results are best illustrated through the following cases that were selected from the study group (fig. 1-26).

Fig. 1. Clinical appearance of a BCC of the medial angle of left eye.

Fig. 2. Excision within oncologic safety margins. The defect was covered using a rotated frontal flap.

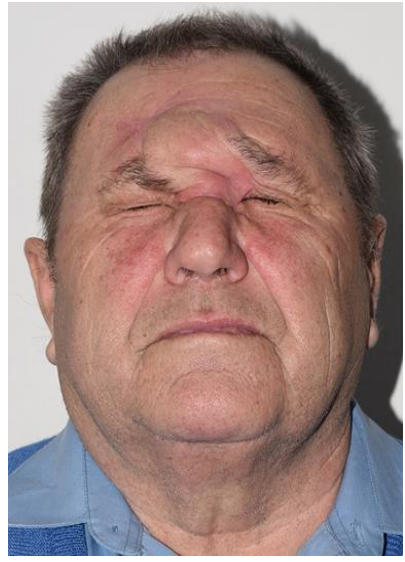

Fig. 3. One-year postoperative appearance proving the favorable functional outcome with accurate eyelid closure.

Fig. 4. One-year postoperative outcome showing the good appearance of the reconstructed area. Although the aesthetic appearance could have been improved, the patient did not want any further corrective surgery.

Fig. 5. Patient with a BCC of the right nostril.

Fig. 6. Intraoperative view of the defect following tumor removal and marking of the paramedian forehead flap.

Fig. 7. Intraoperative appearance of paramedian frontal flap sutured into the defect and closure of the donor area.

Fig. 8. Clinical appearance of the reconstructed area at one year after surgery showing the excellent cosmetic as well as functional outcome due to good nostril patency for an adequate air flow.

Fig. 9. Patient with a BCC of the left nasal-genian region.

Fig. 10. Picture demonstrating the superficial pricking of the island flap used for defect reconstruction. Superficial pricking was performed using a syringe needle during the first 5 days following the surgical procedure for flap decongestion.

Fig. 11. Clinical appearance of island flap reconstruction tunneled into the defect at one month after surgery showing the good functional outcome regarding eyelid closure. There is an obvious increase in volume of the inserted flap due to existing pressure on the return circulation (venous and lymphatic) of the buried flap pedicle.

Fig. 12. Frontal view of the patient one year after surgery proving improved appearance due to the flap volume reduction consecutive to the reestablishing of venous and lymphatic flow.

Fig. 13. Patient with a SCC of the nasal tip and bilateral cervical and intraparotid lymph node metastasis.

Fig. 14. Intraoperative view of the defect resulted after tumor removal and the marking of the paramedian forehead flap.

Fig. 15. Postoperative view at 6 weeks after complex surgery involving tumor resection and plasty with an Indian flap and bilateral neck dissection together with bilateral parotidectomy.

Fig. 16. Clinical appearance of the patient 5 years postoperatively showing the excellent esthetic outcome. The patient received chemotherapy and radiation therapy.

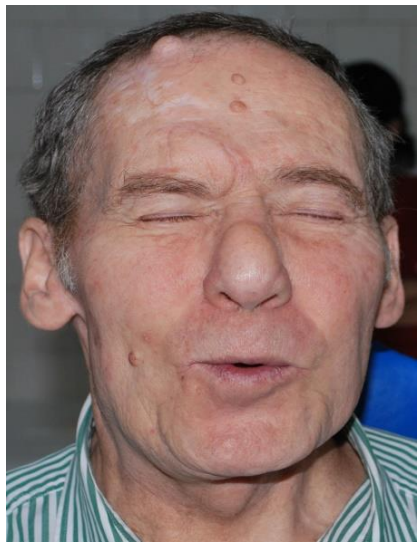

Fig. 17. Five-year postoperative outcome. The cosmetic result is very good and there are no signs of facial nerve damage.

Fig. 18. Patient with BCC recurrence in the left nasal-genian region following incomplete surgical excision done four years before he presented to our clinic with the recurrence.

Fig. 19. Intraoperative view of the resulting defect. A wide excision was performed. The histopathological examination confirmed clear margins in both surface and depth. For an optimal esthetic and functional result, the surgical defect was reconstructed separately for each of the two involved facial units: the nasal and the genian region.

Fig. 20. Intraoperative view of the Indian flap used for the plasty of the nasal and nasal-genian regions.

Fig. 21. Intraoperative aspect of the deep surface of the raised Indian flap.

Fig. 22. Intraoperative view of the left genian flap used for the plasty of the genian defect.

Fig. 23. Intraoperative view of the suspension sutures anchored to the inferior orbital rim used for reducing the tension in the genian flap.

Fig. 24. View of the performed reconstruction at the end of the surgical procedure.

Fig. 25. Frontal view of the patient four years after surgery showing the restored symmetry of the nasal and genian regions with an overall good esthetic outcome.

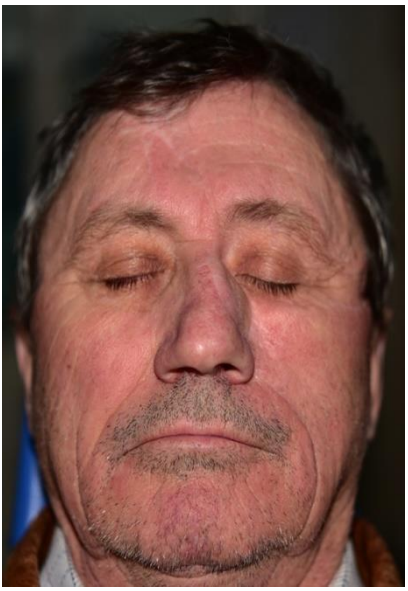

Fig. 26. Frontal view of the patient four years after surgery showing good eyelid closure.

DISCUSSION

The Indian flap (the para-median frontal flap) is an axial flap. The axial vascularization has been demonstrated by Kazanjian in 1930 and it consists of the median branch of the supraorbital artery and the supratrochlear artery. These vessels have a vertical trajectory emerging at 1.7-2.2 cm from the median line (9, 10), initially travelling sub muscular, and then, at approximately 1 cm above the eyebrow they become superficial, located in the subcutaneous tissue.

The Indian flap has undergone numerous innovations and modifications, becoming the first choice for the nasal defects. Although traditionally it is considered "the gold standard" for these sites, the indication has been extended to other sites on the face: the infraorbital region and the upper lip region. Any defect located above the lips or in the medial part of the cheek, may be covered using Indian flaps.

The technical details of the flap dissection are governed by certain principles for achieving viable flaps and good results. Maintaining the axial path is an important operative step, considering the known trajectory of the feeding arteries. The distal extremity of the flap can be extended on the forehead width when needed, but with unpredictable results. The pedicle has to be as narrow as possible avoiding the skin of the glabella, as the excessive bulk of the pedicle passing under the glabellar skin jeopardizes the blood supply and distorts the nasal contour (11). The supratrochlear pedicle can be as narrow as 1.0 to 1.2 cm (12, 13, 14). A subperiosteal dissection is performed at the base of the flap to protect

and include in the flap the initially deep arterial axis.

After extensive resections, especially in cases of recurrence, the frontal flap can be combined with other flaps for functional and cosmetic considerations. Indian flap reconstruction after excision of lesions located in the inner corner of the orbit or on the nasal wing may be difficult. For the medial corner of the eye, the functional result regarding eyelid closure has the same importance as the cosmetic result. When the resection of the nostril wall is wide, and a large part of the alar cartilage is also involved ear cartilage transplantation is needed. A small composite graft consisting of tegument and cartilage is taken from the auricle and used for reconstruction. If the permeability of the nostril is not affected by resection and/or its plasticity, the surgical defect can be covered in good conditions using the Indian flap.

Age is not a contraindication for the frontal flap reconstruction. In the cases presented, there are patients aged over 80 years with comorbidities in which the Indian flap has been safely performed. It is not necessary to modify patient's anticoagulation therapy prior to surgery.

The surgery can be performed under sedation but preferably under general anesthesia. Recurrence after excision of a malignant lesion is due to an imperfect technique. In these situations, the histopathological examination of the specimen reveals the surface margins, and/or the in-depth limits invaded by tumor tissue. Recurrence of BCC is less severe than recurrence of SCC. For recurrent BCC, a correct re-resection solves the problem for good, but for SCC recurrence the re-intervention is precious time lost since the disease usually progresses.

When the recurrence of the SCC develops under the flap, the clinical manifestations have a slower onset due to the thickness of the flap, which may contribute to the spread of the disease (15). To capture an eventual recurrence as early as possible (in unsafe cases and when the histopathological examination of the margin of the resection specimen is not possible), a total skin graft may initially be applied to a favorable receiving site. The skin of the auricular-cephalic groove (from the mastoid and posterior side of the ear) has the same color and texture as the facial skin and is a good option for full thickness skin graft elevation. The graft donor defect is easily covered by suturing the auricular margin at the mastoid edge with an invisible scar. The cosmetic result is often satisfactory, and the resolution can remain definitive. When there are functional or cosmetic reasons requiring a flap reconstruction, the re-intervention does not take place earlier than 6 months. Any recurrence developing during this time is easy to recognize at an early stage.

CONCLUSIONS

As demonstrated by the favorable results of our study, the Indian flap, with all the theoretical specifications and technical improvements over time, remains nowadays an up to date and useful technique in the reconstructive surgery of the face, just as described more than two millennia ago.

Originally, it was designed for the repair of the nasal pyramid, but the indications have been extended to cover defects with other locations on the face. With a correct indication, perfect flap planning, dissection and mobilization, we have found that the functional and cosmetic results can be among the best.

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STUDIU PRIVIND INFLUENŢA FACTORILOR DE MEDIU ŞI INDIVIDUALI ÎN LITIAZA RENALĂ

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STUDY ON THE INFLUENCE OF ENVIRONMENTAL AND INDIVIDUAL FACTORS IN RENAL LITHIASIS (Abstract): The large number of cases with renal lithiasis occurring in the population of the south-east region of Iasi county has determined us to make a study in this region for the identification of environmental and individual factors involved in the etiopathogenesis of this disease. This study is performed to assert the corelation between the clinical and paraclinical patients data with those obtained through water and soil chemical analisys for identification of determinant environmental and individual factors involved in etiopathogenesis of this disease. This study indicates that the environment factors (water, soil) correlated with personal factors, especially the diet and standard of living are the favouring factors of renal lithiasis. **Key words:** RENAL LITHIASIS, KIDNEY STONES, PROPHYLAXIS

Litiaza reno-urinară este consecinţa precipitării în tractul urinar a unor constituenţi care, în urina normală, se găsesc în soluţie fără tendinţă la precipitare. Principalele componentele urinare responsabile în procesul de litogeneză sunt: Ca^{2+}, P^{3+}, Mg^{2+}, oxalat, acid uric, uree, ioni de amoniu şi cistină (1). Urina elaborată de rinichiul normal reflectă starea fiziologică a organismului şi este un lichid supraconcentrat în cristaloizi pe care, însă, îi menţine în stare de soluţie datorită proprietăţilor sale fizico–chimice (1,2); ne referim în primul rând la pH, volumul urinar, concentraţia componenţilor urinari, dar şi la prezenţa unor inhibitori ai cristalizării magneziu, citratul, pirofosfatul, mucopolizaharizii acizi).

Există un context de factori fizici, chimici, metabolici, anatomici, bacteriologici şi de mediu care determină o concentraţie a sărurilor urinare peste punctul de saturaţie, cu formarea calculilor renali.

Mediul înconjurător intervine în mod categoric în geneza calculilor urinari prin deshidratare în atmosferă caldă şi uscată, ingestia de factori litogeni prezenţi în apă sau alimente (3).

Deshidratarea cu oligurie cronică, obiceiurile alimentare unilaterale cu exces proteic, tulburările metabolice (obezitate, denutriţie, gută, cistinurie, hiperoxalurie) tulburări de flux urinar şi încărcarea urinii cu cataboliţi microbieni în infecţiile urinare, medicamentaţie cu potenţial litogenic renal standardul de viaţă scăzut sunt cauze individuale favorizante ale litiazei renale.

Mecanismul de geneză a unui calcul reno-urinar este insuficient cunoscut. Cristalurie există în orice urină normală, dar pentru apariţia litiazei urinare manifestă clinic este necesară intervenţia unor factori multipli, dintre care mulţi rămân încă necunoscuţi (2).

Între stadiul de nucleere şi dezvoltare a calculilor urinari şi evidenţierea acestora întâmplător sau datorită complicaţiilor pe care le detemină, este o perioadă de timp suficient de lungă şi poate fi denumită faza activă metabolic a litiazei urinare (1).

Acest stadiu în care proprietăţile fizico-chimice ale urinii sunt propice litogenezei

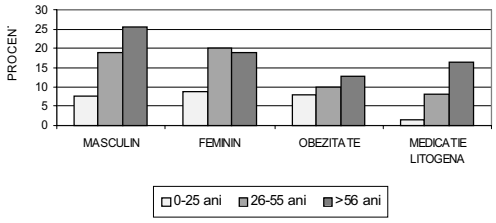

Fig. 1. Structura lotului investigat

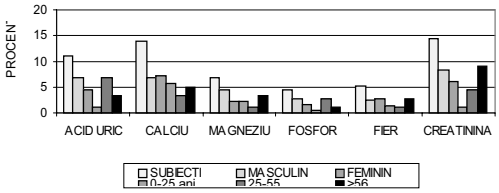

Fig. 3. Parametri biologici cu nivel plasmatic crescut

şi determinate de cauze complexe individuale sub influenţa favorizantă sau indiferentă a factorilor de mediu face obiectul studiului nostru.

MATERIAL ŞI METODĂ

Lotul selectat cuprinde 180 de locuitori ai localităţii Răducăneni aflaţi în evidenţa medicului de familie. Criteriile de selecţie a lotului investigat au avut în vedere circumstanţele de apariţie a litiazei renale şi anume influenţa factorilor individuali şi ai celor de mediu în etiopatogenia acestei afecţiuni. Interesul nostru de a surprinde litiaza renală în faza activă metabolic, ne-a determinat să structuram lotul în 3 categorii : indivizi până în 25 de ani, indivizi între 26 şi 55 de ani şi indivizi peste 55 de ani. In cazul antecedentelor personale am avut în vedere acele afecţiuni însoţite de excreţia în cantităţi anormale a componenţilor litogeni sau unele medicaţii practicate (4). O serie de boli transmise autozomal dominant sau recesiv cum sunt : acidoza tubulară renală, hipercistinuria, litiaza urică familială, hiperoxaluria congenitală, anomalii morfologice congenitale ale aparatului excretor au fost de asemenea luate în consideraţie (2). Alţi factori cum sunt : obiceiurile alimentare, ocupaţia, sedentarismul,

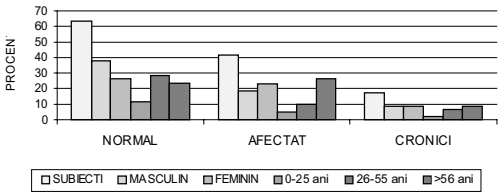

Fig. 2. Profilul metabolic

stressul, nivelul de trai, apartenenţa religioasă diferenţiază subiecţii investigaţi în grupe de risc (3).

Influenţele mediului extern au fost cercetate prin analiza compoziţiei chimice a apei de fântână şi a solului (3).

Aparatura folosită : analizor automat de biochimie AIRONE 200 RA, analizor automat hematologie Mindray, analizor ioni EASY LYTE Ca, microscop optic MICROS.

Investigaţiile de laborator au vizat aprecierea profilului metabolic general, examenul urinii şi dozarea constituenţilor biochimici care sunt implicaţi sau favorizează faza activă metabolic a litogenezei.

Analiza chimică a apei am efectuat-o la Institutul de Igienă „Mihai Ciucă”, Iaşi.

REZULTATE

Analiza rezultatelor obţinute s-a făcut după următoarele criterii :

1. Lotul investigat cuprinde 180 de subiecţi din mediu rural aflaţi în evidenţa medicului de familie : 52.22% subiecţi de sex masculin (7.77%, 0-25 ani, 18.88% 26-55 ani, şi 25.55% peste 56 ani) şi 47.78% subiecţi de sex feminin (8.89% subiecţi până în 25 de ani, 20% subiecţi între 26 şi 55 ani şi 18.89% subiecţi peste 56 de ani). Obezitatea este prezentă la 30.6% subiecţi, iar 16.6% subiecţi sunt subnutriţi. Medicaţie litogenă urmează 25.85% de subiecţi (fig. 1).

2. Profilul metabolic general a fost apreciat prin examene de laborator : hemoleucogramă – metoda Coulter, VSH, glucoză plasmatică şi urinară – metoda Trinder GOD--POD, uree plasmatică şi urinară - metoda urează-GLDH, creatinină plasmatică şi uri-

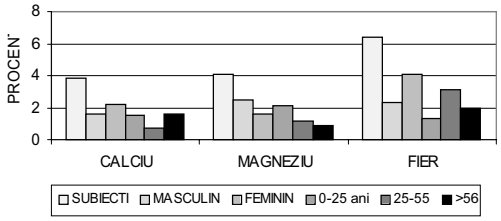

Fig. 4. Parametri biologici cu nivel plasmatic scăzut

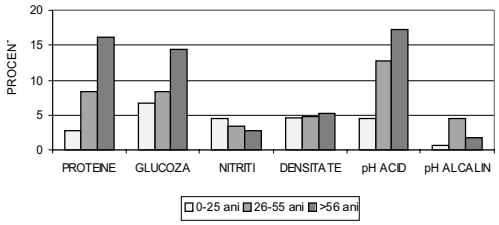

Fig. 5. Examen sumar urină

nară - metoda Jaffé, transaminaze GPT – metoda IFCC - LDH-NADH şi GOT – metoda IFCC - MDH-NADH, colesterol – metoda CHOD-POD, trigliceride - metoda GPO-POD, proteine totale – metoda biuret, fosfataza alcalină metoda p-nitrofenilfosfat, sumar urină şi sediment urinar.

Rezultatele se încadrează în intervalul de valori normale pentru 63.89% de subiecţi, 37.78% subiecţi de sex masculin (SM) şi 26.11% subiecţi de sex feminin(SF) : 11.67% 0-25 ani, 28.89% 26-55 ani şi 23.34% peste 56 ani.

În 41.67% din cazuri, 18.89% SM şi 22.77% SF (5% 0-25 ani, 10% 26-55 ani şi 26.67% peste 56 ani) subiecţii examinaţi prezentau o serie de afecţiuni acute (respiratorii, hepatice, digestive, renale) care au fost confirmate prin examen medical de specialitate.

17.23% de subiecţi, 8.34% SM şi 8.89% SF (2.23% 0-25 ani, 6.11% 26-55 ani şi 8.89% peste 56 ani) sunt bolnavi cronici de hipertensiune arterială, insuficenţă cardiacă, diabet zaharat, insuficenţă renală, infecţii cronice respiratorii, hepatice, renale) ; Aceşti subiecţi, prezenţi în evidenţa medicului de familie au fost investigaţi complementar pentru decelarea constantelor biologice favorizante sau determinante pentru litogeneza renală (fig. 2).

1. Investigaţiile de laborator în sânge şi urină pentru parametri biologici implicaţi în litogeneză : acid uric – metoda uricază–POD, calciu - metoda orto-cresolftaleină, fosfor - metoda cu molibdat de amoniu, magneziu - metoda cu calmagită, fier - metoda ferozină şi creatinina pentru aprecierea orientativă a funcţii excretorii renale.

a) Dozări plasmatice

Un nivel plasmatic crescut a fost decelat pentru (fig. 3) :

- acid uric, 11.1%, (8.34% SM, 8.89% SF, 2.23% 0-25 de ani, 6.11%, 26-55 ani şi 8.89% peste 56 ani) ;
- calciu, 13.9%, (6.67% sex masculin, 7.23% sex feminin, 5.56% 0-25 de ani, 3.34%, 26-55 ani şi 5% peste 56 ani) ;
- magneziu, 6.69%, (4.46% seSM, 2.23% SF, 2.23% 0-25 de ani, 1.12%, 26-55 ani şi 3.34% peste 56 ani) ;
- fosfor, 4.45%, (2.78% SM, 1.67% SF, 0.55% 0-25 de ani, 2.78%, 26-55 ani şi 1.12% peste 56 ani) ;
- fier, 5.12%, (2.43% SM, 2.69% SF, 1.31% 0-25 de ani, 1.2%, 26-55 ani şi 2.61% peste 56 ani) ;
- creatinină, 14.46%, (8.34% SM, 6.12% SF, 1.12% 0-25 de ani, 4.45%, 26-55 ani şi 8.89% peste 56 ani).

Nivel plasmatic scăzut a fost decelat pentru (fig. 4) :

- calciu, 3,8%, (1.67% SM, 2.2% SF, 1.5% 0-25 ani, 0,6%, 26-55 ani şi 1,6% peste 56 ani) ;
- magneziu, 4,1%, (2,5% SM, 1,6% SF, 2.11% 0-25 de ani, 1.12%, 26-55 ani şi 0,87% peste 56 ani) ;
- fier, 6,44%, (2.34% SM, 4,1% SF, 2,36% 0-25 ani, 3,1%, 26-55 ani şi 1,98% peste 56 ani).

b) Analiza urinii

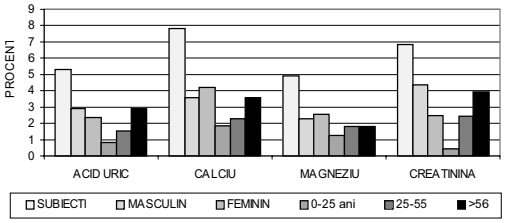

Fig. 6. Dozări biochimice în urină

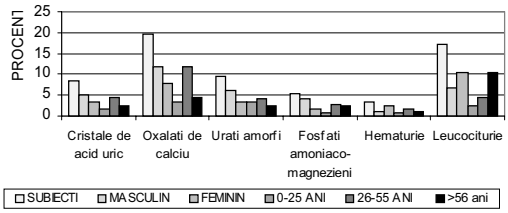

Fig. 7. Examenul sedimentului urinar

La examenul sumar de urina s-a constatat (fig.5) :

- proteinurie prezentă la 15% SM şi la 12.23% SF (2,78% 0-25 ani, 8.34% 26-55 ani şi 16.12% peste 56 ani) ;
- glicozurie prezentă la 13.89% SM şi la 15.86% SF (6,67% 0-25 ani, 8.34% 26--55 ani şi 14.45% peste 56 ani) ;
- nitriţi prezenţi la 5% SM şi la 5.56% SF (4.45% 0-25 ani, 3.35% 26-55 ani şi 2.78% peste 56 ani) ;
- densitatea urinară influenţată la 8.35% SM şi la 6.48% SF (4.72% 0-25 ani, 4.82% 26-55 ani şi 5.29% peste 56 de ani).
- pH-ul urinar este acid la 20.5% SM şi 13.9% SF (4.5% 0-25 ani, 12.8%, 26-55 ani şi 17.3% peste 56 ani) iar alcalin la 3.9% SM şi 2.8% SF (0.6% 0-25 ani, 4.5%, 26-55 ani şi 1.7% peste 56 de ani).

Valori crescute ale concentraţiei în urină s-a constatat pentru (fig. 6) :

- acid uric, 5.3%, (2.92% SM, 2.38% SF, 0.86% 0-25 ani, 1.54%, 26-55 ani şi 2.9% peste 56 ani) ;
- calciu, 7.8%, (3.57% SM, 4.23% SF, 1.87% 0-25 ani, 2.31%, 26-55 ani şi 3.62% peste 56 de ani) ;
- magneziu, 4.9%, (2.31% SM, 2.59% SF, 1.25% 0-25 ani, 1.83%, 26-55 ani şi 1.82% peste 56 ani) ;
- creatinină, 6.85%, (4.37% SM, 2.49% SF, 0.45% 0-25 de ani, 2.42%, 26-55 ani şi 3.98% peste 56 ani).

Examenul sedimentului urinar evidenţiază (fig.7) :

- cristale de acid uric, 8.34%, (5% SM, 3.34% SF, 1.67% 0-25 de ani, 4.45%, 26-55 ani şi 2.23% peste 56 ani) ;
- oxalaţi de calciu, 19.45%, (11.67% SM, 7.78% SF, 3.34% 0-25 ani, 11.67%, 26--55 ani şi 4.45% peste 56 ani) ;
- uraţi amorfi, 9.45%, (6.12% SM, 3.34% SF, 3.34% 0-25 ani, 2.89%, 26-55 ani şi 2.23% peste 56 ani) ;
- fosfaţi amoniaco-magnezieni, 5.56%, (3.89% SM, 1.67% SF, 0.56% 0-25 ani, 2.77%, 26-55 ani şi 2.23% peste 56 ani) ;
- hematurie, 3.34%, (1.12% SM, 2.23%

TABELUL I

Indicatori chimici de calitate a apei

Indicatori chimici de calitatea apei	Valori obţinute		
	Fantâna 1	Fantâna 2	Fantâna 3
Substante organice - mg $KMnO_4/dm^3$	4.32	6.95	4.32
Azotati(NO_3^-) - mg/dm^3	143.13	137.21	113.13
Calciu (Ca^{2+}) - mg/dm^3	92.97	130.04	124.97
Magneziu (Mg^{2+}) - mg/dm^3	174.13	158.57	164.13
Duritate totala - ^oG	53.1	55.1	54.1
Cloruri (Cl^-) mg/dm^3	150	167	160
Bicarbonati (HCO_3^-) - mg/dm^3	811.3	762.5	820.3
Alcalinitate - ml HCl n/10	13.3	12.5	12.3
Reziduu fix - mg/dm^3	1388	1782	1583
Suspensii - mg/dm^3	178	88	98

SF, 0.56% 0-25 de ani, 1.67%, 26-55 ani şi 1.12% peste 56 ani);

- leucociturie, 7.23%, (6.67% SM, 10.56% SF, 2.23% 0-25 ani, 4.45%, 26 - 55 ani şi 10.56% peste 56 ani).

La 13.89% subiecţi investigaţi este prezentă infecţie urinară (5% SM, 8.89% SF, 1.12% 0-25 ani, 2.77%, 26-55 ani şi 10% peste 56 ani). 3.87% din subiecţi au infecţii urinare repetate.

4. Indicatori chimici de calitatea apei: apa este dură, bicarbonatată cu un conţinut apreciabil în săruri minerale (tab.1).

5. Compoziţia chimică a solului - Indicele Hlebnicov de 0,79 arată o poluare medie a solului.

CONCLUZII

O primă concluzie în acest stadiu al studiului ar fi că factorii de mediu studiaţi (apă, sol) corelaţi cu cei personali, în special obiceiurile alimentare şi nivelul de trai sunt factorii favorizanţi ai litiazei reno- urinare.

21 de subiecţi din lotul studiat prezintă risc litogenic renal crescut şi au fost incluşi în programe de monitorizare.

Considerăm că educaţia sanitară în cadrul colectivităţii ar reprezenta una dintre primele măsuri de prevenţie pentru litiaza renală.

Necesitatea adoptării unor măsuri de protecţie a mediului prin programe de identificare şi neutralizare a factorilor poluanţi.

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THE SUCCESS RATE OF TWO TYPES OF ORTHODONTIC BONDED RETAINERS

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THE SUCCESS RATE OF TWO TYPES OF ORTHODONTIC BONDED RETAINERS (Abstract) **Aim**: The aim of this prospective clinical study was to compare the success rate of two types of fixed orthodontic retainers, flat and round wires. **Material and methods:** 53 patients aged between 11 and 53 years old were included in this study. The patients' orthodontic treatment history was bimaxillary fixed appliances for all kind of dento-maxillary malocclusions. The patients were sequentially assigned to receive round or flat retainers. **Results:** No debonding in the first year was considered a success. Retainer failures were monitored and centralized. A statistical test evaluated the success rate by comparing the two types of retainers according to age, gender, and location. **Conclusions:** Bonded retainers maintain incisor alignment following orthodontic treatment. A significant correlation between age and success rate of the flat wire and the round wire retainer was found (p=0.035). The configuration and type of wire have an important role in the failure rate. The success rate of round wire retainer was higher on females and the flat retainers had a greater success on males. **Keywords**: ORTHODONTICS, CONTENTION, ALIGNMENT, RETAINER.

The stability of orthodontic treatment has always been an issue, especially in cases with crowding in the inferior labial segment. There have been numerous studies which revealed relapse cases after aligning the frontal inferior teeth (1). To avoid relapse, every orthodontic treatment is followed by a contention phase. In practice, removable or fixed retainers are used. Nowadays orthodontic mandibular canine to canine retainers are frequently used. The major advantage of the bonded retainer compared to the removable one is that it doesn't need compliance, excepting oral hygiene procedures. One of the studies showed that almost one-third of American orthodontists usually use fixed retainers, representing a bigger proportion than previous reports (2).

Several studies proved that 10 years later after finishing the orthodontic treatment, relapses were found in 40-90% of cases (3). On the other hand, the stability of orthodontic results could last for more than 20 years in 10% of cases (4).

Ten years after orthodontic treatment,

30-50% of patients have the satisfaction of maintaining dental alignment, but after 20 years the percentage drops to 10 %. When relapse occurs, there some efficient strategies are necessary. The treatment methods of solving relapse should be different from the initial treatment, because the periodontal status, the physiological and psychological status are different too. Controlling the relapse is important to avoid wasting time, money and resources to redo the dental alignment (5).

Fixed lingual retainers bonded to mandibular anterior teeth have been introduced in 1970's by Knelrim (6), and have been considered part of the orthodontic treatment, for avoiding relapse or secondary crowding of inferior incisors (7). Hawley introduced the removable retainer: the Hawley removable appliance, an acrylic appliance (8, 9).

The first generation of fixed retainers were made of stainless-steel round wire, with large diameter: 0.030-0.032 inch; they were followed by smaller diameters or coaxial round wires or cross-section rectangular wires having different compositions, bonded on the lingual part of the anterior mandibular teeth. Recently, fiber-reinforced materials and alumina ceramic retainers have been introduced (10, 11).

The conclusion of most orthodontists is that the most efficient method to avoid relapse and secondary crowding of the inferior and superior frontal teeth is the fixed retainer. Because the removable retainers need patient compliance, the golden standard for contention is the fixed retainer.

Zachrisson introduced in 1983 the lingual retainer made of round wire, but nowadays there are many types of wires and diameters and methods of bonding fixed retainers (12, 13, 14).

AIM

The aim of this study was to compare the success rate and debonding rate of 2 types of fixed multistranded retainers: one round 0.0175-inch diameter wire and another flat wire: 0.010 x 0.022 inch. The criterion for evaluating success was: the absence retainer debonding after at least one year from applying it in the oral cavity.

MATERIAL AND METHODS

A number of 53 patients aged between 11 and 53 were included in this study, 33 of them wearing round fixed retainers (fig. 1), and the other 20 flat wire retainers (fig. 2).

The patients' orthodontic treatment history was bimaxillary fixed appliances for all kind of dento-maxillary malocclusions.

The patients were elected from a private clinic from Tîrgu-Mures. History dental files have been used as material to obtain necessary data for our study:

- the total number of patients;
- age;
- gender;
- the type of retainer that has been used (flat or round);
- the retainer's position, lower canine to canine, upper lateral incisor to lateral incisor;
- the date of retainer bonding;
- the date of the first retainer debonding.

The including criteria were:

- fixed orthodontic treatment in history;
- dental alignment and stable occlusion at the end of the orthodontic treatment;
- the possibility of using fixed retainers for upper and lower jaw at the end of the treatment.

The method for applying the retainer included the next steps:

- isolation with rubber dam from the second premolar to the second premolar;
- cleaning the dental surfaces using a brush and a fluoride-free toothpaste;
- fixing the retainer using dental floss (fig. 1 a and b);
- etching using phosphoric acid 37% (fig. 2 a) (15);
- bonding using a special adhesive material (fig. 2 b);
- fixing retainer with a special flowable composite (fig. 3).

The retainers were fixed from canine to canine at the lower jaw and lateral incisor to lateral incisor in the lower jaw. The only difference was the type of multistranded wire: the flat retainer with wire dimensions of 0.010x0.022 inch and the round retainer with 0.0175-inch diameter wire.

The success rate was statistically monitored comparing the two types of retainers by age, gender, and location. The statistical analysis included the descriptive statistics to determine the average value and the chi-squared test to establish the correlation between the age or gender of the patient and the success rate.

The Mantel-Cox log-rank test was used to identify significant differences in survival functions among the groups.

Fig.1. a. Dental isolation using the rubber dam, preparing the dental surfaces **b.** fixing the retainer using dental floss

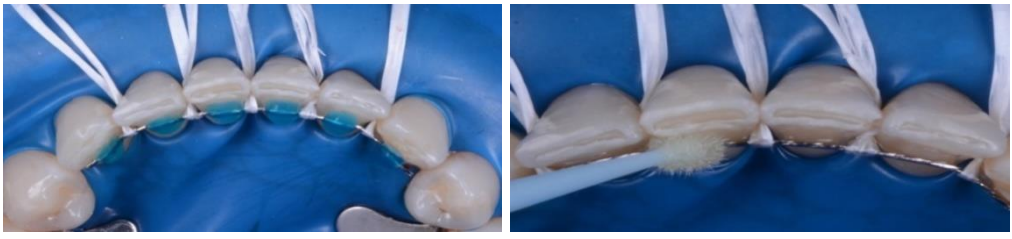

Fig. 2 a. Etching step and **b.** bonding applying

Fig. 3. Retainer composite fixing

RESULTS

A number of 53 patients aged between 11 and 53, 33 females and 20 males were included in the study. A number of 43 retainers on the inferior arch and 37 on the superior arch have been applied. Table 1 shows the success rates of the two types of retainers according to the variables. Overall, significantly ($p< 0.035$) more failures in the multistrand round retainers' group were observed in comparison with the flat multistrand retainers' group (tab. I). According to location, the flat retainers were more successful in the upper arch comparing with the lower arch, but the difference was not statistically significant ($p=0.06$). On the other hand, the round retainers have a significantly lower success rate in the upper jaw comparing to the lower one.

A success rate of 64 % in females with flat wire retainer was observed; in 5 out of 14 patients, retainer detachments were recorded. For the round wire retainers, there has been a rate of success of 86 %, 3 out of 19 patients had retainer detachment. The success rate in males was 80 % for the flat wire retainer when 1 out of 5 patients had a failure. And for the round wire, the success rate was 73%, recording 4 failures out of 15 cases.

TABLE I

The success rate of two types of retainers according to multiple variables: gender, age, location, and general success rate

	Group	Gender		Age		Location		General
		Female	Male	11-18	19-53	Upper	Lower	
Success	Flat retainer	9 (64%)	4 (80%)	8 (62%)	5 (83%)	10 (67%)	9 (64%)	13 (68%)
	Round retainer	16 (84%)	11 (73%)	11 (83%)	16 (76%)	17 (77%)	24 (83%)	27 (79%)

The chi-squared test results demonstrated a significant statistic correlation between the age and the success rate of the round wire and flat wire retainer ($p=0.035$).

DISCUSSION

This study on the success rate of the bonded retainers appears to be the first in the central part of Romania. It was aimed to compare two types of retainers flat and round wire, according to patient gender and age and retainer position.

The general success rate was 68% for the flat wire retainer and 79% for the round wire retainer. This finding is in accordance with the results of Årtun *et al*. (16) and Segner and Heinrici (17).

Analyzing the success rate according to the location of the retainer, the results for the inferior arch showed a success rate of 64% for the flat retainer and 83 % success rate for the round wire. In the upper arch, there has been found a 67% success rate for the flat retainer and a 77% success rate for the round retainer. The failure rate for mandible retainers was lower than the findings of Andrén *et al.* (18) who reported a failure rate of 35% for the mandible. Störmann and Ehmer (19) reported a similar failure rate of 18 % for the 0.0195-inch retainer. The higher success rate might be explained by the use of rubber dam for isolation. Similar studies (Dahl and Zachrisson, 1991 (7); Bearn, 1995 (20); Andrén *et al.*, 1998 (18); Segner and Heinrici, 2000 (17)) have shown higher failure rates for the maxilla compared with the mandible.

The failure rate according to the type of

retainer was between 16-27% for round retainer, smaller than flat retainer 17-38% and comparable with the findings of Årtun *et al.* (16), 27.2 % for the thin, flexible spiral wires. On the other hand, Dahl and Zachrisson reported a smaller failure rate of 10.3% for three-stranded spiral wires and 5.9% with the five-stranded spiral wire (7).

According to patient gender, a success rate of 64% in females with flat wire retainer and 86% with round retainers was observed. The success rate in males was 80 % for the flat wire retainer and 73% for the round wire. These indicated a lower failure rate for flat retainers on males and a lower failure rate for round retainers on females. Lumsden *et al.* (21) study did not show significant differences in failure rate according to gender and age.

The results related to age showed a higher success rate for the round retainer considering both samples: age between 11-18 and 19-53. A slightly increased failure rate was found regarding the patients aged between 11-18 in agreement with the findings of Lumsden *et al.* (21) where the mean age of the subjects was 15.5 years. However, the results were lower than those reported by Dahl and Zachrisson (1991) where the mean age of the patient population was 31.2 years (7).

Although previous investigations (Dahl and Zachrisson, 1991 (7); Årtun *et al.*, 1997 (16); Störmann and Ehmer, 2002 (19); Tacken, 2010 (10); Foek, 2009 (22); Salehi, 2013 (1)) have shown a difference in failure rate when different types of retainers are used. Salehi (2013) showed no significant differences between the wire retainer and the polyethylene fiber retainers for both arches. Tacken (2010), demonstrated that the glass fiber reinforced retainers (GFR) had a lower success rate than the stainless-steel wire retainer. Foek (2009) results were that the stainless-steel wire retainer had greater success than the fiber reinforced retainer.

In other similar studies (Dahl and Zachrisson, 1991 (7); Årtun *et al.*, 1997 (16)) the retainer wires were bonded with chemically polymerized resin composite. The survival rate also depends on the bond strength of the orthodontic materials, Martha K. *et al.* 2013 (23). In the current study, isolation was achieved using rubber dam, according to Audenino *et al.*, 2006 the survival rate of resin-bonded restorations has been higher using rubber dam isolation. In this study, the success rate has been increased by using absolute isolation (24).

Future prospective studies should concentrate on factors such as isolation control, the light intensity of the polymerization device and the composite and adhesive resin used.

CONCLUSIONS

Bonded retainers maintain incisor alignment following orthodontic treatment.

According to statistical data, a significant correlation between age and success rate of the flat wire and the round wire retainer was found, with a p-value < 0.05, $p=0.035$.

The failure causes of wire retainers included fractures, debonding and accidental detachment.

The configuration and type of wire have an important role in the failure rate. The round wire success rate is bigger than the flat retainer success rate. In both cases, there is an average rate of success higher than 60%.

The failure rate of flat retainers is lower on patients aged between 19-53.

On females, the success rate of round wire retainer was higher, and on males, the flat retainers had a greater success.

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EVALUĂRI CLINICO-EPIDEMIOLOGICE, RETRO-PROSPECTIVE ASUPRA INCIDENŢEI ŞI PREVALENŢEI UNOR MALFORMAŢII CONGENITALE DE CORD, PE UN LOT DE 1570 DE COPII, NĂSCUŢI ÎN JUDEŢUL ŞI ÎN MUNICIPIUL IAŞI, ÎN PERIOADA 2000-2009

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CLINICAL EPIDEMIOLOGICAL RETRO PROSPECTIVE STUDIES ON THE INCIDENCE AND PREVALENCE OF CARDIAC CONGENITAL ABNORMALITIES IN A GROUP OF 1570 CHILDREN, BORN IN IAŞI BETWEEN 2000 - 2009 (Abstract): **Material and method**: The study has been conducted on a period of ten years and it included 1570 children with congenital abnormalities (CA), of which 371 (24%) were cardiac abnormalities, 312 (20%) were skeletal abnormalities, 55 (3%) were Down Syndrome and 832 (53%) were other pathologies. **Results**: 48% of the 371 children that were diagnosed with cardiac CA were males, while 52% were females; 52% of the children were from the city, while 48% were from the country-side; 42% of the children have been born prematurely, while 58% of them have been born at normal term. 38% of the children had an APGAR score lower than 7 and 62% of them had an APGAR score higher than 7. Of the total number of births, 72% were caesarian births and 28% were natural births. The different types of Cardiac CA that have been encountered in the study were atrioventricular canal (56%), transposition of the great vessels (18%), common arterial trunk (10%), atrial septal defect (8%), ventricular septal defect (5%) and tetralogy of Fallot (3%). 66% of the total number of deaths were represented by those with cardiac pathology, 21% were caused by hydrocephalus, 7% were caused by diaphragmatic hernia, 4% had renal CA, while 2% were caused by other pathologies. **Key words**: CARDIAC CONGENITAL ABNORMALITIES, PREVALENCE, PREVENTION

Malformaţiile cardiace congenitale (MCC) reprezintă anomaliile structurale cu perturbări funcţionale ale inimii prezente la naştere, ca rezultat al unei alterări a dezvoltării embriologice cardiovasculare, apărute în perioada cardiogenezei (1,2).

Majoritatea MCC îşi au originea în morfogeneza anormală a tubului cardiac primitiv în primele 50 de zile ale vieţii embrionare (3).

Există relaţii strânse între modificările structurale şi funcţia cardiovasculară, iar trecerea de la circulaţia fetală la cea a nou-născutului ameliorează sau agravează unele MCC (4).

Grupul de studiu EUROSCAN a evidenţiat că rata diagnosticului prenatal al malformaţiilor congenitale variază foarte mult în

TABELUL I
Distribuţia cazurilor de malformaţii congenitale, înregistrate la Spitalul Clinic „Cuza Vodă", Iaşi, în perioada 2000-2009

Anul	Nr. Cazuri	Cardiace		Scheletice		Sindrom Down		Altele	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
2000	11	4	36	3	28	0	0	4	36
2001	110	20	18	17	15	9	8	64	58
2002	115	31	27	27	23	3	3	54	47
2003	152	36	24	33	22	3	2	80	53
2004	221	45	20	56	25	6	3	114	52
2005	257	49	19	45	18	7	3	156	61
2006	243	55	23	38	16	9	4	141	58
2007	301	81	27	79	26	9	3	132	44
2008	143	49	34	13	9	7	5	74	52
2009	17	1	6	1	6	2	12	13	76

(*) Pentru valori mai mici de 10, proporţiile sunt orientative.

funcţie de frecvenţa efectuării screening-ului ecografic fetal (5).

În ţările cu experienţă în supravegherea ecografică a sarcinii s-a ajuns la concluzia că registrul de MCC nu aduce date suficiente privind valorile de prevalenţă deoarece unele cazuri subclinice rămân nediagnosticate (bicuspidia valvelor aortice), altele nu sunt înregistrate (malformaţiile grave care au determinat moartea intrauterină a fătului), iar diagnosticul prenatal a condus la întreruperea sarcinilor cu feţi suferind de MCC cu rezultate terapeutice incerte (sindromul de cord stâng hipoplazic). Cu toţi aceşti factori care influenţează prevalenţa MCC, indiferent de locul unde s-a evaluat, aceasta pare să fie de 5-8/1000 naşteri (5).

În România, absenţa efectuării, prin program, a screening-ului ecografic fetal, creează dificultate în a cunoaşte rata diagnosticului prenatal al malformaţiilor congenitale (6).

Astfel se estimează că se nasc anual, aproximativ 1.500-1.600 de copii cu MCC, dintre care, 850-950 necesită corecţie chirurgicală. Doar 30% din cazuri sunt rezolvate în România (7).

MATERIAL ŞI METODĂ

Cercetările au inclus 1570 de copii diagnosticaţi cu malformaţii congenitale (Mcg) în Maternitatea „Cuza Vodă" din Iaşi, în perioada 2000-2009.

Culegerea informaţiilor s-a realizat prin colaborarea cu serviciile de statistică medicală ale clinicii şi prin studierea registrelor de evidenţă a copiilor cu Mcg, din cadrul secţiei de neonatologie.

Cazurile incluse în studiu au fost analizate în raport cu: sexul, mediul de provenienţă (rural, urban), felul naşterii (naturală, cezariană), greutatea la naştere (<2700 g, >2700 g), vârsta gestaţională (<37 săptămâni, >37 săptămâni) şi scorul APGAR (<7, >7), urmat de evidenţierea ponderii fiecărui tip MCC şi decesele induse de acestea.

Prelucrarea şi interpretarea rezultatelor s-a realizat prin metode epidemiologice, clinice, statistico-matematice şi computerizate.

REZULTATE ŞI DISCUŢII

1. Ponderea MCC din totalul Mcg raportate la maternitatea "Cuza Vodă" Iaşi, în perioada 2000-2009.

În cei 10 ani luaţi în studiu au fost raportate 1570 cazuri de Mcg, dintre care 371 (24%) cardiace, 312 (20%) scheletice, 55 (3%) sindrom Down şi 832 (53%) alte patologii. S-a constatat o preponderenţă anuală a MCC. Astfel, am observat că cele mai multe afecţiuni de natură cardiacă au fost înregistrate în anul 2000 (36%) şi în 2008 (34%), pentru ca în ceilalţi ani prevalenţa

TABELUL II

Distribuţia anuală a cazurilor de malformaţii congenitale cardiace, în funcţie de mediul de proveniență şi sex, raportate la Spitalul Clinic „Cuza Vodă", Iaşi, în perioada 2000-2009

Anul	Nr. Cazuri MCC	Mediul de proveniență				Sex			
		Urban		Rural		M		F	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
2000	4	1	25	3	75	0		4	100
2001	20	9	45	11	55	9	45	11	55
2002	31	15	48	16	52	18	58	13	42
2003	36	19	53	17	47	19	53	17	47
2004	45	27	60	18	40	17	38	28	62
2005	49	25	51	24	49	23	47	26	53
2006	55	24	44	31	56	21	38	34	62
2007	81	45	56	36	44	44	54	37	46
2008	49	28	57	21	43	25	51	24	49
2009	1	1	100	0	0	1	100	0	0

(*) Pentru valori mai mici de 10, proporţiile sunt orientative.

cazurilor să înregistreze valori de 20%.

Raportându-mă la Mcg scheletice, acestea ocupă un loc imediat următor celor cardiace, însumând 20% din total, cu un maxim în anul 2000 (28%) şi un minim în 2009 (6%). Cele mai frecvente au fost displaziile congenitale de şold, piciorul strâmb congenital şi polidactilia sau sindactilia. Sindromul Down a înregistrat o creştere a prevalenţei în anii 2001(8%) şi 2009 (12%). Printre celelalte Mcg înregistrate, în ordinea frecvenţei s-au situat : hidrocefalia, cele renale, herniile diafragmatice (tab. I).

2. Repartiţia pe sex şi mediul de proveniență.

În cei 10 ani luaţi în studiu, din cei 371 de copii cu MCC, 194 (52%) au fost de sex feminin şi 177 (48%) masculin, 194 (52%) proveneau din mediul urban şi 177 (48%) din rural (tab. II).

3. Analiza repartiţiei în relaţie cu greutatea înregistrată la naştere şi vârsta gestaţională.

Din totalul de 371 de copii diagnosticaţi la naştere cu MCC, am observat că 205 (55%) au avut o greutate < 2700 grame, cu o prevalenţă crescută in anul 2008 (37%). Din cei 166 (45%) care au avut o greutate > 2700 grame, s-a înregistrat o creştere semnificativă în anul 2001 (90%).

În aceeaşi perioadă de 10 ani, 154 (42%) din copii au fost prematuri (< 37 de săptămâni) şi 217 (58%) cu naşteri la termen (> 37 de săptămâni). Cele mai multe cazuri de prematuritate s-au înregistrat în anul 2006 (69%) şi cele mai puţine în 2004 (20%), iar pentru copiii născuţi la termen valorile prevalenţei au fost de 31% în anul 2006 şi 80% în 2004 (tab. III).

3. Repartiţia în funcţie de felul naşterii şi scorul APGAR acordat la naştere.

Luând în consideraţie evaluarea medicală a nou-născutului la 60 de secunde după expulzie, care apreciază respiraţie spontană, frecvenţa cardiacă, culoarea tegumentelor, excitabilitatea reflexă şi tonusul muscular, au fost analizate nivelurile Scorului Apgar.

În cazul nostru copiii care de la naştere au prezentat suferinţă fetală (APGAR <7), au înregistrat o prevalenţa de 10 % în anul 2001 şi 64 % în 2004.

Din totalul copiilor diagnosticaţi cu MCC, 267 (72%) au fost născuţi natural, iar 104 (28%) prin cezariană, diferenţa fiind puternic semnificativă ($p<0,005$), deoarece în anul 2003 s-au înregistrat 92% de naşteri naturale şi 8% prin cezariană. Totuşi, în anul 2008, prevalenţa naşterilor prin cezariană a fost de 73% faţă de 27% cea a naşterilor naturale ($p=0,05$).

TABELUL III

Distribuţia anuală, în raport de greutatea la naştere şi vârsta gestaţională a cazurilor de malformaţii congenitale cardiace, raportate la Spitalul Clinic „Cuza Vodă", Iaşi, în perioada 2000-2009

Anul	Nr. Cazuri MCC	Greutate la naştere (gr.)				Vârstă gestațională (săpt.)			
		< 2700		> 2700		< 37		> 37	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
2000	4	2	50	2	50	2	50	2	50
2001	20	2	10	18	90	8	40	12	60
2002	31	6	19	25	81	11	35	20	65
2003	36	7	19	29	81	11	31	25	69
2004	45	3	7	42	93	9	20	36	80
2005	49	4	8	45	92	28	62	21	38
2006	55	18	33	37	67	38	69	17	31
2007	81	18	22	63	78	26	32	55	68
2008	49	18	37	31	63	21	43	28	57
2009	1	0	0	1	100	0	0	1	100

(*) Pentru valori mai mici de 10, proporţiile sunt orientative

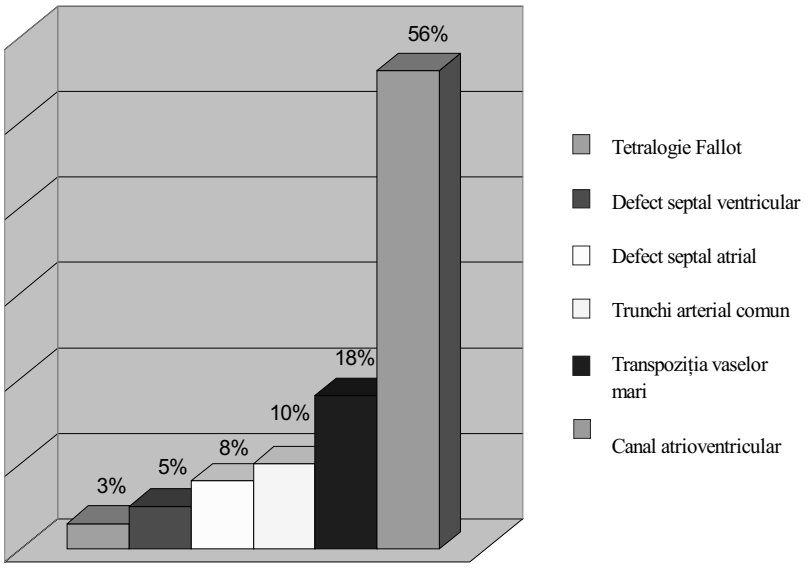

Fig. 1. Repartiţia pe tipuri a malformaţiilor congenitale înregistrate în serviciul de neonatologie al Spitalul Clinic „Cuza Vodă", Iaşi, în perioada 2000-2009

4. Distribuţia cazurilor de malformaţii congenitale cardiace în relaţie cu natura acestora.

Cele mai frecvente cazuri de MCC studiate în cei 10 ani, au fost: canal atrioventricular (56%), transpoziţia vaselor mari (18%), trunchi arterial comun (10%), defect septal atrial (8%), defect septal ventricular (5%) şi Tetralogia Fallot (3%)(Fig.1).

6. Decesele înregistrate la copiii cu malformaţiilor congenitale.

Din totalul deceselor înregistrate în cei 10 ani, majoritatea au fost reprezentată de cele cu patologie cardiacă (66%), urmate

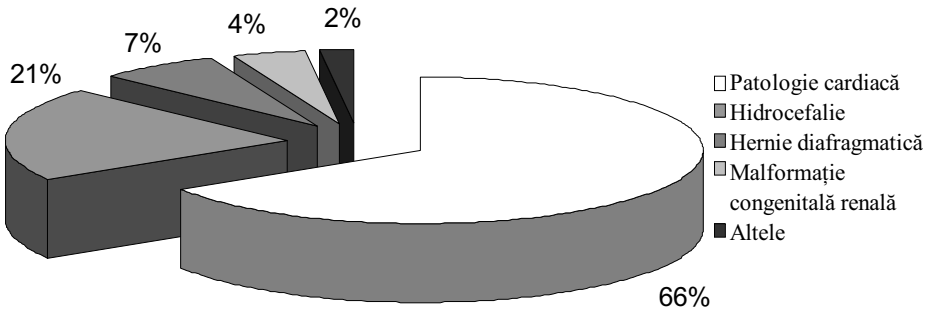

Fig. 2. Frecvenţa deceselor în cazul copiilor cu malformaţii congenitale înregistrate în serviciul de neonatologie al Spitalul Clinic „Cuza Vodă", Iaşi, în perioada 2000-2009

de hidrocefalie (21%), hernie diafragmatică (7%), malformaţie congenitală renală (4%) şi altele (2%) (Fig. 2).

CONCLUZII

În perioada 2000-2009 au fost înregistraţi 1570 copii cu malformaţii congenitale, dintre care 371 (24%) cardiace, 312 (20%) scheletice, 55 (3%) sindrom Down şi 832 (53%) alte patologii.

Din cei 371 de copii diagnosticaţi cu MCC, 48% au fost de sex masculin si 52% de sex feminin, 52% din mediul urban şi 48% din rural. S-a evidenţiat o prevalenţă de 42% în cazul naşterilor premature, 58% la termen; 55% copii cu greutate < 2700 grame şi 45% copii cu greutate > 2700 grame; scorul APGAR acordat la naştere a fost de 38% în cazul celor cu o valoare < 7 şi de 62 % la cei cu valoare > 7; din totalul naşterilor 72% au fost naturale şi 28% prin cezariană.

MCC mai frecvent întâlnite printre cazurile studiate, au fost: canal atrioventricular (56%), transpoziţia vaselor mari (18%), trunchi arterial comun (10%), defect septal atrial (8%), defect septal ventricular (5%) şi Tetralogie Fallot (3%). Din totalul deceselor înregistrate în cei 10 ani, 66% au fost cele cu patologie cardiacă, 21% hidrocefalie, 7% hernie diafragmatică, 4% malformaţie congenitală renală şi 2% altele.

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MORUSIN AND KUWANON G – PROMISING ANTI-MRSA AGENTS

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MORUSIN AND KUWANON G-PROMISING ANTI-MRSA AGENTS (Abstract): Synergy between natural products and antibiotics represent a promising strategy in the development of new antimicrobial therapies. **Aim:** The present work investigated possible synergistic interactions between prenylated flavones morusin and kuwanon G and antibiotics (tetracycline, ciprofloxacin) against methicillin-resistant *Staphylococcus aureus* (MRSA). **Material and Methods**: The minimum inhibitory concentrations (MICs) of prenylated flavones and antibiotics against MRSA (MRSA ATCC 33591 and four MRSA isolates of animal origin) were determined by the broth microdilution method. The interactions between prenylated flavones and antibiotics were evaluated by the checkerboard and time-kill assays. **Results:** In the broth microdilution assay, morusin and kuwanon G showed very good antibacterial activity (MICs ranging 3.13 and 12.50 µg/mL). The checkerboard assay demonstrated that morusin and kuwanon G act synergistically with both antibiotics; significant reductions in antibiotic MICs (2 to 512-fold) were recorded. The time-kill assay (at ½MIC) indicated bactericidal synergy for the combinations of morusin or kuwanon G with ciprofloxacin against *S. aureus* ATCC 33591. **Conclusions:** The combination of morusin or kuwanon G with antibiotics are good candidates for the development of novel treatments for MRSA infections in humans and animals. **Keywords**: METHICILLIN – RESISTANT *STAPHYLOCOCCUS AUREUS*, SYNERGY, ANTIBIOTICS, MORUSIN, KUWANON G.

Nowadays the treatment of infectious diseases has to overcome a serious problem – antibiotic resistance. One great concern is methicillin-resistant *Staphylococcus aureus* (MRSA) due to its resistance to beta-lactams, including cephalosporins, but also aminoglycosides, macrolides, tetracyclines and fluoroquinolones (1, 2, 3).

MRSA transmission from animals to humans and *vice versa* is of great interest (4, 5). Considering the human exposure to livestock (6) and pet animals (7), but also the consumption of animal products (8), many researches focused on animals as sources of antimicrobial-resistant bacteria (9). The potential transfer of resistance genes from animals to humans is facilitated by the irrational antibiotic use not only for

medical purpose (treatment of animal infections), but also for growth promotion and prophylactic purposes (sub-therapeutic antibiotic doses) (10). In both humans and animals, MRSA strains are difficult to treat pathogens.

A promising strategy in the management of MRSA infections is the synergism between natural products and conventional antibiotics (11).

The present work investigated possible synergistic interactions between two prenylated flavones, morusin (MO) and kuwanon G (KG), and tetracycline (TE) or ciprofloxacin (CIP) against MRSA strains. Both prenylated flavones (fig. 1) are active constituents of *Morus* spp. (mulberry) and antioxidant (12), anti-inflammatory (13) and antibacterial (14, 15) activities have been reported for them.

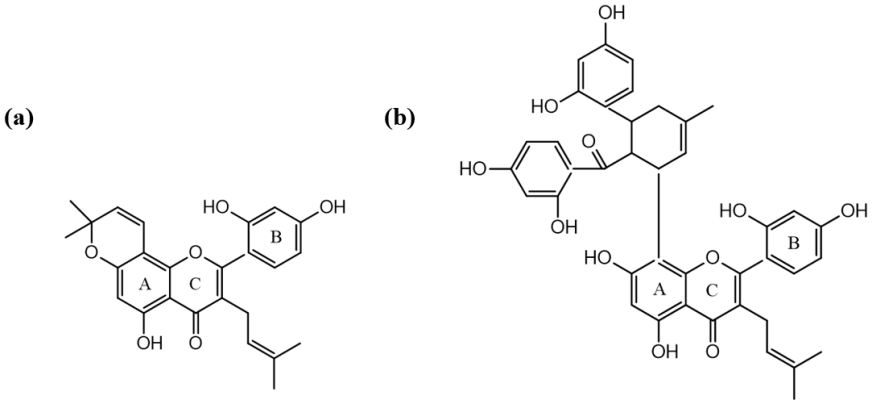

Fig. 1. Morusin (a) and kuwanon G (b)

MATERIAL AND METHODS

Methicillin-resistant *S. aureus* ATCC 33591 strain and four MRSA isolates (MRSA T1–T4) collected from various infections (recurrent otitis, pyoderma, laryngo-pharyngitis) in dogs were used in this study. The isolates were identified and characterized phenotypically by culture, hemolysis, catalase and coagulase tests and other enzymatic tests; their identity was confirmed by molecular tests (Polymerase Chain Reaction) (16). Minimum inhibitory concentrations (MICs) of prenylated flavones and antibiotics were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) (17) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (EUCAST) (18). Checkerboard method (19) and time-kill assay (20) were performed in order to evaluate the interactions between prenylated flavones and antibiotics.

The results in checkerboard method were interpreted on the basis of the fractional inhibitory concentration index (FICI) values, isobolograms and response surface approach (ΔE model) (19). For each combination, FICI was calculated as follows: $FICI = FIC_{antibiotic} + FIC_{prenylated\ flavone}$ where $FIC_{antibiotic} = MIC_{antibiotic\ in\ combination\ with\ prenylated\ flavone} / MIC_{antibiotic\ alone}$ and $FIC_{prenylated\ flavone} = MIC_{prenylated\ flavone\ in\ combination\ with\ antibiotic} / MIC_{prenylated\ flavone\ alone}$.

A combination is deemed to have a synergistic effect if $FICI \leq 0.5$, additive effect if $0.5 < FICI \leq 1$, indifferent effect if $1 < FICI \leq 4$ and antagonistic effect if $FICI > 4$ (20).

In isobolograms, FIC values of antibiotics and prenylated flavones were represented on the *x* and *y* axis, respectively. The type of interaction is defined depending on the area the ratio points fall in. Synergistic, additive and indifferent interactions are expressed if the ratio points fall below the 0.5 : 0.5, 1 : 1 and 4 : 4 lines, respectively. The ratio points falling above the 4 : 4-line express antagonistic interactions (11).

The results of the checkerboard assay were also interpreted using the response surface approach (ΔE model) which compares the experimental data with theoretical data (predictive data) indicating the statistical significance of the interactions (19, 21).

The time-kill assay was performed for the combinations showing synergistic interactions in the checkerboard assay; the prenylated flavones and antibiotics were tested alone and in combination at sub-inhibitory concentrations (½MIC). The bacterial colonies (CFU/mL) were counted after 0, 4, 24 and 48 hrs. incubation at 37°C. An interaction is defined synergistic if the increase in bacterial killing induced by the combination in comparison to the most active component of the combination, tested alone, after 24 hrs. incubation (ΔLC_{24}), expressed as log10 CFU/mL, is equal or higher than 2 ($\Delta LC_{24} \geq 2$ log10 CFU/mL). Addition, indifference and antagonism are expressed if $\Delta LC_{24} = 1 - 2, \pm 1$ and > -1 log10 CFU/mL, respectively (22). Killing curves were also represented by plotting the viable colony counts (log10 CFU/mL) against time.

RESULTS AND DISSCUSION

In this study, both MO and KG showed very good antibacterial activity against *S. aureus* ATCC 33591 with MIC values of 6.25 and 12.50 µg/mL, respectively. In previous investigations, we assessed the antibacterial effects of MO and KG against MRSA T1 – T4 isolates and found MIC values ranging from 3.13 to 12.50 µg/mL (23, 24).

TABLE I
The median values of MIC (µg/mL) for antibiotics and susceptibility* of bacterial strains

Strain	MIC_{CIP}	Susceptibility to CIP	MIC_{TE}	Susceptibility to TE
S. aureus ATCC 33591	0.50	S	128	R
MRSA T1	0.25	S	0.25	S
MRSA T2	0.13	S	16	R
MRSA T3	0.50	S	2	S
MRSA T4	0.50	S	32	R

*according to CLSI 2017; CIP – ciprofloxacin, TE – tetracycline, (S) – susceptible, (R) – resistant

The antibacterial effects of prenylated flavones in combination with antibiotics were further evaluated using the checkerboard method. According to FICI interpretation, combinations of MO or KG with TE showed synergistic and additive interactions against *S. aureus* ATCC 33591 and MRSA isolates (tab. II and III). The corresponding isobolograms are depicted in figure 2a and 2b. According to CLSI susceptibility breakpoints, S *aureus* ATCC 33951, MRSA T2 and T4 isolates proved to be resistant to TE (tab. I). It is worth mentioning that KG reversed TE resistance of *S. aureus* ATCC 33591 and MRSA T4, whereas MO reversed TE resistance of MRSA T2; in combination with prenylated flavones, MIC of TE was decreased below the CLSI susceptibility breakpoint of 4 µg/µL (17). The prenylated flavones increased the susceptibility to TE of MRSA T1 and T3 causing 2 to 4-

fold reduction in MIC value of TE. With respect to combinations with CIP, MO and KG showed synergistic and additive interactions; the prenylated flavones increased susceptibility of all strains causing 2 to 16-fold reduction in MIC of CIP (tab. II and III; fig. 2c and 2d).

All synergisms found by FICI interpretation were confirmed by ΔE model. The additive interactions assed by FICI model were interpreted as indifference in ΔE model (tab. II and III).

TABLE II

Interactions between MO and antibiotics

Agent	Checkerboard assay							Time-kill assay		
					ΔE model*					
	[A]MIC	DRI	FICI	INT	∑SYN (n)	∑ANT (n)	INT	Ma	ΔLC24**	INT
➢ *S. aureus* ATCC 33591										
TE	16	8	0.25	S	3703.08 (62)	0 (0)	S	MO	1.17 ± 0.03	Ad
MO	0.78	8								
CIP	0.03	16	0.19	S	4285.14 (66)	0 (0)	S	CIP	2.02 ± 0.09	S
MO	0.78	8								
➢ MRSA T1										
TE	0.06	4	0.38	S	120.17 (12)	0 (0)	S	TE	0.60 ± 0.10	I
MO	0.78	8								
CIP	0.13	2	0.52	Ad	40.46 (16)	0 (0)	I	ND		
MO	0.10	64								
➢ MRSA T2										
TE	1	16	0.19	S	117.96 (10)	0 (0)	S	MO	0.55 ± 0.05	I
MO	0.78	8								
CIP	0.03	4	0.31	S	124.98 (40)	0 (0)	S	CIP	0.87 ± 0.00	I
MO	0.39	16								
➢ MRSA T3										
TE	0.50	4	0.28	S	153.92 (16)	0 (0)	S	TE	0.90 ± 0.03	I
MO	0.10	32								
CIP	0.13	4	0.37	S	236.10 (33)	0 (0)	S	CIP	1.06 ± 0.03	Ad
MO	0.39	8								
➢ MRSA T4										
TE	16	2	0.52	Ad	40.02 (40)	0 (0)	I	ND		
MO	0.10	64								
CIP	0.25	2	0.53	Ad	76.32 (29)	0 (0)	I	ND		
MO	0.20	32								

Note: [A]MIC in combination (μg/mL), DRI – dose reduction index, FICI – fractional inhibitory concentration index (median of three FICI calculated for three independent experimental determinations), INT – interpretation, Ma – most active agent of combination, Ad – addition, S – synergism, I – indifference, TE – tetracycline, CIP – ciprofloxacin, MO – morusin, ND – not determined

*n, number of combinations (amongst the 70 combinations for each strain) with statistically significant synergism ∑SYN or antagonism ∑ANT

**The increased killing colony counts of the combination between antibiotic and prenylated flavone compared to the most active single agent expressed in $\log_{10}$ CFU/mL at 24h of incubation (mean ± standard deviation)

TABLE III

Interactions between KG and antibiotics

Agent	Checkerboard assay							Time-kill assay		
					ΔE model*					
	[A]MIC	DRI	FICI	INT	∑SYN (n)	∑ANT (n)	INT	Ma	ΔLC24**	INT
➢ *S. aureus* ATCC 33591										
TE	0.25	512	0.50	S	4000.00 (15)	0 (0)	S	KG	0.18 ± 0.03	I
KG	6.25	2								
CIP	0.13	4	0.28	S	3602.06 (60)	0 (0)	S	CIP	2.63 ± 0.21	S
KG	0.39	32								
➢ MRSA T1										
TE	0.13	2	0.52	Ad	0 (0)	- 30.38 (10)	I	ND		
KG	0.02	64								
CIP	0.02	16	0.31	S	172.78 (44)	0 (0)	S	CIP	1.17 ± 0.05	Ad
KG	3.13	4								
➢ MRSA T2										
TE	8	2	0.53	Ad	10.03 (4)	0 (0)	I	ND		
KG	0.20	32								
CIP	0.03	4	0.50	S	110.18 (3)	0 (0)	S	CIP	1.07 ± 0.01	Ad
KG	1.56	4								
➢ MRSA T3										
TE	0.50	4	0.27	S	102.04 (6)	0 (0)	S	TE	0.79 ± 0.03	I
KG	0.20	64								
CIP	0.25	2	0.52	Ad	21.78 (4)	0 (0)	I	ND		
KG	0.20	64								
➢ MRSA T4										
TE	0.25	128	0.51	Ad	28.06 (12)	0 (0)	I	ND		
KG	6.25	2								
CIP	0.25	2	0.52	Ad	76.32 (7)	0 (0)	I	ND		
KG	0.20	64								

Note: [A]MIC in combination (μg/mL), DRI – dose reduction index, FICI – fractional inhibitory concentration index (median of three FICI calculated for three independent experimental determinations), INT – interpretation, Ma – most active agent of combination, Ad – addition, S – synergism, I – indifference, TE – tetracycline, CIP – ciprofloxacin, KG – kuwanon G, ND – not determined

*n, number of combinations (amongst the 70 combinations for each strain) with statistically significant synergism ∑SYN or antagonism ∑ANT

**The increased killing colony counts of the combination between antibiotic and prenylated flavone compared to the most active single agent expressed in $\log_{10}$ CFU/mL at 24h of incubation (mean ± standard deviation)

The time-kill assay confirmed only the synergisms between MO or KG and CIP against *S. aureus* ATCC 33591 (tab. II and III; fig. 3a and 3b). The other combinations, found to be synergistic in the checkerboard method, showed additive or indifferent kinetics in the time-kill assay.

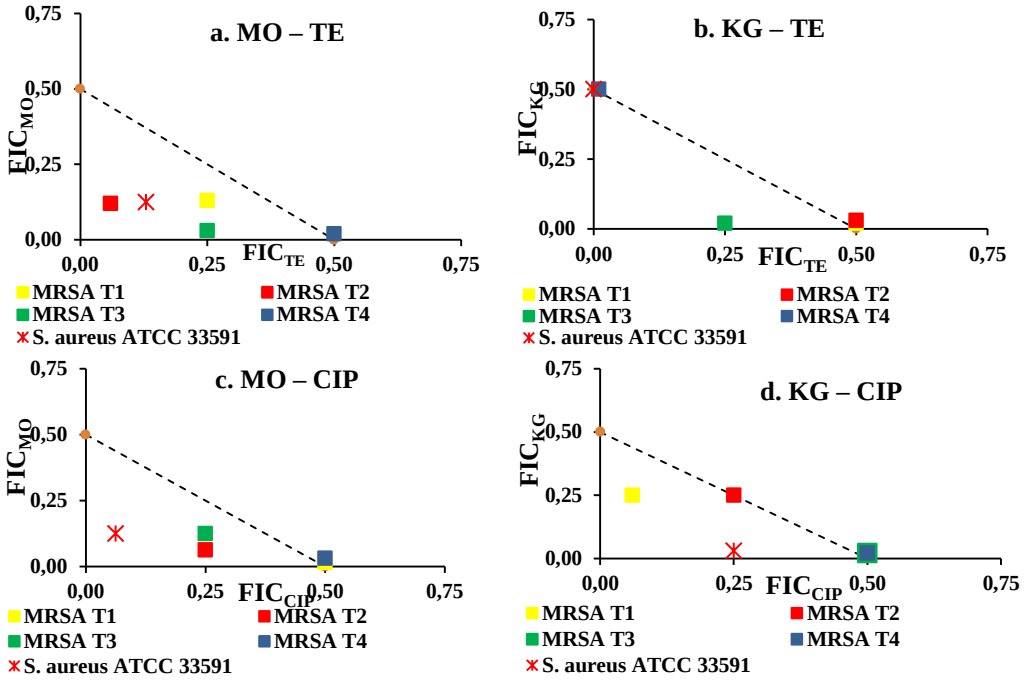

Fig. 2. Isobolograms depicting the interactions between MO or KG and antibiotics

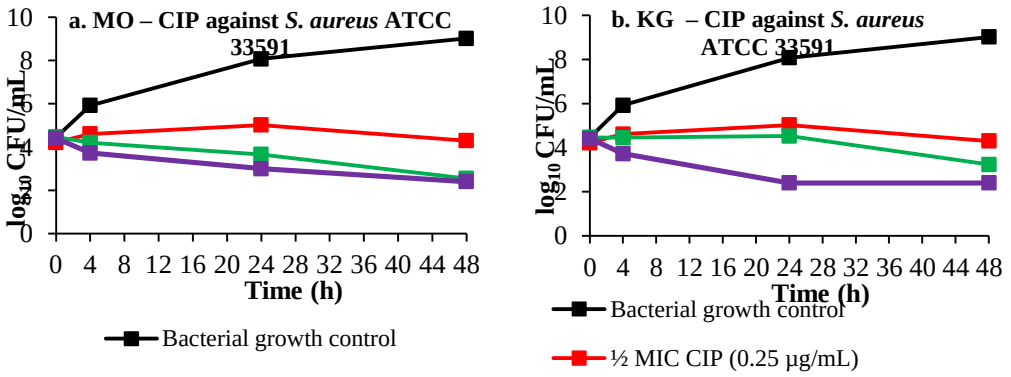

Fig. 3. Time-kill curves of MO or KG and CIP alone and in combination against *S. aureus* ATCC 33591

The interactions between prenylated flavones and antibiotics have been rarely mentioned in literature. Zuo *et al*. (2018) have recently found that four prenylflavonoids isolated form ethanolic mulberry root bark (cyclocommunol, morusinol, morusin and kuwanon E) reversed MRSA resistance to aminoglycosides, especially to amikacin (25). We have previously reported synergism between

MO or KG and other antibiotics (oxacillin, amoxicillin, erythromycin, gentamicin) against the same MRSA isolates collected from infections in dogs (MRSA T1-T4) (23, 24).

CONCLUSIONS

The combination of MO or KG with antibiotics is a promising strategy for the development of novel treatments for MRSA infections in both animals and humans.

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NEWS

12-YEAR FOLLOW-UP STUDY ON LONG-TERM EFFECT OF OUTDOOR AIR POLLUTION ON MORTALITY AND MORBIDITY IN FRANCE

It has been shown that air pollution has a short and long-term impact on human's health. Air pollution is a major risk factor for respiratory and cardiovascular morbidity and mortality. The impact on the respiratory system: exacerbations in chronic respiratory diseases like bronchitis, COPD, asthma, and reduced lung function due to exposure to various ambient air pollutants. This study evaluated the long-term association of the major air pollutants (PM10, PM2.5, NO2, O3) with morbidity and mortality in metropolitan France, based on preexisting data. The results show that cardiovascular and respiratory morbidity were related to NO2, along with PM2.5 and PM10. Also, exposure to NO2 and O3 could also lead to increased health risks (Shreosi Sanyal, Thierry Rochereau , Cara Nichole Maesano, *et al*. Long-Term Effect of Outdoor Air Pollution on Mortality and Morbidity: A 12-Year Follow-Up Study for Metropolitan France. http://dx.doi.org/10.3390/ijerph15110000).

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CORELATIONS BETWEEN THE ENT MODIFICATIONS AND BMI IN PATIENTS WITH SLEEP BREATHING DISORDERS

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CORELATIONS BETWEEN THE ENT MODIFICATIONS AND BMI IN PATIENTS WITH SLEEP BREATHING DISORDERS (Abstract): **Aim**: To show if between the BMI index of weight and the oro and hypopharingeal modifications we can establish a link for the same value of the index of AHI hypoapnea. **Material and method**: At the patients with sleep breathing pathology of an obstructive type we conducted nasal, oro- and hypopharingeal endoscopic examinations, measurements and calculation of the weight index, followed by the polygraphic evaluation of the sleep, the establishment of the degree of sleep obstructive apnea; we used a form of ENT examination. The classification of patients according to the value of the apnea index of AHI hypoapnea in 3 groups: AHI < 10, 10 < AHI < 50, and AHI > 50. According to the value of the BMI weight index we analyze 3 groups of patients : BMI < 27, 27 < BMI < 40, BMI > 40. **Results**: For AHI < 10, habitual snoring we can notice that normal amygdales appear in an increased percent for BMI < 27 or BMI > 40 and AHI < 10 the increased webbing is specific for 27 < BMI < 40. For AHI > 50 the occurring oropharingeal modifications are: normal amygdales, long and broad uvula and webbing spanning between 5-10 mm. **Conclusion**: Oropharingeal modifications and the index of BMI weight are essential elements, taken individually in the preoperatory evaluation of patients with syndrome of obstructive sleep apnea. **Key words**: OBSTRUCTIVE SLEEP APNEA, BMI INDEX, AHI INDEX

The partial or total obstruction of the upper breathing system during sleep determines the apparition of the syndrom of obstructive sleep apnea. The reduction of muscular tonus represents an important factor in the apparition of this pathology (1).

The oro and hypopharingeal structural modifications are essential elements in explaining the etiology of the syndrome of obstructive sleep apnea. To this we can add the influence played by the neck's greasy tissue in the link of the upper breathing ways following the external compression of this upon the pharyngeal walls (2,3,4). Obesity represents an important factor in the apparition of the obstruction of upper breathing ways during sleep, hypoventilation and desaturation, as well as in the apparition of arterial hypertension in patients with the syndrome of sleep apnea.

It is known that both BMI index as well as oropharingeal modifications represent

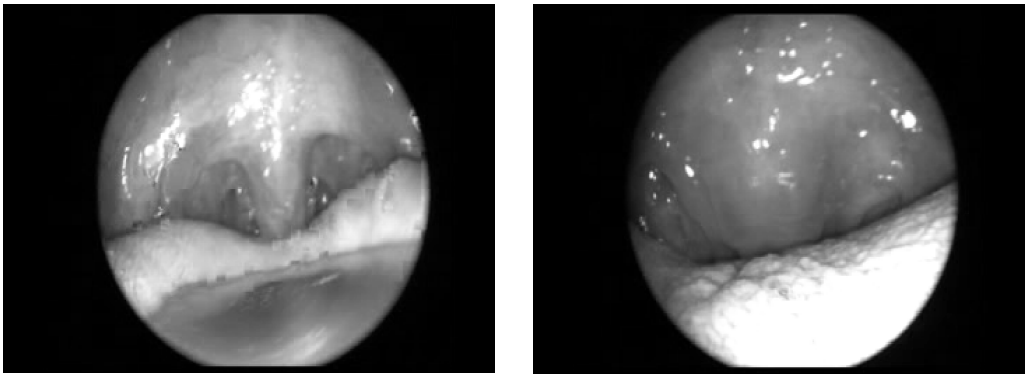

Fig. 1. Endoscopic aspect of the oropharingeal modifications

Fig. 2. Polygraphic recording

individually determining factors of the syndrome of sleep apnea (5,6,7,8). What is not known is whether between the two parametres for the same value of the apnea index there is or is not a link worth taking into consideration.

The aim of this study was to show if between the BMI index of weight and the oro and hypopharingeal modifications we can establish a link for the same value of the index of AHI hypopneous apnea, or if the two parametres have an importance only individually and a significant relationship cannot be established between them.

MATERIAL AND METHOD

The realisation of this study had at its base the examination of some patients with sleep breathing pathology of an obstructive type. In these patients we conducted nasal, oro and hypopharingeal endoscopic examinations, measurements and calculation of the weight index, followed by the polygraphic evaluation of the sleep and the establishment of the degree of sleep obstructive apnea. In view of the evaluation of oro and hipopharingeal modifications, we used a form of ENT examination. Through the filling of this examination questionnaire, in view of the study we selected patients according to the growth of the amygdales, this way : normal ones, hypertrophied ones and massively hypertrophied ones, according to the growth of the uvula – long and broad uvula, as well as according to the value of the webbing : webbing between 5-10 mm and webbing greater than than 10 mm (fig. 1).

The polygraphic recording done allowed the classification of patients according to the value of the apnea index of AHI hipoapnoea in 3 groups : $AHI < 10$, $10 < AHI < 50$, and $AHI > 50$ (fig. 2).

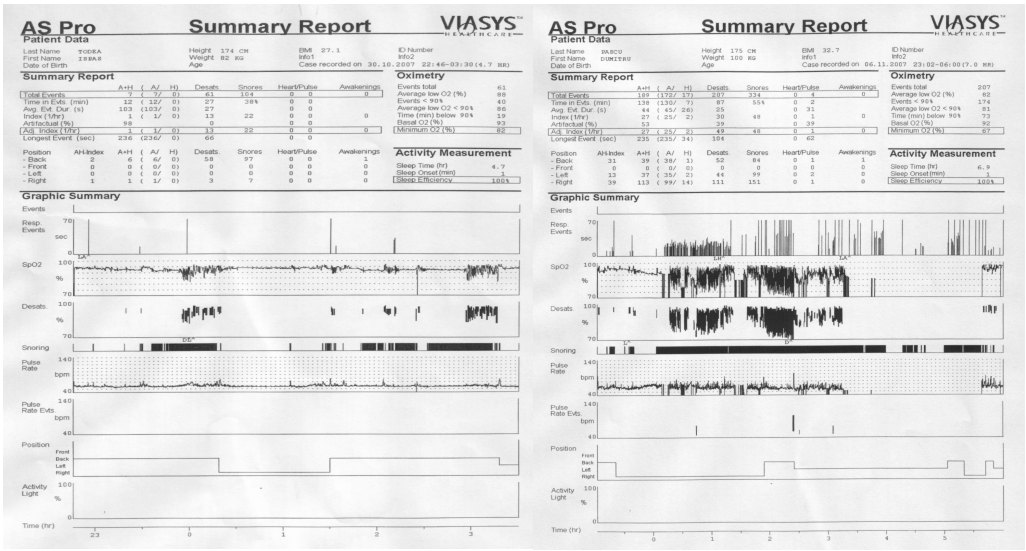

Fig. 3. Polygraphic result of the evaluation of the BMI index

The classification of the patients was also done according to the value of the BMI weight index in 3 groups: BMI < 27, 27 < BMI < 40, and BMI > 40 (fig. 3).

For the same value of the AHI index the values of the BMI weight index were correlated with the frequency in percents of the growth of amygdales, uvula and the webbing index.

RESULTS AND DISCUSSIONS

For AHI < 10, habitual snoring (fig. 4) we can notice that normal amygdales appear in an increased percent only for BMI < 27 or BMI > 40.

The long and broad uvula is specific for BMI < 27 and in a smaller percentage for 27 < BMI < 40.

For 10 < AHI < 50 (fig. 5) equivalent to the syndrome of incipient and moderate obstructive apnea we can conclude the following: normal amygdales as growth appear both for patients with BMI < 27 as well as for patients with BMI > 40, amygdaline hypertrophy being more frequent in patients with 27 < BMI < 40.

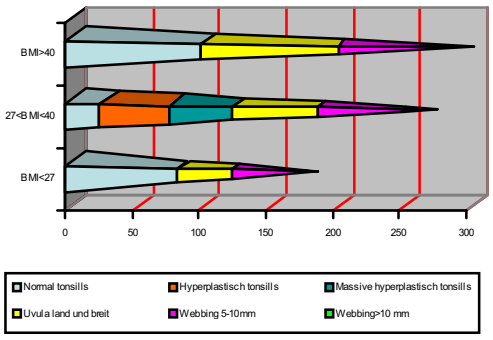

Fig. 4. The correlation between the oropharingeal modifications and the BMI index for value AHI < 10

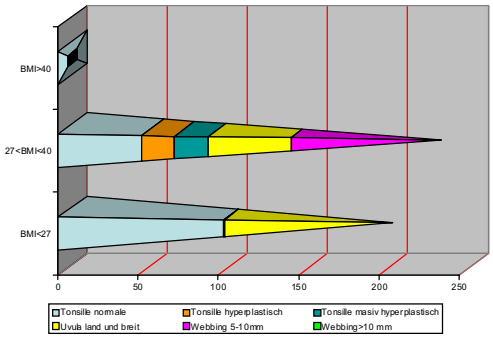

Fig. 5. The correlation between oropharingeal modifications and the BMI index for value 10 < AHI < 50

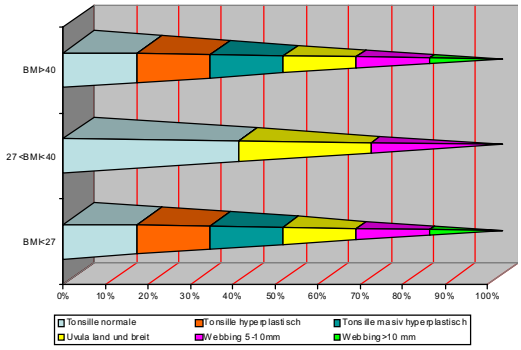

Fig. 6. The correlation between the oropharingeal modifications and the BMI index for value AHI > 50

The long and broad luette appears more frequently in patients with BMI : 40, the webbing between 5-10 mm occurring in a comparatively equal percentage with BMI and more increased at BMI > 40, while the webbing > 10 mm is specific for 27 < BMI < 40.

In such situations for AHI < 50 corresponding to a syndrome of severe obstructive apnea making a comparative analysis of the results we can say that between the growth of the amygdales and the value of the BMI index there is not a direct link, while the webbing spanning from 5-10 mm increases proportionally with the frequency with BMI.

For AHI > 50 (fig. 6) the occurring oropharingeal modifications are : normal amygdales, long and broad uvula and webbing spanning between 5-10 mm. Their values are significant only for 27 < BMI < 40.

CONCLUSIONS

Oropharingeal modifications and the index of BMI weight are essential elements, taken individually in the preoperatory evaluation of patients with syndrome of obstructive sleep apnea.

For the same value of the AHI index of apnoea-hipopnoea thus for the same pathological clinical status there are great variations between the oropharingeal modifications and the BMI weight index without being able to say that in patients with snoring and syndrom of severe obstructive apnea once with the growth of BMI index the oropharingeal parametres are modified proportionally, increasing in frequency. For the syndrome of incipient and moderate obstructive apnea these modifications pass through a quite constant modification which is essential in the preoperatory evaluation of this category of patients.

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O COMPARAŢIE ÎNTRE VALOAREA PSA TOTAL, SCORUL GLEASON ŞI REZULTATELE SCINTIGRAFIEI OSOASE LA DIFERITE GRUPE DE VÂRSTĂ

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A COMPARAISON BETWEEN THE TOTAL PSA, THE GLEASON SCORE AND THE BONE SCINTISCAN RESULTS FOR DIFFERENT AGE GROUPS (Abstract): The aim of the study is to compare de results of the bone scintigraphy of prostate cancer patients in different age groups with their total PSA (PSAt) and with their histopathological test results. **Materials and metods**: Of the 180 patients with prostate cancer who were analyzed by Scintiscan in the last five years in our laboratory, 86 have a known PSAt value, and of these, 55 have a known Gleason Score. The 180 patients were divided into three age groups: 1) < or equal 60 years; 2) 60-70 years and 3) >70 years. The 86 patients with a known PSAt were also divided into five groups according to their PSAt value and the 55 patients with a known Gleason Score were divided in three groups. The scintigraphy results included all the patients in 3 groups, according to the presence, the absence or the likelihood of having bone metastasis. The 33 patients with proven bone metastasis were divided, according to their numbers, into the four Soloway groups. **Results**: Among the patients with PSAt > 20 ng/mL, considered high risk for bone metastases, according to the Recomandations of CCAF, 21 (32.81%) of the 64 patients do not show the presence of bone metastases. For PSAt > 50 ng/mL, all 5 patients < 60 years of age have metastases, while only 15 (62.5%) of the 24 patients over 70 years old have metastases and 1 (4%) has low likelihood. 6 (21.42 %) of the 28 patients with PSAt > sau egal 100 ng/mL do not have metastases. 10 (43.4 %) of the 23 patients with a Gleason Score <7, considered low risk, do have metastases (6 patients – 26%) or low probability of metastases (4 patients – 17.4%). **Conclusions**: Our study confirms that the probability of bone metastasis for a high PSAt value is reversely proportional to age. In our study there is no direct correlation between Gleason Score and the bone scan results.
Key words: PROSTATE CANCER, BONE SCAN, PSAt, GLEASON SCORE

KEY FACTORS FOR MINI-IMPLANT SUCCESS: THE CRITICAL ROLE OF CORTICAL BONE THICKNESS AND ORTHODONTIC FORCES IN ENHANCING STABILITY AND PREVENTING FAILURE

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KEY FACTORS FOR MINI-IMPLANT SUCCESS: THE CRITICAL ROLE OF CORTICAL BONE THICKNESS AND ORTHODONTIC FORCES IN ENHANCING STABILITY AND PREVENTING FAILURE (Abstract): This study **aims** to analyze the biomechanical performance of titanium (Ti6Al4V) mini-implants (MIs) under orthodontic forces using finite element analysis (FEA) and to evaluate stress, strain, and displacement behavior within cortical and spongy bone under realistic clinical conditions. **Materials and methods:** A three-dimensional finite element model of a mini-implant (2.0 mm diameter, 12 mm length) was developed using CT-based reconstruction of the mandible and periodontal ligament. The model was meshed with tetrahedral elements and simulated in ANSYS Workbench 19.2. Material properties for bones, teeth, ligament, and implant were defined as linear elastic, homogeneous, and isotropic. Orthodontic forces of 2N and 10N were applied at a 30° angle to replicate molar intrusion scenarios. Stress, strain, and displacement distributions were analyzed for cortical and spongy bone, with special attention to localized areas of maximum impact. **Results:** Maximum von Mises stress and equivalent strain values were localized around the implant insertion site. Cortical and spongy bone demonstrated high resistance to applied forces, with stress and strain levels remaining below their fracture thresholds. The results highlighted the critical role of cortical bone thickness in ensuring primary stability. Displacement was minimal, with a maximum value of 0.028948 mm at the implant head under a 2N force, demonstrating functional stability. Forces exceeding 3N were identified as the threshold for potential spongy bone failure, reinforcing the appropriateness of 2N for orthodontic applications. **Conclusions:** The study confirms that mini-implants can effectively withstand orthodontic forces when inserted into adequate cortical bone thickness. The integration of finite element modeling with clinical insights provides valuable guidance for optimizing mini-implant performance and minimizing adverse effects. These findings contribute to improving treatment strategies and ensuring the longevity of orthodontic anchorage systems. **Keywords:** FINITE ELEMENT METHOD (FEM); FRACTURE; MINI-IMPLANT; ORTHODONTIC FORCES; STRESS DISTRIBUTION; BONE-IMPLANT INTERACTION.

INTRODUCTION

Cortical bone serves as a structural barrier during orthodontic tooth movement, and its thickness can significantly affect the mechanical stress experienced by the bone when orthodontic forces are applied. For

instance, studies have shown that the stress in cortical bone can increase dramatically in response to orthodontic forces, potentially leading to microfractures and bone loss, especially in areas where the bone is already thin (1). The remodeling of alveolar bone around moving teeth is a well-documented phenomenon in orthodontics, yet the specific impact of cortical bone thickness on this process remains underexplored (1). Understanding this relationship is essential, as inadequate bone support can lead to mini-implant failure, which has been reported to occur in up to 28% of cases due to insufficient cortical bone quality and quantity (2). Moreover, the application of orthodontic forces must be carefully calibrated to avoid overloading the cortical bone, which can result in adverse outcomes such as periodontal tissue loss and compromised tooth stability (3, 4). The interaction between orthodontic forces and cortical bone thickness is particularly relevant when considering the use of temporary anchorage de-vices (TADs), where the primary stability of mini-implants is heavily influenced by the surrounding bone characteristics (5, 6). Research indicates that the quality and thickness of cortical bone at the insertion site are critical determinants of the success of these devices (7, 8).

The simultaneous study of cortical bone thickness and orthodontic forces is critical for advancing orthodontic practices and improving patient outcomes. A significant gap in current orthodontic research lies in the understanding of how variations in cortical bone thickness influence the response to orthodontic forces, particularly in the context of mini-implants used for anchorage.

Moreover, patient-related factors such as age, oral hygiene, and systemic health conditions can significantly influence the success rates of mini-implants (9). The impact of mechanical factors on bone stress and strain in orthodontic mini-implants is a critical area of study that integrates biomechanics, material science, and biological responses. Experimental research in orthodontics today aims to investigate the usefulness of recent technology and advancements into predictable methodology by incorporating tools such as finite element analysis (FEA), 3D CAD CAM, low-level laser therapy (LLLT) and digital simulations (10-12). These technologies enable more accurate predictions of treatment outcomes, better understanding of biomechanical forces, and the development of customized, patient-specific approaches.

Through these advancements, researchers can refine diagnostic techniques and optimize the performance of orthodontic appliances, making treatments more efficient, reliable, and less invasive. Research has shown that the insertion angle of mini-implants plays a crucial role in stress distribution within the bone. For instance, Popa *et al.* (13) demonstrated that increasing the insertion angle from 30° to 120° results in decreased stress on the bone, indicating that a more oblique insertion can lead to better stress distribution and potentially lower the risk of implant failure. An increase in insertion angle can influence stress levels, the torque required for removal does not consistently correlate with stress distribution across all angles, suggesting a more complex interaction (14). The mechanical properties of the materials used for mini-implants also significantly affect their performance. The use of titanium alloys, particularly Ti6Al4V, is prevalent due to their favorable mechanical properties and biocompatibility, which are essential for maintaining stability during orthodontic treatment (15).

However, discrepancies in Young's

modulus between the implant material and the surrounding bone can lead to stress-shielding effects, potentially resulting in implant loosening over time (15). This highlights the importance of selecting materials that closely match the mechanical properties of bone to optimize the performance of mini-implants. Furthermore, the dimensions of mini-implants, including their diameter and length, have been shown to influence their mechanical stability. Kovuru's finite element study indicated that larger diameter and longer mini-implants resulted in decreased mobility, which correlates with reduced stress on the adjacent bone (2). This finding is consistent with other studies that emphasize the importance of implant design in minimizing stress concentrations in the peri-implant bone (16, 17). The biological response of the periodontal ligament (PDL) to mechanical forces is also a critical factor in understanding bone remodeling around mini-implants. Mechanical stress applied during orthodontic treatment induces various cellular responses in the PDL, which are essential for bone remodeling. For example, moderate mechanical loading has been shown to promote osteogenesis, while excessive forces can lead to cellular damage and dysregulation of bone remodeling processes (18, 19). The balance of mechanical forces is vital; while moderate stress supports bone formation, excessive stress can trigger bone resorption and negatively impact the stability of mini-implants (20, 21). The Finite Element Method (FEM) has emerged as a pivotal tool in the biomedical field, especially in orthodontics and the design of medical implants such as hip prostheses, surgical instruments, and external fixators, offering the advantage of simulating complex biological systems and enabling detailed analysis of stress and strain distributions in tissues like the periodontal ligament (PDL), alveolar bone, and surrounding structures, as well as predicting the mechanical behavior of medical devices under various loading conditions to enhance their design and functionality . (22-24). An increase in cortical bone thickness is hypothesized to significantly improve the primary stability of mini-implants in ortho-dontic treatments, reducing the likelihood of implant failure under standard orthodontic forces. Conversely, thinner cortical bone is expected to result in higher stress concentrations, which may decrease implant longevity and increase the likelihood of failure when subjected to higher orthodontic forces. Optimal loading conditions are hypothesized to increase the mechanical stability of orthodontic mini-implants by reducing stress, strain, and displacement in both the implant and surrounding bone, thereby enhancing long-term success in orthodontic applications.

MATERIALS AND METHODS

In this study, a titanium (Ti6Al4V) mini-implant, with dimensions of 2.0 mm in diameter and 12 mm in length, was modeled using the finite element method (FEM) (Figure 1). The mandible's geometry was derived from scanned data, with computed tomography (CT) images being processed and digitized (Figure 1). Three-dimensional solid models of both the mini-implants and the mandible were created and integrated using SpaceClaim, a commercial computer-aided design (CAD) software (Figure 1). The periodontal ligament was represented with a 0.25 mm thickness, corresponding to the outer geometry of the dental roots. The mini-implant's insertion point was positioned midway along the depth of the roots of both premolars, specifically in the space between the premolar and molar. The entire

model was imported into the finite element analysis software (version 2021; ANSYS, Inc., Canonsburg, Pennsylvania, USA) and discretized using automatically generated 10-node tetrahedral structural elements (fig. 4). The materials for the bones, teeth, periodontal ligament, and mini-implant were modeled as linear elastic, homogeneous, and isotropic, with mechanical properties sourced from published literature. To ensure convergence in the finite element model, various element sizes, ranging from 0.3 mm to 1.2 mm, were tested. It was concluded that an element size of 0.3 mm was optimal for the mini-implant insertion area, while 0.8 mm was suitable for the rest of the model.

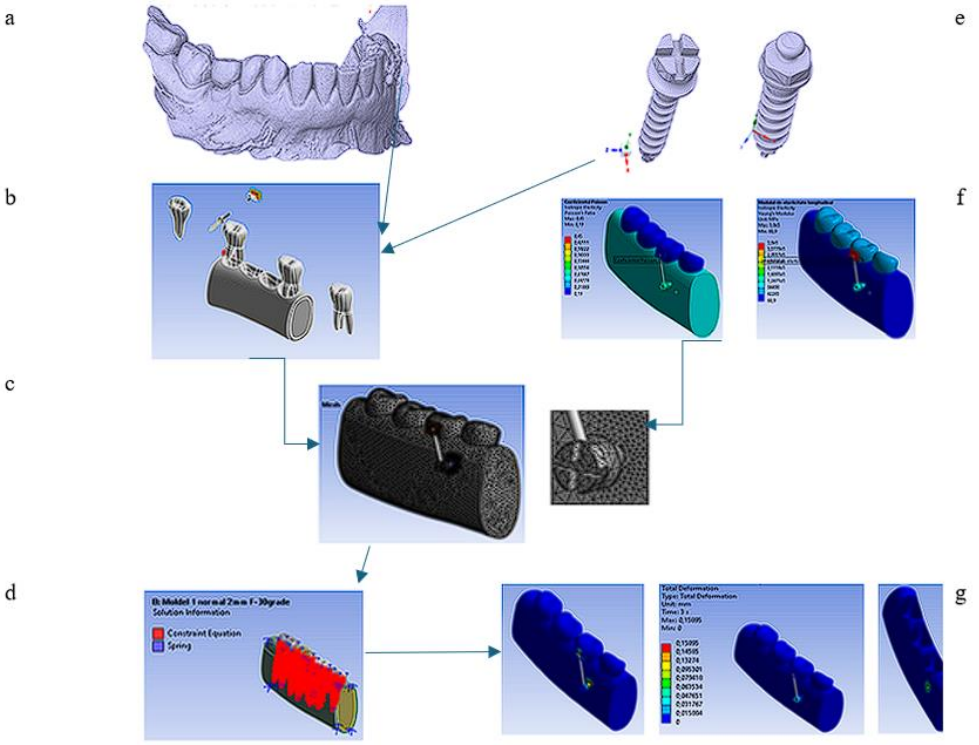


Fig. 1. Digital workflow for structural analysis of mini-implants using CT data: a. Mandible STL file; b. Geometric model (CAD): Teeth, PDL, Adhesive, Mini-implant, Mandible (cortical bone and spongy bone), Bracket; c. Discretized model; d. Analysis settings; e. Mini-implant STL file; f. Material model; g. Obtained results.

The interfaces between the teeth and the periodontal ligament were assumed to be bonded, while a frictional contact (friction coefficient = 0) was assumed between the bone and the mini-implant. Orthodontic forces ranging from 0.1 N to 10 N were applied. A force of 2 N and an additional 10 N force were applied at a 30° angle relative to the vertical axis (Y) to simulate real-world orthodontic conditions. These forces were directed from the mini-implant toward the molar via the connector tube, accurately representing the biomechanics involved in a molar intrusion scenario with

skeletal anchorage. This angled application reflects clinical load transfer during orthodontic treatments and allows for a realistic assessment of stress and strain distributions within the periodontal ligament, alveolar bone, and surrounding tissues.

The inclined force direction plays a crucial role in controlling the intrusion of the first molar, making this model a precise simulation for evaluating biomechanical responses during this specific orthodontic movement. These models were created using SpaceClaim software, (19.2, ANSYS, Inc., Canonsburg, Pennsylvania, U.S) which allowed for the import and remodeling of CT images in STL format. The models were geometrically simplified to ensure stability during finite element analysis without compromising realism (fig. 1). Finite element modeling involved discretizing the 3D models into a large number of elements and nodes (fig. 1). The mesh was finer in critical areas, such as the insertion site of the implant and the adjacent region. The CAD model was automatically meshed into finite elements using ANSYS Workbench 19.2 software (ANSYS Workbench 19.2, Canonsburg, Pennsylvania, U.S). Material properties, such as Young's modulus and Poisson's ratio, were specified for each component of the implant system (fig. 1). Contact conditions were also defined to simulate interactions between the different parts of the model.

RESULTS

Results obtained for the loading scenario with 2N, 30° to the normal

Figure 2 provides a visual representation of the results from the finite element analysis for the loading scenario where a 2N force is applied at a 30° angle to the normal. This analysis is crucially important in understanding the stress, strain, and displacement behavior of the bone-implant system under orthodontic loading conditions.

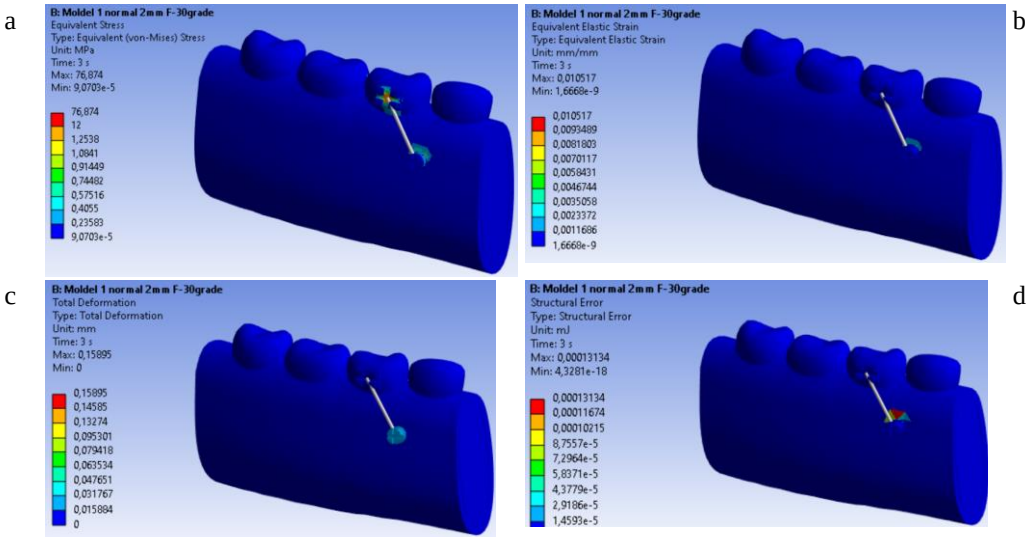


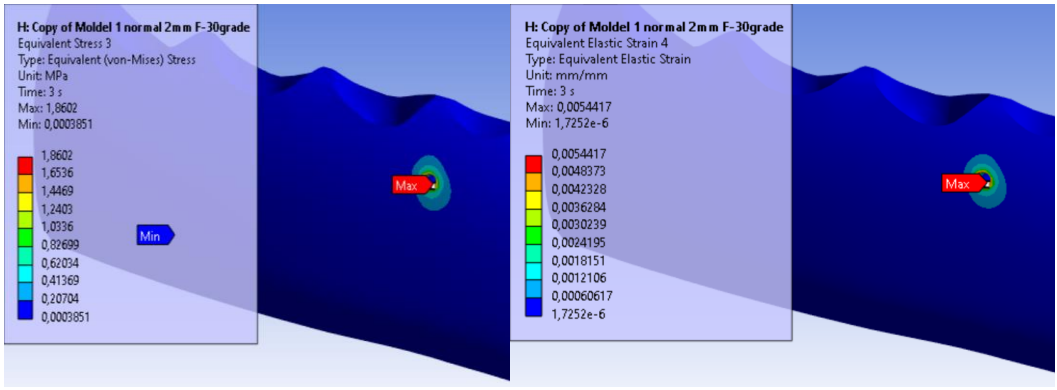
Fig. 2. The von Mises equivalent stress state, strain state, total displacements, and structural error on the global model: a. The von Mises equivalent stress state on the global model; b. The equivalent strain states on the global model; c. Total displacements on the global model; d**.** Structural error on the global model.

Figure 2 (a) displays the von Mises equivalent stress state on the global model. This indicates the distribution of stress throughout the model. Areas with higher stress concentrations can be observed, which are essential in predicting potential failure points or areas of concern regarding structural integrity. Figure 2 (b) shows the equivalent elastic strain state on the global model. This provides insights into how the material deforms under load, with higher strain regions indicating areas where the material undergoes significant deformation. Figure 2 (c) represents the total displacements on the global model. This gives an overall picture of how much the mini-implant and surrounding structures move or displace in response to the applied force, crucial for assessing the functional stability of the mini-implant in a clinical setting. Figure 2 (d) highlights the structural error on the global model. Structural error maps are essential for verifying the reliability of the simulation, ensuring that the model's results fall within acceptable error margins and the analysis is valid.

Results obtained in cortical bone

In Figure 3a, the von Mises equivalent stress state on the cortical bone is presented. The maximum value obtained is 5.29 MPa in the area immediately surrounding the orthodontic anchorage. It can be observed that relatively high values are obtained in a relatively small volume of material, with the area indicated as "Max" in the image. The stress values are five times lower or more for the areas adjacent to the recorded maximum.

Based on the results obtained for the compressive strength of the cortical bone (200 MPa) and the tensile strength (110 MPa), it can be concluded that the bone is not affected by the orthodontic anchorage. The value obtained is much lower than the fracture limit of the cortical bone.



a b

Fig. 3. Results obtained in the cortical bone in the mini-implant insertion area: a. The von Mises equivalent stress state in the cortical bone in the mini-implant insertion area; b. The equivalent strain state in the cortical bone in the mini-implant insertion area.

In Figure 3b, the equivalent linear strain state on the cortical bone is presented. The maximum value obtained is 0.016378 mm/mm in the area immediately surrounding the orthodontic anchorage, at the node with the maximum von Mises stress value.

Relatively high values are observed in a relatively small volume of material, with the area indicated as “Max” in the image.

Based on the results obtained for the cortical bone’s resistance to compression (0.0085 mm/mm) and to tension (0.007 mm/mm), it can be concluded that the bone is affected by the orthodontic anchorage only at the node where the maximum value was recorded. The value obtained for the rest of the material in the area immediately surrounding the anchorage is lower than the specific linear deformations obtained experimentally for cortical bone.

In Figure 4, the graph of maximum von Mises equivalent stresses as a function of loading force in the cortical bone section is presented. The maximum value obtained is 17.972 MPa in the most stressed area, which is the same area shown in Figure 3a for a force magnitude of 10N, while the value door 2N orthodontic force is 5.2914 MPa. The nonlinear graph is a result of the application of nonlinear contact between the mini-implant and the cortical bone. The graph shows that the fracture limit values of the cortical bone are not exceeded for all applied force values, indicating that the cortical bone withstands forces greater than 10N

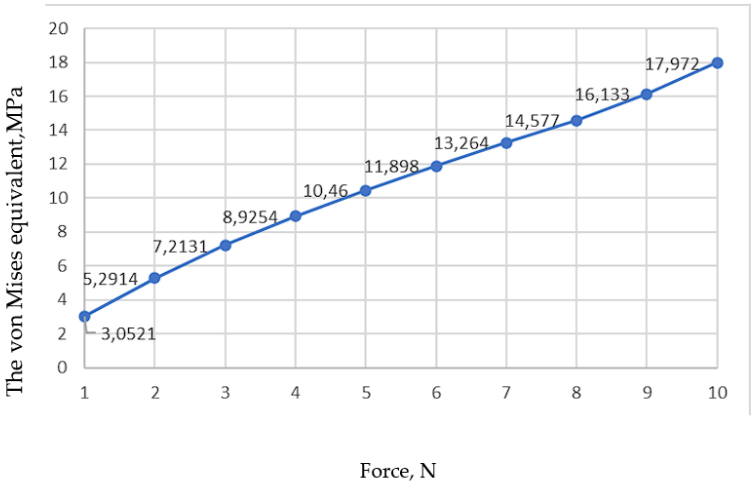
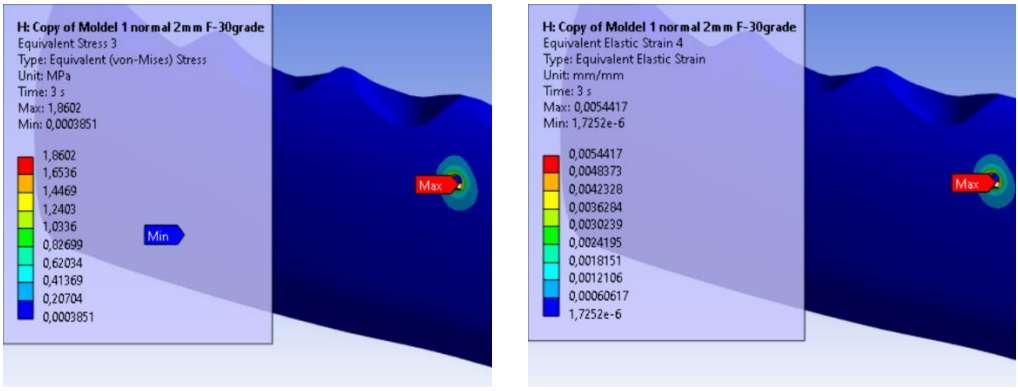


Fig. 4. The von Mises equivalent stress state in the cortical bone.

Results obtained in the spongy bone

In Figure 5a, the von Mises equivalent stress state in the spongy bone is presented. The maximum value obtained is 1.86 MPa in the area immediately surrounding the orthodontic anchorage. It can be observed that relatively high values are concentrated in a relatively small volume of material, with the area indicated as "Max" in the image. The stress values are five times lower or more for the areas adjacent to the recorded maximum. Based on the results obtained for the compressive strength of the spongy bone (2.6 MPa) and the tensile strength (2.3 MPa), it can be concluded that the bone is not affected by the orthodontic anchorage. The value obtained is much lower than the fracture limit of the spongy bone. In Figure 5b, the equivalent linear strain state in the spongy bone is presented. The maximum

value obtained is 0.0054417 mm/mm in the area immediately surrounding the orthodontic anchorage, at the node with the maximum von Mises stress value. Relatively high values are observed in a relatively small volume of material, with the area indicated as "Max" in the image. Based on the results obtained for the compressive strain resistance of the spongy bone (0.08 mm/mm) and tensile strain resistance (0.13 mm/mm), it can be concluded that the bone is affected by the orthodontic anchorage only at the node where the maximum value was recorded. The value obtained in the rest of the material in the area immediately surrounding the anchorage is lower than the specific linear deformations obtained experimentally for spongy bone.



a b

Fig. 5. Results obtained in the spongy bone in the mini-implant insertion area: a. The von Mises equivalent stress state in the cortical bone in the mini-implant insertion area; b. The equivalent strain state in the cortical bone in the mini-implant insertion area.

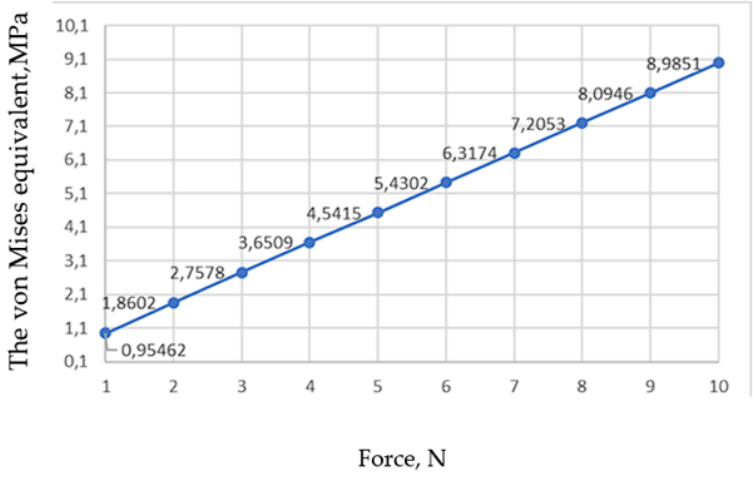


Fig. 6. The von Mises equivalent stress state in the cortical bone.

In Figure 6, the graph of maximum von Mises equivalent stresses as a function of loading force in the spongy bone section is presented. The maximum value obtained is 8.98 MPa in the most stressed area, which is the same area shown in Figure 14 a for a force magnitude of 10N, while the minimum value is 1.8602 MPa for an orthodontic force of 2N. The graph indicates that the fracture limit values of the spongy bone are exceeded for applied forces greater than 3N, suggesting that the spongy bone withstands forces up to 3N. If this force is exceeded, there is a likelihood of cracks forming. It is also observed that a force of up to 2N, chosen for orthodontic treatment, is appropriate, as this value is supported by most studies.

TABLE I.
Results for cortical and spongy bone.

Parameter	Result	Threshold/Limit
Maximum Von Mises Stress	5.29 MPa near anchorage	Compressive: 200 MPa, Tensile: 110 MPa
Maximum Equivalent Linear Strain	0.016378 mm/mm near anchorage	Compression Resistance: 0.0085 mm/mm, Tension Resistance: 0.007 mm/mm
Maximum Von Mises Stress	1.86 MPa near anchorage	Compressive: 2.6 MPa, Tensile: 2.3 MPa

The results suggest that both cortical and spongy bone (tab. I) largely remain within the safe limits of their mechanical strength. However, the slight deformation exceeding the permissible limit in the cortical bone at maximum stress may indicate a vulnerable area, especially under repetitive loading scenarios.

DISCUSSION

FEA is a powerful computational tool used to predict the mechanical behavior of structures under various loading conditions. However, the accuracy of FEA results is highly dependent on the assumptions made during modeling. The presumption of 100% osseointegration simplifies the model but neglects the variability inherent in biological systems. This can lead to erroneous predictions regarding stress distribution, load transfer, and potential failure modes of implants (25).

Impact of von Mises equivalent stress on cortical bone: The maximum values of von Mises equivalent stress are recorded in the area immediately surrounding the orthodontic anchorage. These high values indicate a concentration of stresses in this region, which may be associated with the risk of deformation or bone damage. However, it is important to note that the relative stress values are lower in the areas adjacent to the recorded maximum, suggesting that the orthodontic impact on the cortical bone is localized and does not significantly affect the entire bone. Equivalent linear strain in cortical bone: The maximum values of equivalent linear strain are also recorded in the area immediately surrounding the orthodontic anchorage.

These strains may indicate excessive loading or a weakness in the bone in this region. However, similar to von Mises stress, the strains are localized and do not significantly affect the entire cortical bone. Cortical bone resistance to compression and tension: By comparing the cortical bone's resistance to compression and tension with the deformations and stresses induced by the orthodontic anchorage, it can be observed that the bone is not significantly affected. The resistance values of the cortical bone are much higher than the deformations and stresses induced by the orthodontic anchorage, indicating the bone's ability to withstand the applied

orthodontic forces. Importance of the localization of maximum stress and strain: The fact that maximum stress and strain are localized in a small area of the cortical bone suggests that orthodontic treatment has a concentrated impact, which can be effectively managed and monitored. This aspect can be considered in developing and implementing orthodontic treatment strategies that minimize adverse effects on the cortical bone.

Impact of von Mises equivalent stresses on spongy bone: The maximum values of von Mises equivalent stresses are recorded in the area immediately surrounding the orthodontic anchorage on the spongy bone. These high values indicate a concentration of stresses in this region, which may be associated with the risk of deformation or bone damage. However, the relative stress values are lower in the areas adjacent to the recorded maximum, suggesting that the orthodontic impact on the spongy bone is localized and does not significantly affect the entire bone. Equivalent linear strain in spongy bone: The maximum values of equivalent linear strain are also recorded in the area immediately surrounding the orthodontic anchorage on the spongy bone. These strains may indicate excessive loading or a weakness in the bone in this region. However, similar to von Mises stresses, the strains are localized and do not significantly affect the entire spongy bone. Spongy bone resistance to compression and tension: By comparing the spongy bone's resistance to compression and tension with the deformations and stresses induced by the orthodontic anchorage, it can be observed that the bone is not significantly affected. The resistance values of the spongy bone are much higher than the deformations and stresses induced by the orthodontic anchorage, indicating the bone's ability to withstand the applied orthodontic forces. Importance of contact pressure distribution: The distribution of contact pressure between the mini-implant and the spongy bone can influence the stability and durability of the orthodontic anchorage. The maximum contact pressure highlights areas of maximum load and can provide information on the potential for bone damage or weakening in the anchorage area. Evaluation of stress and strain in cross-section: The analysis of stresses and strains in the anchorage section provides detailed information on the structural behavior of the bone in the mini-implant insertion area. The maximum values of stress and strain are localized in the areas adjacent to the mini-implant and can be considered in the design and adjustment of orthodontic treatments to minimize adverse effects on the bone.

The maximum displacement value of 0.028948 mm is observed at the head of the mini-implant, in the area where the orthodontic force is applied. This information is useful for evaluating the structural behavior of the orthodontic anchorage system and identifying areas of greatest mobility during treatment. Von Mises equivalent stress in cross-section: This analysis is important for evaluating the mechanical stress to which the orthodontic anchorage system is subjected and for identifying critical areas concerning material resistance and deformation. To accurately model the interactions between mini-implants and bone tissue, several crucial factors must be considered. Research has indicated that the insertion angle of mini-implants into the bone significantly influences stress reduction and stability (5). It is recommended that mini-implants be inserted at a 30° angle to achieve optimal results. The fit of the mini-implant threads into the bone is vital for

stability and the ability to withstand applied forces (26).

Cortical bone thickness is a crucial factor in determining the primary stability of mini-implants. Thicker cortical bone has consistently been associated with increased initial stability of mini-implants, while thinner cortical bone has been linked to a higher risk of implant failure (5). Studies have highlighted the importance of cortical bone thickness in influencing primary stability, with some research emphasizing it as the most critical factor affecting the success rate of mini-implants (6). Research has shown that increasing cortical bone thickness significantly increases the pull-out force, underscoring the importance of bone quality for primary stability. Studies have indicated that mini-implants achieve greater primary stability in denser cortical bone (5, 27-31). The choice of mini-implant design and size is crucial for establishing sufficient primary stability, particularly considering the insertion region and local bone quality (30, 32).

Cortical bone thickness has been identified as a critical factor in stabilizing mini-implants, with some studies suggesting that it may be the most important factor in ensuring stability (33, 34). The influence of cortical bone thickness on primary stability has been further supported by research showing that greater cortical bone thickness increases the initial stability of the implant (35, 36). The primary stability of mini-implants is significantly affected by the quality and thickness of the cortical bone at the insertion site. Thicker cortical bone is associated with increased stability and a higher success rate for mini-implants, emphasizing the importance of considering both bone quality and quantity when planning mini-implant placement. There is a consensus in various studies that cortical bone thickness is a key determinant of the initial stability of mini-implants in orthodontics.

Thin cortical bone presents a risk of implant failure, while adequate cortical bone thickness -typically greater than 1 mm- is essential for ensuring the stability and success of orthodontic mini-implants. The mechanical properties of biological tissues, including bones and the periodontal ligament, are well-known to be viscoelastic and highly dependent on the rate of deformation. Orthodontic treatment is a progressive process that uses small-magnitude orthodontic forces, as the deformation and displacement of dental tissue require time to modulate. Based on this condition and assuming that alveolar bone is a ductile material, von Mises stress has been widely accepted in orthodontic research.

The selection of this stress index has been advantageous because it is a scale designed to quantify stress without directional orientation. This stress index makes it easier for clinicians to predict where stress concentrators may appear; this is important because higher stress levels can lead to potential mini-implant failure. The results of our study indicated that the equivalent stress and strain state in the cortical bone was primarily affected by the exposed length of the mini-implant, the type of mini-implant, and the material from which the mini-implant is made. Previous research has mainly focused on the insertion length of mini-implants. In 2011, Chatzigianni *et al.* reported that the displacement of longer mini-implants under a force of 2.5 N was greater than that of shorter implants (37). Several studies have shown that longer mini-implants have higher success rates than shorter ones. Sometimes, the head of the mini-implant may become covered by the alveolar mucosa. In such cases, intervention with ortho-

dontic devices such as elastic chains or coil springs could lead to inflammation of the alveolar tissue, which is strongly correlated with mini-implant failure. Partial insertion of longer mini-implants could minimize the possibility of the alveolar mucosa covering the head of the mini-implant, thereby reducing the chances of failure. Studies have shown that variations in cortical bone thickness can influence the stress distribution around mini-implants during orthodontic loading. For instance, Hirai *et al.* utilized 3D FEA to demonstrate that the insertion depth of miniscrews affects the stress distribution in both the screws and the surrounding bone, indicating that optimal insertion depth is crucial for minimizing stress concentrations and enhancing stability (38).

Clinical implications

These results can be discussed in the context of clinical orthodontic practice, highlighting the importance of understanding the mechanical behavior of mini-implants and identifying strategies to improve their performance during orthodontic treatment. This could include modifications to insertion techniques, improvements in mini-implant materials, or adjustments to the treatment protocol to minimize the risk of complications or failures.

Future research directions: Based on these results, future research directions could be suggested to further investigate the mechanical behavior of mini-implants and develop new strategies or technologies to improve their stability and performance in clinical practice. This could include additional computer simulation studies or experimental tests to validate and expand upon the current findings.

Reasons for the selection of 12 mm length in mini-implants. The decision to utilize a 12 mm length for the mini-implants (MIs) was made based on several key considerations: *(i)Enhanced stability*: longer mini-implants have the potential to offer superior primary stability, particularly in cases where bone density is lower (32); *(ii)Improved bone anchorage*: in certain clinical scenarios, the thickness of both the cortical and spongy bone layers may necessitate the use of longer mini-implants to achieve adequate depth of anchorage (12)*; (iii) Supporting literature*: while the minimum recommended length for mini-implants is typically around 6 mm, existing studies indicate that longer implants can lead to improved long-term performance, particularly in regions with varying bone density (1). The selection of the 12 mm length was informed by both theoretical evidence and practical applications observed in prior research. *(iv) Clinical experience*: our clinical experience further supports the use of 12 mm mini-implants, as they have consistently demonstrated favorable outcomes in terms of stability and osseointegration, particularly in more complex cases where additional length may provide a critical advantage.

CONCLUSIONS

The findings demonstrated that von Mises stress and equivalent strain were localized around the mini-implant anchorage, without significantly affecting the surrounding bone structure. Both cortical and spongy bone showed resistance levels far exceeding the applied forces, confirming their capacity to withstand orthodontic loading. However, areas of maximum stress and strain require careful consideration to avoid localized damage or potential failure. Additionally, the role of cortical bone thickness as a key determinant of primary stability was reinforced, with thicker bone consistently linked to better outcomes. this

study underscores the value of understanding the mechanical behavior of mini-implants to ensure their effective use in orthodontic practice. By integrating FEA insights with clinical observations, orthodontic treatments can be tailored to maximize outcomes while minimizing complications, paving the way for more predictable and reliable MI applications.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interests.

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NOISE POLLUTION IN REHABILITATION SETTINGS

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NOISE POLLUTION IN REHABILITATION SETTINGS (Abstract): Noise pollution is one of the main pollutants in the urban areas and a major challenge for rehabilitation units. Noise affects both patients during the healing process and doctors during healthcare activities. Noise comes from the exterior and the interior of the medical ward. Due to the use of powered instruments, orthopedic surgery is the loudest medical setting (between 66 and 100 dB A). Intensive care units may reach sound levels between 55 and 70 dB A. Neurosurgery, urology, cardiology and gastrointestinal surgery produce between 62 and 65 dB A. WHO has provided guidelines for specific noise levels within hospital units, recommending noise levels not exceeding 30 dB A at night and 35 dB A during the day. One of the main sources of noise in hospital activity is represented by the conversations undertaken by medical professionals, doctors and patients, patients and visitors. However, medical rehabilitation is an area in which such discussions are more necessary, as long-term therapies require active involvement of the patient and family members or caregivers. Besides family and patient, the rehabilitation process requires a well-connected multidisciplinary team made of doctors, nurses, psychologists, physical therapists, occupational therapists, speech and respiratory therapists, dietitians and social workers, who share medical information almost entirely verbally. That is why, rehabilitation doctors and nurses should be aware of the need to maintain a muffled voice tone. Minimizing the sound levels of all technological devices used in the rehabilitation units should be also considered. When possible, the design of new rehabilitation units must aim not only at earthquake safety, functionality and cleanliness, but also at the reduction of hospital noise. **Keywords**: NOISE POLLUTION, PREVENTION, REHABILITATION, SOUND LEVELS.

As patients in medical rehabilitation are mostly prone to painful diseases, they are more sensitive to noise than the general population. Noise affects both patients during the healing process and doctors during healthcare activities. Noise comes from the exterior and the interior of the medical ward. The renovations of the Romanian hospitals carried out while continuing to provide healthcare and the noise produced during medical procedures have a negative impact on the quality of medical staff performance and on the duration of patient's convalescence.

A hierarchy of the level of noise produced by medical activities was provided by numerous studies (1). To better understand the noise levels, it should be mentioned that the sounds produced by the take-off of an airplane are the equivalent of 100 dB A, a vacuum cleaner yields 70 dB A, a restaurant conversation equals 60 dB

A, the noise of a quiet urban neighborhood at night represents 35 dB A, a whispered conversation has 20 dB A and the sound breathing has 10 dB A (2). Due to the use of powered instruments, orthopedic surgery is the loudest medical procedure (between 66 and 100 dB A). Intensive care units may reach sound levels between 55 and 70 dB A. For instance, burn intensive care units have an average sound level of 70 dB A (3). In pediatric intensive care units, the average sound level was about 63 dB A (4). Neurosurgery, urology, cardiology and gastrointestinal surgery produce between 62 and 65 dB A. But this has not always been the case. In 1960, hospitals recorded an average sound level of 57 dB A during the day and of 42 dB A at night. In 2005, sound level averages reached 72 dB A during the day and 60 dB A at night. Above all, current technology was responsible for this increase (5), but also the continuous growth of population in urban areas, which makes hospitals more crowded than ever.

The diversity of clinical manifestations induced by pollution request individual and group-specific interventions for rehabilitation and the most successful possible professional reinsertion. That is why, the World Health Organization (WHO) has provided guidelines for specific noise levels within hospital units, recommending noise levels not exceeding 30 dB A at night and 35 dB A during the day. The International Noise Council recommends a maximum of 45 dB A during the day, 40 dB A in the evening and 20 dB A at night. The United States Environmental Protection Agency (EPA) recommends a sound level limit of 45 dB A during the day and 35 dB A at night for hospital patient rooms (6). The National Institute for Occupational Safety and Health (NIOSH) in the United States recommends noise levels which not exceed 40 dB A during the day and 35 dB A at night (3). Unpredictable disparate noise is regarded as more stressful than continuous noise (5). Sound sources may be mobile (relocating patients and equipment) or static (air conditioning and alarming systems).

One of the main sources of noise in hospital activity is represented by the conversations between medical professionals, between the doctor and the patient, between the patient and the visitors. However, medical rehabilitation is an area in which such discussions are more necessary, as long-term therapies require active involvement of the patient and family members or caregivers. Besides family and patient, the rehabilitation process requires a well-connected multidisciplinary team made of doctors, nurses, psychologists, physical therapists, occupational therapists, speech and respiratory therapists, dietitians and social workers. Medical information is delivered almost entirely verbally. Despite this, healthcare professionals should be aware of the need to maintain a muffled voice tone. On the other hand, the more crowded the hospitals become, the harder it is to maintain verbal communication at normal voice levels. Therefore, another critical issue is having to deal with the conflicting requirements of clear communication and the duty of confidentiality.

Further noise-limiting measures include restricting the number of visitors and isolating highly circulated areas with thick curtains and closing the doors, wearing soft-soled shoes, avoiding non-urgent activities at night, adjusting the television volume in hospital wards or the use of audio headphones (which may be quite uncomfortable and may even represent yet

another cause of hearing loss), setting mobile phones on silent mode, reducing the number of false alarms, which only desensitize the medical staff (7), repairing scraping doors and soundproofing of walls.

Hospital architecture should pursue to create a more silent environment, by taking the following measures: providing single patient rooms, replacing the right angles with curves, which produce less sound vibrations, sticking sound absorbing materials or panels on corridors, positioning the nursing ward in the center, with patient rooms surrounding it (8, 9). There are also causes of noise which are more difficult to limit and control, such as air conditioning and ventilators, noise-generating beds, computers, alarm systems for medical personnel, mobile phone conversations, opening and closing of cabinets and doors, crying and calls of patients in pain.

The negative effects of noise were acknowledged even by the ancient Greeks, who prohibited the noisy metal work in cities. Florence Nightingale, the founder of modern nursing, considered unnecessary noise as the cruelest absence of care (3).

Prolonged exposure to noise may induce temporary or permanent hearing loss, due to damaging the sensory cells in the organ of Corti. Although it was long argued that only industrial noise can affect the ear, numerous studies have reported the onset of hypoacusia in the general urban population, as well as the decrease of the onset age of presbycusis (age-related hearing loss, which is otherwise considered a physiological phenomenon). In professions in which noise reduction cannot be achieved, such as the army, conditioning or toughening of the ear through prior exposure to lower sound levels before exposure to harming noise, such as gunshots and explosions, was successfully used (10), but this is not an option in healthcare, where noise pollution is caused rather by repetitive and cumulative sounds than by acute and powerful sounds. The only piercing sounds are produced by the alarms of medical devices.

The treatment of adults having hearing loss is impaired by doctor-patient communication issues, as doctors have difficulties understanding the symptoms of the patients and patients do not understand treatment and prescription instructions (11). Auxiliary aids and services needed for effective therapy may have a major impact on treatment expenses, which are anyway high in medical rehabilitation, due to the long duration of therapy. Lip reading or written notes are merely ineffective and time-consuming solutions (12). However, influencing patient's decision to obtain hearing aids in form of equipment or services might be a major goal of healthcare workers, because people are not aware of the importance of the sense of hearing as compared to sight. Statistics show that the hearing aids are used in only 50% of the patients with hearing loss (13), which may not be the case in the more fashionable matter of wearing glasses. Rehabilitation doctors should also consider regular ear and hearing checks.

Noise can damage the body not only through the hearing process, but also by affecting skin, bones, joints and muscles. As noise requires even further raising of the tone of voice, it can also lead to voice disturbances (14). Noise was found to cause disturbances in the activity of the nervous system and in the function of the brain, by modifying the normal electrical pathways and overlapping some new disruptive pathways, as shown by electroencephalography.

One of the most severe negative effects on the health status of patients is sleep disturbance, which occurs at sound levels exceeding 50 dB A. During sleep, all physiological activities are minimized and even low-intensity noise can cause significant changes. The prolongation of time needed to fall asleep and the decrease of the period of deep sleep lead to fatigue, irritability, disorientation, the need for increased sedation and delayed healing and rehabilitation, due to the negative effects of sleep deprivation on the immune system (15). Quite frequently, rehabilitation patients need not only a sound night sleep, but also daytime rest.

Noise activates the sympathetic nervous system and the auditory–hypothalamic–pituitary axis. The exposure to powerful or prolonged noise leads to cardiovascular stimulation and vasoconstriction (hypertension, increased systemic vascular resistance, ischemic heart disease, cardiac arrhythmia, increased blood cholesterol), pituitary and adrenal gland stimulation, gastrointestinal system problems, immunosuppression and poor immune response to infection, impaired wound healing, a negative effect on cognitive tasks and altruistic behaviors, vertigo, agitation and irritability, insomnia and fatigue, headache and inappropriate conduct. Noise is a risk factor for neurosis, psych asthenia, hypertension, gastritis and gastric and duodenal ulcers, colitis, diabetes and hyperthyroidism. In pregnant women, prolonged exposure to noise leads to an increased risk of preterm birth and delivery of infants with low birth weight (15, 16). Noise decreases attention and concentration, speed and precision of movements, leading to poor labor productivity, especially in intellectual activities.

Due to the active role played by patients in medical rehabilitation, they are not immune to noise like the sedated patients in intensive care units, but rather strongly affected by noise. Doctors and nurses are also at risk for noise-induced changes in behavior, such as low interpersonal engagement, aggressive conduct and noncommunicative attitudes (5). Noise exposure may even lead to burnout in healthcare workers, as noise is experienced as a stressful situation, due to the evolution process, when sounds were a possible source of danger (17).

As more than half of all acoustic signals perceived in hospitals are related to human activities and speech, it is possible to control this phenomenon by changing human behavior. Hospital noise was sometimes replaced by music or by beneficial forms of sounds, such as relaxing white noise, which may be used for therapeutic purposes, especially for the cognitive rehabilitation of stroke patients (18, 19, 20).

Traffic noise outside the hospital is also a critical issue. Many studies have focused on controlling noise pollution within the hospital setting, neglecting the major impact of external noise caused by street traffic. Moreover, rehabilitation patients require long-term medical care at home, where they are affected by the urban noise experienced all over the city. All these negative effects of noise require a more accurate study of the sources of noise in cities, to help establish the existing levels and of accepted levels, as well as the necessary measures for reducing noise. Measures for the control of urban noise pollution include regulations and laws setting noise limits in human communities. Some of them are as follows: the functional zoning of populated areas (with special interest in the location of industrial facilities that are important sources of noise),

improving the road network and road traffic and, where appropriate, regulations on air travel to avoid the loudness discomfort caused by overflying human settlements, especially at nighttime (21, 22). Building rehabilitation clinics outside the congested urban areas in quieter locations might also solve the problem of street noise.

CONCLUSIONS

Medical staff working in rehabilitation units must be trained to limit the impact and the level of noise, by lowering the tone of voice and minimizing the sound levels of all technological devices. The purchase of new equipment must consider quieter medical appliances. On the eve of building the new Regional Hospital of Iasi, the reduction of hospital noise should be an objective equally important for the building design as earthquake safety, functionality and cleanliness.

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NEWS

HISTOPATHOLOGIC FEATURES OF PROGNOSTIC SIGNIFICANCE IN HIGH-GRADE OSTEOSARCOMA

Osteosarcoma is a malignant mesenchymal neoplasm characterized by bone production. Several types of primary high-grade osteosarcoma are recognized by the World Health Organization, including the "conventional" types encompassed by the chondroblastic, fibroblastic, and osteoblastic subtypes, among others. Despite heterogeneity in histomorphology, most high-grade osteosarcomas receive similar treatment, namely, neoadjuvant chemotherapy—typically consisting of doxorubicin, cisplatin, and high-dose methotrexate, followed by surgical resection and maintenance chemotherapy. Although a number of groups have assessed the contribution of clinical parameters—namely, patient age, sex, anatomic site of primary tumor, tumor size, and metastasis at presentation—in predicting survival outcomes for patients with osteosarcoma, there are limited data concerning histopathologic attributes. Histopathologic prognostic factors represent an essential component to the treatment stratification of many types of cancer. The authors suggest that detailed microscopic examination (tumor pleomorphism, mitotic activity, and morphologic subtype) yields useful prognostic information in high-grade osteosarcoma. In biopsy samples, necrosis and chondroblastic differentiation are adverse features. In resections, quantitation of the extent of residual viable tumor may be of greater clinical relevance than attempting to distinguish necrosis from other noncellular elements. In summary, the integration of clinical and microscopic features is a feasible and effective means for improving prognostication in patients with osteosarcoma. The effective stratification of patients using the proposed scoring system merits further investigation, ideally in prospective cohorts, to validate interobserver reproducibility and predictive ability (Chui MH, Kandel RA, Wong M, et al. Histopathologic Features of Prognostic Significance in High-Grade Osteosarcoma. *Arch Pathol Lab Med* 2016, 140 (11):1231-1242).

Doina Butcovan

PARTICULARITIES OF THE ETIOLOGICAL STRUCTURE OF NOSOCOMIAL INFECTIONS IN HOSPITALS OF ORTHOPEDICS AND TRAUMATOLOGY PROFILE

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PARTICULARITIES OF THE ETIOLOGICAL STRUCTURE OF NOSOCOMIAL INFECTIONS IN HOSPITALS OF ORTHOPEDICS AND TRAUMATOLOGY PROFILE (Abstract): Determination of etiological structure of septic-purulent nosocomial infections (SPNI) in hospitals of orthopedics and traumatology profile. **Materials and methods**: there were analyzed 2013 strains of microorganisms isolated from the patients with SPNI. **Results**: it was established that Gram-positive microorganisms (63.19%) prevail in hospitals of Orthopedics and Traumatology profile, including *S. aureus* (39.19%), *E. faecalis* (12.76%), *S. epidermidis* (10,13%), followed by Gram negative microorganisms (35.56%), including *P. aeruginosa* (10.68%), *E. aerogenes* (8.64%), *E. coli* (4.32%), *C. diversus* (4.37%), *P. vulgaris* (2.58%), *Kl. pneumoniae* (1.54%). It was found that Gram positive strains prevail in young population, but Gram negative bacteria and fungi prevail in old population. Also, it has been shown that pathologic foci with associations of microorganisms predominate among old population, while monocultures prevail among young population. During the last years, it was observed an increase of SPNI caused by associations of microorganisms. **Conclusions**: The septic-purulent pathology has a polyetiological character in hospitals of orthopedics and traumatology profile, both in general population, as well in the hospital outbreaks among patients. **Keywords**: SEPTIC-PURULENT, NOSOCOMIAL INFECTIONS, GRAM-POSITIVE, GRAM-NEGATIVE, ETIOLOGICAL STRUCTURE.

Septic-Purulent Nosocomial Infections (SPNI) is one of the main problems of contemporary medicine (1, 2, 3), being caused by a variety of microorganisms. Thus, studying the etiological peculiarities per stationary profile, by age, methods of treatment is an important element in the epidemiological surveillance system (3-11). Also, the increase of frequency of association of two or more causative agents in the development of SPNI was underlined (1). The peculiarities of etiological structure of SPNI patients of orthopedics and traumatology profile are determined in the current study.

MATERIAL AND METHODS

This research is a descriptive transversal epidemiological study based on retrospective analysis of 2013 strains, a result from microbiological investigations performed in bacteriological laboratory of Traumatology and Orthopedics Clinical Hospital during years 2009-2010 and 2013-2014. The identification of microorganisms

in SPNI was performed per classical methods (Galeţchi 1997, Buiuc 1999), collecting the material from the wound was carried out using the moistened in physiological solution (0.9%) buffer.

RESULTS

The study of those 2013 strains of microorganisms, emphasized that the etiological structure of the traumatological septic-purulent infections (SPI) includes 18 species of microorganisms, the majority are opportunistic bacteria (tab. I). The Gram-positive microorganisms prevail, which is (63.2%). The causative agents in trauma SPI were represented by *S. aureus*-62.02%, *S. epidermidis*-15.02% and *S. saprophyticus*-1.10%. *Enterococcus* is 21.86%, prevailed *E. faecalis*-20.20%, followed by *E. faecium*-1.5% and *S. pyogenes*-0.07%.

Gram negative microorganisms group constituted 35.56% of the total number of strains detected from the patients with trauma SPI. *P. aeruginos*a-30.02%, and *E. aerogene*s-24.3%, followed by *E. coli*-12.30% prevailed. Microorganisms of the *Citrobacter* genus -17.59%, *Proteus*-10.06% and *Klebsiella*-5.30%. From 25 patients with SPI with infected wounds were isolated- *C. albicans* (1.24%).

TABLE I

The structure of microorganisms isolated from the patients with SPI in hospitals of orthopedics and traumatology profile

Groups	*Microorganism species*	Number of strains	% from the group	% of total strains
Gram-positive Microorganisms	*Staphylococcus aureus*	789	62.02	39.19
	Staphylococcus epidermidis	191	15.02	9.49
	Staphylococcus saprophyticus	14	1.10	0.69
	Enterococcus faecalis	257	20.20	12.76
	Enterococcus aecium	20	1.57	1.0
	Streptococcus pyogenes	1	0.07	0.05
	Total Gram positive	**1,272**	**100.0**	**63.20**
Gram-negative Microorganisms	*Escherichia coli*	88	12.3	4.40
	Enterobacter aerogenes	174	24.3	8.64
	Enterobacter cloacae	1	0.14	0.05
	Klebsiella pneumoniae	31	4.33	1.54
	Klebsiella oxytoca	7	0.97	0.35
	Pseudomonas aeruginosa	215	30.02	10.68
	Proteus vulgaris	52	7.26	2.58
	Proteus mirabilis	20	2.80	1.0
	Citrobacter freundii	39	5.44	1.89
	Citrobacter diversus	87	12.15	4.32
	Klebsiella oxytoca	7	0.97	0.35
	Acinetobacter calcoaceticus	2	0.28	0.1
	Total Gram negative	**716**	**100.0**	**35.56**
Fungi	*Candida albicans*	25	100.0	1.24
TOTAL		**2,013**	**100.0**	**100.0**

The distribution of the isolated strains taking into consideration the occupied rank in the total clinical picture (tab. I) demonstrates that in the general etiological structure of SPNI in hospitals of orthopedics and traumatology profile is as it follows: on the first place are the microorganisms of the *Staphylococcus* genus (*S. aureus*-39.19% and *S. epidermidis*-12.76%), followed by *P. aeruginosa*-10.61%, *E. faecalis*-9.49%, *E. aerogenes*-8.64%, *E.coli*-4.40%, *C. diversus*-4.32%, *P. vulgaris*-2.58%, *C. freundi*-1.89%, *Kl. pneumoniae*-1.54%, other microorganisms-3.46%.

There are 8 species of microorganisms that prevail in mixt infections, including the Gram-positive ones: *S. aureus*-in 31.52% cases of infections, *S. epidermidis*-5.35%, *E. faecalis*-19.48%; from the Gram-negative microorganisms *P. aeruginosa* in 10.86%, *E. aerogenes*-10.25%, *E. coli*-5.04%, *Citrobacter*-7.35%, *Proteus*-5.04%, *Klebsiella*-2.01%; other microorganisms 2.87% (fig. 1).

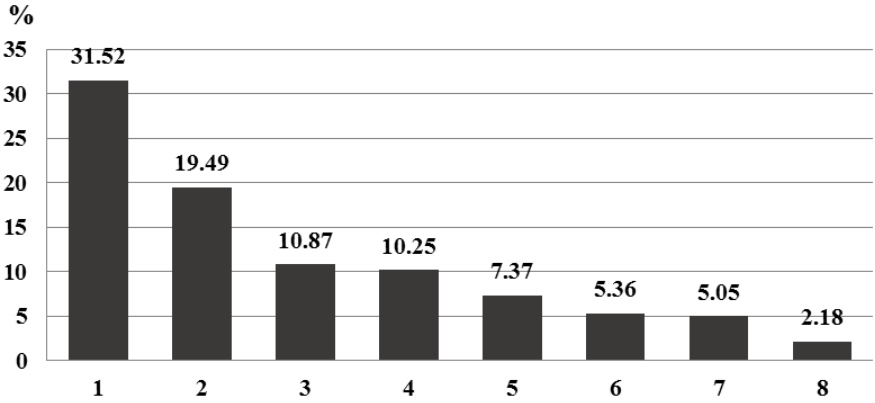

Fig. 1. The species of microorganisms prevalent in associations of pathogens according to the occupied rank: 1- *S.aureus; 2- E. faecalis; 3- P. aeruginosa; 4- E. aerogenes; 5- Citrobacter; 6- S.epidermidis; 7- E.coli and Proteus; 8- Klebsiella*

Peculiarities in the structure of microorganisms in relation with the patients' age. It has been found that Gram-positive microorganisms prevail among young age groups, the share part of which diminishes with age, from 74.68% in people up to 20 years, up to 55.17% in 70 years and older persons. And vice versa, Gram negative microorganisms and fungi prevail in the old age groups, with an increasing rate from 25.3% and 0.0%, respectively, among patients under the age of 20 years, up to 42.67% and 2.15% respectively, among patients aged 70 years and older (tab. II).

At same time, it has been found that the number of isolated strains from pathological foci varies and is in direct relation to the age of patients (tab. III, fig. 2). Thus, monocultures were isolated from pathologic foci in young patients, while the associations from 2-5 strains of microorganisms in elderly. This phenomenon is demonstrated both by the growing share of associations of microorganisms, detected in patients with SPNI according to the age, and by calculating to 1,000 patients in each age category. One strain was isolated predominantly in patients aged 16-39 years that is

1303,7 per 1000 patients. While associations from 2-5 strains are prevalent in patients over 40 years and more-1648,3 strains per 1,000 patients, the statistics difference being confirmed ($t=3.95$, $p<0.001$).

The analysis of evolutionary changes in the structure of monocultures and associations of microorganisms. It is interesting both the epidemiological and clinical evolution of the number of strains isolated from patients with SPNI-in associations or monocultures. During years 2009-2010 and 2013-2014, 2013 respectively 1,857 strains of microorganisms from 1,280 and 1,089 patients respectively were isolated, the average number of isolate strains from a patient being 1.57 and 1.71 respectively some foci, which demonstrates the increased number of patients with SPNI affected by associations of microorganisms (tab. IV).

TABLE II

The structure of microorganisms isolated from the patients with SPI, per the patients' age

The group of micro-organisms	Indices	Age category (years)						
		≤20	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**≥70**
Gram positive	No.	59	156	205	207	315	202	128
	%	74.68	67.82	65.70	64.68	62.25	60.29	55.17
Gram negative	No.	20	74	104	117	185	127	99
	%	25.3	32.17	33.33	36.67	36.56	37.91	42.67
Fungi	No.	-	-	3	5	6	6	5
	%	0.0	0.0	0.96	1.56	1.18	1.79	2.15
Total	**No.**	**79**	**230**	**312**	**320**	**506**	**335**	**231**
	%	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

TABLE III

Frequency of strains number isolated from pathological foci according to the age of patients

Number of strains	Indices	Age category (years)						
		≤20	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**≥70**
1 strain	No.	48	108	114	110	150	130	66
	%	716.40	620.68	609.62	582.01	474.68	610.32	485.07
2 strains	No.	8	40	54	67	112	70	39
	%	238.80	459.77	346.15	708.99	708.86	657.27	582.08
3 strains	No.	5	14	27	21	40	19	27
	%	223.88	241.37	433.15	333.33	379.74	267.60	604.47
4-5 strains	No.	-	-	2	2	3	3	2
	%	-	-	21.39	63.49	37.97	37.55	59.70
Total patients	**No.**	**61**	**162**	**197**	**200**	**305**	**222**	**134**
	%	**1,179.1**	**1,321.8**	**1,668.4**	**1,693.1**	**1,601.2**	**1,572.7**	**1,723.8**

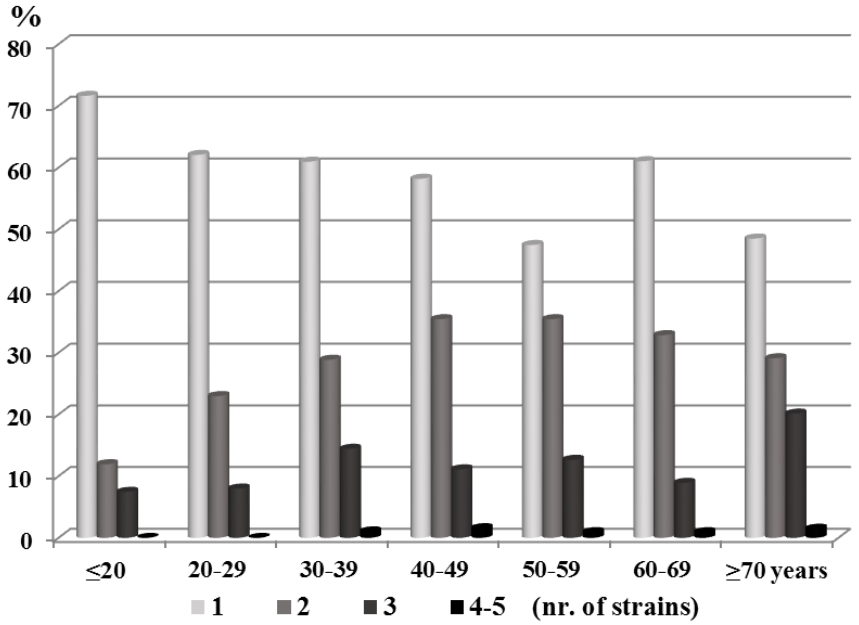

Fig. 2. Strains' number distribution isolated from pathologic foci according to the age of patients

TABLE IV

Distribution of cases of traumatology SPI according to the number of strains isolated from the pathologic focus

Periods	Indices		Number of strains of microorganisms detected in patients with SPNI					Total
			1	2	3	4	5	
Years 2009-2010	No. of patients	Abs.	725	390	153	11	1	1,280
		%	56.64	30.47	11.95	0.86	0.78	100.0
	No. of strains	Abs.	725	780	459	44	5	2,013
		%	36.01	38.74	22.80	2.2	0.25	100.0
Years 2013-2014	No. of patients	Abs.	526	372	178	12	1	1,089
		%	48.30	34.16	16.35	1.10	0.09	100.0
	No. of strains	Abs.	526	744	534	48	5	1,857
		%	28.33	40.06	28.76	2.58	0.27	100.0

It was emphasized that the mono-etiological nature of septic-purulent infections foci, in the multiannual dynamics, is decreasing from 56.64% of investigated patients or 36.01% of isolated strains-between 2009-2010, up to 48.30 % of patients or 28.33% of detected strains-in 2013-2014, being confirmed the statistical difference ($t=2.89$, $p<0.01$). And vice versa, we found an increase of polyetiological aspect. There were isolated from 2 to 5 septic-purulent strains 43.36% of those

investigated, that is 63.99% of all the isolate ones. During the next period, 2013-2014, there were found associations of 2-5 strains of microorganisms in 51.70% of the investigated ones with septic-purulent infections, being 71.67% of the total number of strains (tab. IV, fig. 3).

The number of patients with associations of microorganisms increased by 8.34% in multi-annual dynamics, but the number of microorganisms isolated in associations increased by 7.68%.

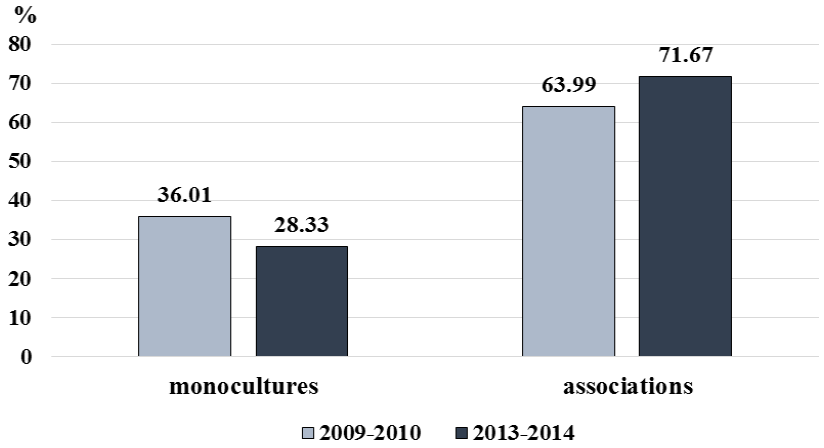

Fig. 3. The distribution of patients with SPI in hospitals of ortopedopedic and trauma profile with monocultures and associations of microorganisms

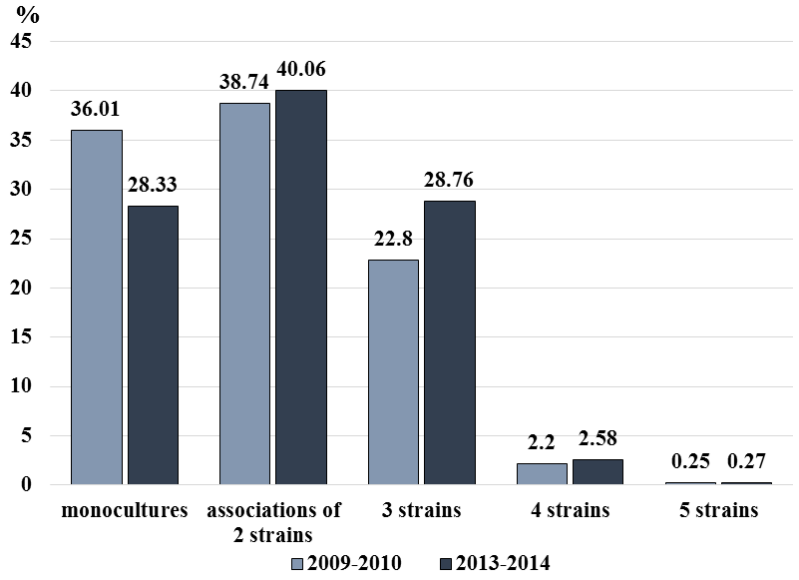

Fig. 4. Comparative distribution of SPI cases according to the number of strains isolated from the pathologic focus, over periods

Therefore, from the total number of isolated strains 63.99% (years 2009-2010) and 71.67% were proven to be in various associations (t=2.75, p<0.01), including 38.74% and respectively 40.06%-associations of two species-1.32% more in the second period; 22.80% and respectively 28.75%-associations from three species-increasing by 5.95% compared to the previous stage; 2.2% and 2.58% respectively-associations from four species-growing by 0.38%; and 0.25% and respectively 0.27%-associations from five species (tab. IV, fig.4).

CONCLUSIONS

Our researches showed that the septic-purulent pathology in hospitals of orthopedics and traumatology profile has a polyetiological character, both in general, and in the infectious foci in patients. The Gram-positive microorganisms prevail (63.19%) compared to Gram-negative ones (35.56%).

We have established that the SPNI are caused mainly by monocultures and Gram-positive bacteria in patients of young age, while SPNI with Gram-negative, fungi and associations with 2-5 strains, prevail in old age.

We have determined that the rate of patients with monocultures decreases in multi-annual dynamics and, *vice versa*, we have registered an increasement of cases caused by the associations of multiple microorganisms.

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IMPORTANCE OF DENTAL MAXILLOFACIAL AESTHETICS IN DENTAL THERAPY

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IMPORTANCE OF DENTAL MAXILLOFACIAL AESTHETICS IN DENTAL THERAPY (Abstract): The recent development of aesthetic dentistry reflects a concentration of the dentists' concern for satisfying patients' requests for an aesthetic appearance of their teeth. Modern dentistry combines medical treatment with the art of aesthetic restorations. Over the years, the restoration of the facial harmony has changed its character, from random to necessary, and from optional to compulsory, as the aesthetic defects have become less and less accepted. **Aim**: Contributions to understanding the principles that must be respected in dental-facial aesthetics and their application in various accessible prosthetic techniques that lead to significant improvement of the aesthetic appearance of the final restoration. **Material and methods:** The study included 22 (aged 40 to 80 years) patients (mean age: 60 years), presenting to the Department of Clinical Dentistry for different types of prosthetic restorations. **Results and discussions:** Taking into consideration the aesthetic principles and criteria, the prosthetic restorations in two similar clinical situations were performed using a different methodology. **Conclusions:** To grant the actual interventions the necessary provisions of true clinical success, the dentist must have and develop the skills that allow him/her to be valued as both a scientist, and an artist. **Keywords:** THERAPY, AESTHETICS, DENTAL-FACIAL, RESTORATION.

Aesthetics is defined as "the science of beauty in nature and art." Although easy to be accepted, such an interpretation contradicts several famous statements of giants of the universal cultural heritage: Plato – "Beauty is virtual" -, Hegel – "beauty cannot be an exact science" -, or Leonardo de Vinci – "Human beings represent the sensitive vector that gives life to essential beauty" (1,2,13).

The well-known Frush and Fischer's SPA triad: sex, personality, age (3) is critical in personalization and choice of the optimal therapeutic solution, as well as other criteria of dental-facial and dental-somatic harmony and, through symmetry, harmony of proportion of the rhythms (repeating, at equal intervals, a certain element of a certain kind), by harmonizing the form with the function, by dissimulating the prosthetic artifice.

Evaluation are made by taking into con-

sideration the components of the aesthetics of the smile: facial, dental-facial, and dental.

Regardless of definition, aesthetics is a reality that reaches all fields of life. Even if the aesthetic emotion is specific to art, for modern people it became necessary in daily environment, daily life, because, as Darwin, Herder, Nietzsche believed (4), the beauty and aesthetic feeling are very important for the development of individual and social life (14).

Dental conditions, aggravated with advancing age, essentially change the aesthetic appearance, which impacts the psychic of the patients, sometimes triggering psychoses (3, 6).

Dental-maxillofacial aesthetics needs attention, so that the treatment is individualized per the patient's wishes.

The goal of the study was to understand the basic principles that must be respected in dental-facial aesthetics and to prove the mastering of these principles by applying them in dental-facial aesthetics, including various prosthetic techniques that help to significantly improve the aesthetic appearance of the final restorations.

MATERIAL AND METHODS

We conducted this study in a group of 22 patients, aged 40 to 80 years (mean age: 60 years) presenting to the Department of Clinical Dentistry for different types of prosthetic restorations.

Physical examination was performed in all subjects, having medical records containing details related to involution of physiognomy and results of complementary examinations (orthopantomography, study model, biometric measurements), useful in making the diagnosis.

During clinical facial examination, we paid attention to several parameters, useful for restorations: contour of the face, with dominant features (nose, cheekbones, forehead, lips etc.); profile features; existing facial asymmetries; mandible-cranial relationship.

Intraoral examination aimed at highlighting the elements that can contribute to alteration of physiognomic function: teeth, periodontium, edentulous ridges, and occlusal ratios. Special attention was paid to bone reorganization, size of alveolar ridges and the relations between these, and potential prosthetic space. Examination of the old dentures revealed elements that might compromise the physiognomic appearance: arches, false gums, damaged margins (length, depth, casting), hygiene status.

For statistical analysis ANOVA and T-student tests were used, and the database was managed with MS Office Excel and Epi Info 2000.

RESULTS

It was proven that, next to the other dental specializations, dental prosthetics brings substantial contributions to morphological and functional restoration of the stomatognat system of the elderly, compromised by these conditions (5). The last decade brought an impressive volume of research in dental prosthetics, which determined reorientations in therapeutic concepts, together with new treatment means (6).

General principles of dental-facial restorations are individualized, per age and gender (7).

Age-group and gender distribution of patients showed that most patients were female (7 male patients – 31.81% and 15 female patients – 68.19%) (fig. 1).

Patient distribution by dental syndromes revealed a high prevalence of total (mono-

or bi-maxillary) and subtotal edentation.

Patient distribution by syndromes: total edentation - 3 cases (13.63%); subtotal edentation - 6 cases (27.27%); partial edentation - 2 cases (9.09%); dental crown lesions - 5 cases (22.72%); periodontal disease - 4 cases (18.18%); malocclusions - 2 cases (9.09%) (fig. 2).

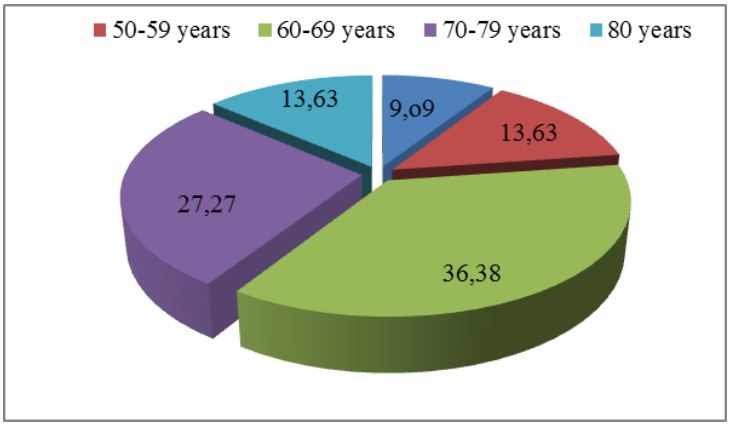

Fig.1. Age-group distribution of patients (years)

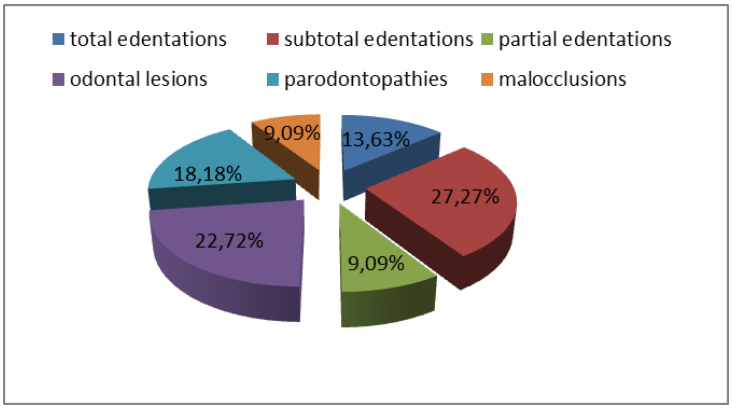

Fig. 2. Distribution of patients by syndromes

These values are irrelevant as in most of the cases the syndromes were associated.

History indicated the reasons for requesting the dental treatment and a preponderance of the female gender presenting for restoring their physiognomic function.

Of the 22 patients, in 8 elderlies (36.38%) with damaged removable dentures and 6 (27.27%) with conservative dental restorations the most common complaints were related to functional dissatisfaction related to the material, manufacturing technology requesting their restoration or re-optimization. Some of the patients did not have dentures – 3 (13.63) not trusting their wishes would be met.

Disorders of the stomatognat system frequently have a negative effect on dental-facial aesthetics. The impactation of physiognomic appearance in elderly is deter-

mined by dental, coronary lesions, with or without loss of substance, periodontal conditions, integrity loss of dental arch due to edentation and dental migrations, occlusion disorders, and disturbance of the mandibular-cranial relationship.

In all 22 cases included in the study, local clinical examination revealed important clinical changes of stomatognat system, prosthetic field, providing useful information for choosing and positioning frontal teeth, distributing edentulous spaces, placing the occlusal plane, moulding the vestibular side or the margins, in removable dentures.

Intraoral examination revealed the existing oral pathology, in some cases with important consequences on the patient's physiognomy (fig. 3).

Fig. 3. Aspect of oral pathology in elderly (Collection of the Clinic)

The dental and periodontal status proved to be heterogenous, a consequence of involution phenomena and/or associated pathology: there were patients with more or less complete dentition, - 4 cases (18.18%) with coronary odontal lesions, periodontal diseases, malocclusions, and partial or total edentation.

Examination of old dentures brought useful information related to technology and functional satisfaction, which served as an important guide for new dentures – 11cases (50.00%) (fig. 4).

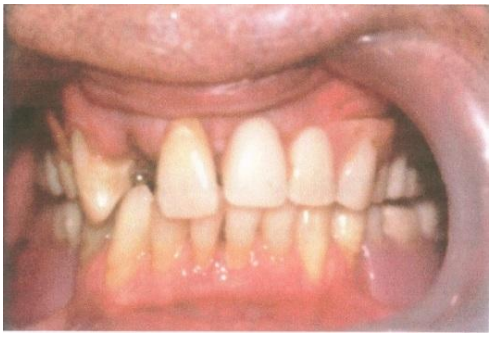

Fig. 4. Examining old dentures (Collection of the Clinic)

Dental and prosthetic treatment offered in 9 cases (40.90%) multiple possibilities of recovery of functions of the stomatognat system, physiognomic appearance, influenced by the oral pathology, but also by the involutive process. Although the patient was helped to rebuild self-esteem by the restoration of his physiognomic appearance, it should not prevail over the other fundamental principles of dental practice (7, 8).

The aesthetic principles and criteria of prosthetic restoration therapy were applied in all 22 cases included in this study. Thus, we used the ceramic metal procedure in 7 cases (31.81%), in which the aesthetic success depended on specific problems related to the form of preparation and the nature of the dental-prosthetic joint.

Since the ceramic metal restorations were manufactured correctly, their durability is better than the one of conventional ceramic, due to their metal infrastructure and low fusing porcelain, very durable, with better binding forces than the cohesion forces of porcelain itself – 11 cases

(50.00%) cases.

Of the 22 cases included in the study, 5 (22.72%) cases suffered from dental crown lesions at different levels (11, 17, 18) with rapid caries progression, not allowing the preservation of dental crowns and requiring immediate ceramic metal therapy, this time using the substitution method and two-piece prosthetic technique (fig. 5).

Fig. 5. Dental crown lesions (Collection of the Clinic).

In general, the preparation technique resembled that of any other type of ceramic metal crown. On vestibular facet, we chose the 90° angle threshold preparation, as well as Jacket crowns made of porcelain.

The use of porcelain margin technique in bevel preparations, with tilted or knife-edge thresholds was contraindicated, since the marginal porcelain would be very thin, impossible to fit properly and would easily fracture (11, 12, 14). This technique allows the use of any modern materials for manufacturing the models, including epoxy resins, copper, plaster, and improved plaster.

DISCUSSION

As other authors stated (1, 2, 9), our aesthetic goals were to reproduce all parameters, except for the ones related to placing the gingival margin, gingival zenith and the cervical coronary third technique.

In terms of restoration, from depth to surface, we applied a preparation to the cervix, especially at vestibular level where it should be an optimal space for applying metal and porcelain layers, and especially opaque, in a sufficiently thick layer, that can effectively mask the subjacent metallic structure.

As other authors stated (10, 11) provisional dentures helped us reach our aesthetic goals, which, besides proper protection of organic structures, provided the restoration of morphological and functional integrity of the dental arch, with the possibility of immediate social reinsertion (9, 11).

CONCLUSIONS

Contributions to understanding the principles that must be respected in dental-facial aesthetics and their application in various accessible prosthetic techniques that lead to significant improvement of the aesthetic appearance of the final restoration. The general principles based on dental facial aesthetic restoration/of aesthetic restorative dentistry were individualized according to age and gender, while dental syndromes allowed an analytical and critical estimation of such elements as: proportions, symmetry and diversity.

Elderly patients with damaged removable dentures complained of functional problems and expressed dissatisfaction with the material and manufacturing technology, which created physiognomic problems, requesting their restoration or re-optimization (.

Although the use of metal and porcelain gave acceptable results, better safety was accomplished by using a combination in which the difference between the thermal expansion coefficients was higher.

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XRCC1 Arg194Trp AND Arg399Gln POLYMORPHISMS AND RISK OF NON-HODGKIN LYMPHOMA IN A ROMANIAN POPULATION

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XRCC1 Arg194Trp AND Arg399Gln POLYMORPHISMS AND RISK OF NON-HODGKIN LYMPHOMA IN A ROMANIAN POPULATION (Abstract): *XRCC1* polymorphisms may alter the individual's capacity to repair DNA damages and may increase the risk for developing different types of cancer, including Non-Hodgkin Lymphoma (NHL). The purpose of our study was to investigate the association between *XRCC1* Arg194Trp and Arg399Gln polymorphisms and risk of NHL, in a case-control study consisted of 81 patients with NHL and 113 healthy controls. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods were performed to determine the *XRCC1* genotypes. A statistically significant association was observed between the *XRCC1* Arg194Trp polymorphism and risk of NHL ($p < 0.0001$, OR = 7.01, 95% CI = 2.488-19.753) in both male and female. We found no association between NHL and control groups with respect to *XRCC1* Arg399Gln polymorphism ($p = 0.4867$, OR = 1.346, 95% CI = 0.5822-3.111). Also, we did not find differences between the *XRCC1* polymorphisms and NHL risk taking into account the IPI score, ECOG performance status, Ann Arbor stage and the treatment outcome ($p > 0.05$, for all comparisons). Our results suggested an increased risk for NHL regarding the *XRCC1* Arg194Trp polymorphism in a Romania population, while *XRCC1* Arg399Gln polymorphism is not associated with NHL. Further work is needed to validate these results on a larger sample study. **Keywords:** *XRCC1*, GENE POLYMORPHISM, NON-HODGKIN LYMPHOMA.

Non-Hodgkin lymphoma (NHL) represents a heterogeneous group of malignancies. According to GLOBOCAN (1) the incidence of NHL for both sexes is 2.7 per 100,000 persons and in the past 50 years, the incidence has increased significantly, being higher in men than in women (2). In Romanian population the incidence of NHL is estimated at 1566 cases men and women per year (1).

A number of exogenous and endogenous exposures, such as virus infections, immune dysfunction, benzene and other solvents and alteration in capacity to repair DNA, have been proposed as risk factors for NHL (2-6).

Recent studies have shown the association between single-nucleotide polymorphisms (SNPs) in DNA repair genes and susceptibility to NHL (7-18). X-ray repair

cross-complementing group 1 (*XRCC1*) protein plays an important role in the DNA repair mechanisms, specifically in base excision repair pathway and produces amino acid substitutions (2-3, 5-6). *XRCC1* is involved in the repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents (6, 14).

Many polymorphisms of the *XRCC1* gene on chromosome 19q13.2 have been identified and have been registered in the SNP database (http://www.ncbi.nlm.nih.gov/SNP). These polymorphisms might contribute to the risk of cancer, including NHL (9-11, 15-17). Thus, the most studied polymorphisms in hematological malignancies in *XRCC1* gene are Arg194Trp, Arg399Gln and Arg280His (2-18).

Our aim was to evaluate the effect of *XRCC1* Arg194Trp and *XRCC1* Arg399Gln polymorphisms on the risk of NHL within a case-control study conducted in Romania. We also investigated the influence of *XRCC1* polymorphisms on treatment outcome and disease stage patients with NHL.

MATERIAL AND METHODS

The patients' group consisted of 81 Caucasian adults diagnosed clinically and histologically with NHL, according to World Health Organization classification (19), at the Hematology Clinic I from Emergency Clinical County Hospital Tîrgu Mureș, Romania. Medical reports and laboratory data of the patients with NHL were reviewed to evaluate histologically subtypes of NHL, the International Prognostic Index (IPI) score, The Eastern Cooperative Oncology Group (ECOG) performance status, Ann Arbor stage, treatment outcome and survival rate.

The control group comprised 113 healthy individuals with no history of cancer or other chronic diseases. The mean age in NHL group was 59.96 years and in the control group was 65.26 years.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy Tîrgu Mureș, Romania (no. 111/12.14.2015) and written informed consent was given according to the principles of the Declaration of Helsinki.

DNA samples were extracted from fresh peripheral blood using the Quick-gDNA MiniPrep kit (Zymo Research, USA) and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (20) were performed to determine the *XRCC1* genotypes.

All statistical analysis were performed using GraphPad InStat software. The Chi-square test and Fisher's exact test (two-sided) were used to compare genotype frequencies ($p < 0,05$). To estimate the associations between genotypes and risk of NHL were also calculated the odds ratios (OR) and 95% confidence interval (95% CI) for OR.

RESULTS

The allele and genotype distribution of the *XRCC1* Arg194Trp and Arg399Gln polymorphisms among the patients and controls are shown in table I.

A significant association was observed between the *XRCC1* Arg194Trp (Arg/Trp) polymorphism and risk for developing NHL ($p < 0.0001$, OR = 7.01, 95% CI = 2.488-19.753) in both male and female.

No significant associations were found in females between controls and patients for *XRCC1* Arg399Gln (Arg/Gln, Gln/Gln) and *XRCC1* Arg194Trp (Trp/Trp) polymorphisms and NHL risk.

TABLE I

Genotype distribution of the *XRCC1* gene polymorphisms in NHL patients and controls

	Controls No. (%)	Patients No. (%)	OR (95% CI)	p value
XRCC1 194				
Arg/Arg	107 (94.69)	58 (71.60)	Ref.	Ref.
Arg/Trp	5 (4.42)	19 (23.46)	7.01 (2.488-19.753)	<0.0001
Trp/Trp	1 (0.88)	4 (4.94)	7.379 (0.8054-67.607)	0.0598
Arg/Trp+Trp/Trp	6 (5.31)	23 (28.40)	7.072 (2.724-18.356)	<0.0001
Arg allele	219 (96.90)	135 (83.33)	Ref.	Ref.
Trp allele	7 (3.10)	27(16.66)	6.257 (2.651-14.768)	<0.0001
XRCC1 399				
Arg/Arg	47 (41.59)	40 (49.38)	Ref.	Ref.
Arg/Gln	50 (44.25)	30 (37.03)	0.705 (0.3797-1.309)	0.2674
Gln/Gln	16 (14.16)	11 (13.58)	0.8078 (0.3364-1.940)	0.6644
Arg/Gln+Gln/Gln	66 (58.41)	41 (50.62)	0.7299 (0.4110-1.296)	0.282
Arg allele	144 (63.72)	110 (67.90)	Ref.	Ref.
Gln allele	82 (36.28)	52 (32.0)	0.8302 (0.5416-1.272)	0.3926

No.- number, Ref. – reference category

In this study, a decreased risk for developing NHL was observed between NHL male patients and male controls with respect to *XRCC1* Arg399Gln SNP (OR = 0.5102, 95% CI = 0.2153-1.209), but not statistically significant. However, no association was obtained in women regarding *XRCC1* Arg399Gln combined heterozygous and variant homozygous genotypes and NHL risk (p = 0.4867, OR = 1.346, 95% CI = 0.5822-3.111).

In table II are summarized demographic and clinical characteristics of the NHL cases according to the genotype of the *XRCC1* Arg194Trp and Arg399Gln polymorphism.

Furthermore, we observed that an III or IV Ann Arbor stage, an increased ECOG performance status and increased IPI score were associated with poor survival time for NHL and a higher resistance to treatment. The lowest median survival time in our study was 31.2 months in *XRCC1* Trp194Trp polymorphism and 29.62 months in *XRCC1* Gln399GLn polymorphism.

There were no significant differences between *XRCC1* Arg399Trp and *XRCC1* Arg399Gln polymorphisms and ECOG performance status and IPI score, respectively (p > 0.05, for all comparisons). Also, no differences were found regarding NHL patients' outcome (resistant, partial remission and complete remission to treatment) and investigated SNPs (p = 0.3482, p = 0.6959).

We noticed that 8 of our patients had the variant genotype for both studied polymorphisms of the *XRCC1* gene, 5 of them

were females (4 patients with Arg/Trp + Arg/Gln, 3 with Arg/Trp + Gln/Gln and 1 patient with Trp/Trp + Arg/Gln). In the control group, we found that a male patient (70 years old) had the Arg/Trp + Arg/Gln genotype.

The frequency of *XRCC1* combined variant heterozygous and homozygous genotypes were higher in NHL patients than in controls. We may presume that presence of combined variant genotype may be associated with the risk of developing NHL in the investigated cohort.

DISCUSSION

In the present study, we investigated a possible association between the *XRCC1* polymorphisms (Arg194Trp and Arg399Gln) and risk for developing NHL in a population from Transylvania region. To our knowledge, our study is the first to analyze the association of the *XRCC1* Arg194Trp and Arg399Gln polymorphisms with NHL in a Romanian population.

Defects in repair pathways induced by endogenous and exogenous carcinogens can lead to mutations implicated in cancer pathogenesis (2-6). However, previous studies reported contradictory results (2, 6-18). Thus, we hypothesized that DNA base excision repair gene *XRCC1* Arg194Trp and Arg399Gln polymorphism may increase or decrease the risk of NHL.

In this study, we found that *XRCC1* Arg194Trp polymorphism is associated with an increased risk of NHL. This association is confirmed in a study performed by Kim I et al. (7) in a Korean population, showed a significantly increased the risk of diffuse large B-cell lymphoma (DLBCL) and Hill D et al. (8) revealed that the *XRCC1* 194Trp allele was associated with a slightly increased risk (1.4-fold) of developing NHL.

TABLE II

Patients' demographic and clinical characteristics according to the *XRCC1* genotypes

	***XRCC1 194* no. (%)**				***XRCC1 399* no. (%)**			
	Arg/Arg	**Arg/Trp**	**Trp/Trp**	**Arg/Trp+ Trp/Trp**	**Arg/Arg**	**Arg/Gln**	**Gln/Gln**	**Arg/Gln+ Gln/Gln**
Gender								
Male	20	8	2	10	15	11	4	15
Female	38	11	2	13	25	19	7	26
Age, mean	61	60	59.5	59.75	60.5	61	59	60
NHL								
Ann Arbor stage III-IV	22 (70.97)	7 (22.58)	2 (6.45)	9 (29.03)	14 (45.16)	13 (41.94)	4 (12.90)	17 (54.84)
LDH, increased levels	36 (66.67)	14 (25.92)	4 (7.41)	18 (33.33)	30 (55.56)	18 (33.33)	6 (11.11)	24 (44.44)
Extranodal dissemination	10 (62.5)	6 (37.5)	0 (0.00)	6 (37.5)	8 (50.00)	5 (31.25)	3 (18.75)	8 (50.00)
ECOG PS								
1,2	38(65.5)	14(73.68)	3(75.00)	17(73.91)	30(75.00)	27(90.00)	7(63.64)	34(82.93)
3,4	20(34.48)	5(26.31)	1(25.00)	6(26.09)	10(25.00)	3(10.00)	4(36.36)	7(17.07)

	XRCC1 194 no. (%)				*XRCC1 399* no. (%)			
	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Trp+ Trp/Trp	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Gln+ Gln/Gln
IPI score								
0,1,2	34 (58.62)	9 (47.36)	2 (50.00)	11 (47.83)	25 (62.50)	14 (46.66)	7 (63.64)	21 (51.22)
3,4,5	24 (41.37)	10 (52.63)	2 (50.00)	12 (52.17	15 (37.50)	16 (53.33)	4 (36.36)	20 (48.78)
Treatment outcome								
Resistant	25 (45.45)	11 (68.75)	2 (50.00)	13 (65)	20 (52.63)	15 (53.57)	3 (33.33)	18 (48.65)
Partial remission	18(32.72)	4 (25)	2 (50.00)	6 (30)	12 (31.57)	7 (25.00)	5 (55.55)	12 (32.43)
Complete remission	12 (21.81)	1 (6.25)	0 (0.00)	1 (5)	6 (15.78)	6 (21.42)	1 (11.11)	7 (18.92)
Survival								
Living	40 (80.00)	14 (82.35)	3 (75.00)	17 (80.95)	28 (75.67)	19(79.16)	10 (100.00)	29 (85.29)
Deceased	10 (20.00)	3 (17.64)	1 (25.00)	4 (19.05)	9 (24.32)	5 (20.83)	0 (0.00)	5 (14.71)

No. – number, NHL – Non-Hodgkin Lymphoma, LDH - Lactate dehydrogenase, ECOG PS- Eastern Cooperative Oncology Group performance status, IPI - International Prognostic Index

In a meta-analysis performed by Li Y et al. (9) in 2010 on 13 case-control studies, variant genotypes of *XRCC1* Arg194Trp polymorphism was associated with an increased risk of NHL in the Asian population, while *XRCC1* Arg194Gln polymorphism had a decreased risk for this disease.

In contradiction, in a recent meta-analysis conducted by Tang L et al. (21) of 20 case-control studies no significant association was observed between NHL and *XRCC1* Arg194Trp polymorphism. Similar findings were observed in a small children cohort with B-cell lymphoma (33 patients and 52 controls) that frequency of *XRCC1* Arg194Trp polymorphism was significantly lower in patients than in the control group ($p = 0.005$). No association was reported between *XRCC1* Arg194Trp allele and DLBCL in Australian population (2).

In addition, no SNP variants of *XRCC1* Arg194Trp, Arg280His and Arg399Gln alone were found to be related with the risk of NHL or histologic subtypes of NHL in a Chinese population (10). However, Liu J et al (10) reported that two combinations of SNP variants were associated with an increased risk of NHL. Moreover, they revealed that Arg399Gln variant allele was associated with risk of follicular lymphoma among heavy smokers.

In the present study, *XRCC1* Arg399Gln polymorphism was no associated with the risk of NHL. The lack of association for the *XRCC1* ArgGln399 variants with NHL was also observed in a Japan population described by Matsuo K et al. (22) (372 cases with malignant lymphoma and 500 controls) and in a comprehensive meta-analysis conducted by Du L et al. (11) in 2015 on 27 case-control studies. Moreover, Du L et al. (11) suggested that *XRCC1* Arg399Gln polymorphism might be a risk factor for leukemia in the Asian population, but not in malignant lymphoma or in the European population. Therefore, we can assume that the variant heterozygous and homozygous genotypes for *XRCC1*

Arg399Gln polymorphism has a protective role in NHL and other types of malignancies (9, 11, 21-22).

In contrast to our findings, Veropaeva E et al. (12) demonstrated that the *XRCC1* Arg399Gln polymorphism (Gln/Gln) was associated with a significantly increased risk of aggressive NHL in 42 cases. On the other hand, in a study conducted in America, El-Zein R et al. (14) observed that *XRCC1* Arg399Gln SNP had a significantly increased risk of Hodgkin's disease.

According to Kim H et al. (23), *XRCC1* Arg399Gln polymorphism had a decreased risk for NHL in a Korea population in a case-control study consisted in 694 cases and 1700 controls (OR = 0.80, 95% CI = 0.66–0.96, p = 0.02).

Our results suggested the presence of 8 cases of combined genotypes with NHL and another one in the control group. In a study from 2014 performed by Li SX et al. (15) on 282 cases and 232 controls it was shown that combined genotypes *XRCC1* 399-280-194 were not associated with NHL risk. According to Kim I et al. (7), *XRCC1* haplotype A (194Arg-280Arg-399Arg) was associated with a decreased risk of DBCL (OR = 1.38; 95% CI = 1.05-1.80; P = 5 0.019).

The current study is the first to evaluate the differences between analyzed *XRCC1* Arg194Trp and *XRCC1* Arg399Gln polymorphisms and risk of NHL taking into account the IPI score, ECOG performance status, Ann Arbor stage and treatment outcome and there were no significant differences (p > 0.05, for all comparisons).

There are few data that evaluated the response to treatment or survival chemotherapy or radiotherapy associated with *XRCC1* polymorphisms. For example, Kim K et al. (24) reported an increased association between variant genotypes of the *XRCC1* Arg194Trp polymorphism in patients with cervical cancer and treatment response (platinum-based neoadjuvant chemotherapy).

According to Kim I et al. (7) no relation was noticed between *XRCC1* Arg399Gln genotypes and response to treatment, results similar to our findings.

However, our study has following limitations. First, the sample size group is relatively small compared to previous studies and could influence the statistical power. And the second, the unknown environmental factors were not considered, this could modify the effect of the *XRCC1* SNPs.

CONCLUSIONS

The present study demonstrates that the variant genotypes of the *XRCC1* Arg194 Trp polymorphisms were associated with an increased risk of NHL in a Romanian population. On the other hand, we can assume that *XRCC1* Arg399Gln does not represent a risk factor for NHL.

Thus, further investigations on larger sample groups are needed to clarify the association between *XRCC1*polymorphisms and susceptibility to NHL.

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ADENOCARCINOMA AND TUBERCULOSIS OF THE SIGMOID COLON AND FALLOPIAN TUBE – A RARE ASSOCIATION. A CASE REPORT AND REVIEW OF THE LITERATURE

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ADENOCARCINOMA AND TUBERCULOSIS OF THE SIGMOID COLON AND FALLOPIAN TUBE – A RARE ASSOCIATION. A CASE REPORT AND REVIEW OF THE LITERATURE (Abstract) Association of adenocarcinoma and tuberculosis (TB) of the sigmoid colon is a rare clinical condition even in an endemic country as Romania, with challenging diagnosis and treatment. **Case report**. We present the case of a 57-year-old female patient who was admitted on emergency basis for a diagnosis of obstructive sigmoid adenocarcinoma. The patient was operated on and it an obstructive sigmoid tumor with serosal invasion, adherent (invading) to the body of uterus and left adnexa and urinary bladder serosa, no liver or peritoneal metastases. A sigmoidectomy was performed *"en bloc"* with subtotal hysterectomy, left adnexectomy and extramucosal cistectomy. The histopathological exam showed a moderately differentiated, ulcerated adenocarcinoma, widely infiltrating the colon wall invading the myometrium. Ziehl Neelsen (ZN) stain identified the presence of metachromatic bacillary structures in the colonic wall, lymph nodes and adnexal areas. Postoperative course was uneventful and the patient was discharged 10 days postoperatively in good clinical condition. After one year when the patient completed the full course of antitubercular drugs, a thorough work-up was performed. Colonoscopy, CT of the thorax, abdomen, pelvis showed no signs of recurrence while tumoral marker CEA (1,62 ng/ml – n<3,4) and QFT (Quantiferon-TB Gold) test were within normal range. **Discussion and conclusion**. Although digestive tuberculosis is included in differential diagnosis for those patients presenting abdominal pain or obstructive digestive symptoms in endemic regions, in this case the absence of TB infection criteria and positive endoscopic biopsy for colonic adenocarcinoma did not allow a complete pre- or perioperative diagnosis. **Keywords**: ADENOCARCINOMA, TUBERCULOSIS, SIGMOID, FALLOPIAN TUBE.

Association of adenocarcinoma and tuberculosis (TB) of the sigmoid colon is a rare clinical condition even in an endemic country as Romania. As preoperative diagnosis of colon cancer routinely poses no difficulties, the association of malignancy and TB may be challenging, both in term of diagnosis and treatment. Due to the intricate presentation of these two disorders, in most of the cases pathology examination of

the surgical specimens ultimately reveals the accurate diagnosis in most of these cases. It is to be elucidated if the association of these lesions is a coincidence or there is an etiological relationship, as ulcerative colitis and Crohn's disease are proved to be a premalignant condition for colon cancer.

CASE REPORT

We present the case of a 57-year-old female patient who was transferred in our department on emergency basis from the gastroenterology department. The patient had no personal or collateral relevant history (including TB) and was initially admitted in the gastroenterology unit for persistent lower quadrants abdominal pain, change in bowel habit (diarrhea followed by constipation) fatigue and altered general status. A diagnosis of sigmoid carcinoma was established by colonoscopy that revealed a tumor at 40 cm from the anal verge, with macroscopic and microscopic features of an adenocarcinoma (fig. 1).

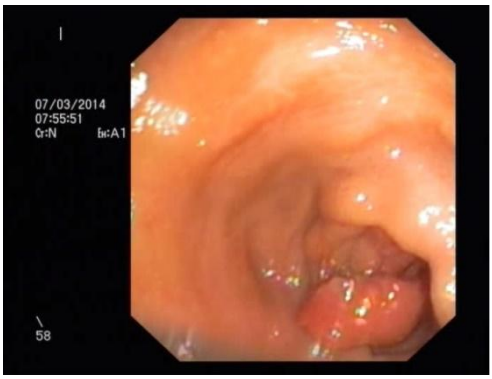

Fig. 1. Colonoscopy aspect

The thorax radiography was normal whereas ultrasound examination of the abdomen revealed no liver or peritoneal deposits. Shortly after colonoscopy the patient developed symptoms of lower intestinal obstruction and was referred to surgery. The physical examination at admittance in our unit revealed a distended abdomen, tenderness in the left lower quadrant where an imprecisely defined mass was palpable and exaggerated bowel sound; rectal touch and vaginal examination were inconclusive. Except for a mild microcytic anemia, the lab tests were within normal range. Hidroaeric levels were present on plain abdominal X-ray.

Following a swift preoperative preparation, the patient was operated on through a median laparotomy; the exploration of the peritoneal cavity revealed an obstructive sigmoid tumor with serosal invasion, adherent (invading) to the body of uterus and left adnexa and urinary bladder serosa, no liver or peritoneal metastases. Under these circumstances, a sigmoidectomy was performed *"en bloc"* with subtotal hysterectomy, left adnexectomy and extramucosal cistectomy. Postoperative course was uneventful and the patient was discharged 10 days postoperatively in good clinical condition.

Resection pieces were examined at the Pathology Department of "Sf. Spiridon" Hospital. The anatomic and pathological examination consisted in the macroscopic evaluation of the colonic tumor and subsequently the fragments were fixed in 10% neutral formalin and processed for histological techniques including the classic paraffin, cutting on microtome 4µm sections and stained with Hematoxylin-Eosin (HE) and Ziehl Neelsen (ZN) for tuberculous bacillary identification.

The histopathological exam showed a moderately differentiated, ulcerated adenocarcinoma, widely infiltrating the colon wall (fig. 2) complicated with parietal fis-

tula and granulation tissue (in which also found food debris). The infiltrative tumor interested the external myometrium about 2 mm (fig. 3). In the lymph nodes, the adherent colon and the omentum numerous granulomatous lesions, with central caseous necrosis and tendency to confluence were identified (fig. 4). The tuberculous lesions were also found in the left fallopian tube with second involvement of the ovary. Ziehl Neelsen (ZN) stain identified the presence of metachromatic bacillary structures in the colonic wall, lymph nodes and adnexal areas (fig. 5).

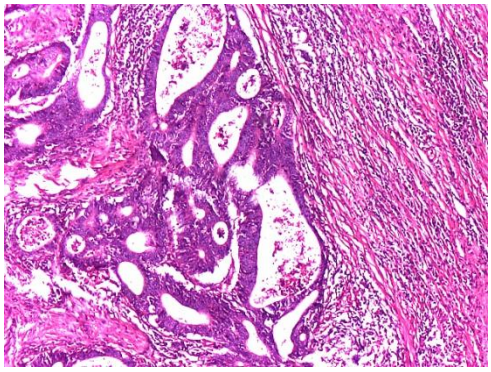

Fig. 2. Moderately differentiated, ulcerated adenocarcinoma, HE, x 4

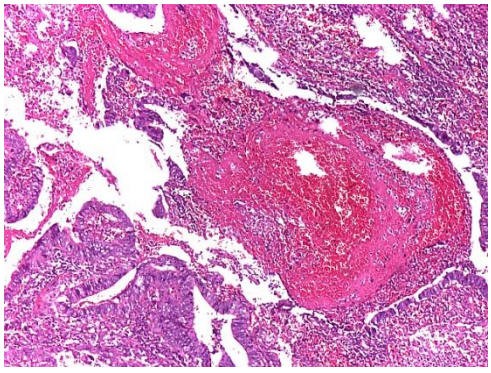

Fig. 3. Infiltration of the uterus, HE, x 4

The interdisciplinary meeting between surgeon, oncologist and pneumologist recommended anti TB treatment and reassessing the patient on regular basis. After one year when the patient completed the full course of anti-tubercular drugs, a thorough work-up was performed. Colonoscopy, CT of the thorax, abdomen, pelvis showed no signs of recurrence while tumoral marker CEA (1,62 ng/ml – n<3,4) and QFT (Quantiferon-TB Gold) test were within normal range.

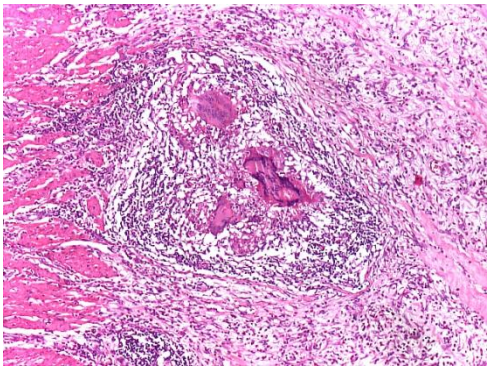

Fig. 4. Tuberculous granulomas in colonic wall, HE, x 4

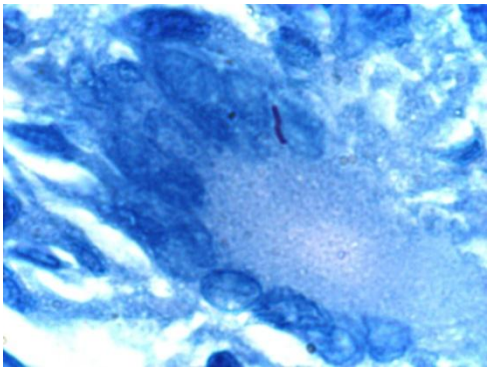

Fig. 5. Koch bacillus, ZN, x 100

DISCUSSION

Since the initial mentioning almost 200 years ago by Bayle, association of tuberculosis and carcinoma is well documented in

lungs, skin and larynx (1). However, the coexistence of carcinoma and tuberculosis is rare, especially when located on the same site on the colon. Less than 70 cases are reported in the literature and to our knowledge this is the first to be mentioned in Romania. In an extensive review, Falagas and col established three different types of association between malignancy and TB: (i) the development of cancer on a background of a previous tuberculous infection; (ii) the concurrent existence of TB and malignancy in the same patient(s) or clinical specimen(s); and (iii) the diagnostic challenges due to multi-faceted presentations of these two disorders (2).Experimental research found that *Mycobacterium tuberculosis* can induce DNA damage and increased ant apoptotic activity, which along with prolonged chronic inflammation may facilitate carcinogenesis (3-7).

As demonstrated that pulmonary scarring of TB etiology may generate lung cancers, chronic inflammatory mucosal changes from GERD and H. pylori gastritis are initiating a sequence of metaplasia and dysplasia results in neoplastic change. It is also universally accepted that severe weight loss or malnutrition related to an advanced neoplastic disease disturbs host immunity and increase susceptibility to activate tubercular infection, possibly by tumor peptides or antigens. On the other hand, according to some Indian authors, the association of carcinoma and tuberculosis is coincidental, the argument being that compared to the high incidence of abdominal tuberculosis in India, the cases of coexisting tuberculosis and carcinoma are very few (8,9).

A particular aspect of the case we report is the association with genital tuberculosis and a question to be asked is if TB lesions originated from the uterus or fallopian tube and subsequently involved the sigmoid or vice versa. Genital TB is almost always secondary to TB elsewhere in the body and the mode of spread is usually hematogenous or lymphatic and occasionally occurs by way of direct contiguity with an intraabdominal or peritoneal focus (10). Although only one tube appears infected, in more than 90% of patients with genital TB, the tubes are involved bilaterally.

CONCLUSIONS

In our study, although digestive tuberculosis is included in differential diagnosis for those patients presenting abdominal pain or obstructive digestive symptoms in endemic regions, in this case the absence of TB infection criteria and positive endoscopic biopsy for colonic adenocarcinoma did not allow a complete pre- or perioperative diagnosis.

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NEWS

THE EFFECTS OF CHLORHEXIDINE AND SODIUM HYPOCHLORITE ON THE SETTING TIME OF CALCIUM-ENRICHED MIXTURE CEMENT

The aim of a study realized by a group of Iranian researchers was to evaluate whether adding 2% chlorhexidine and 2.6% sodium hypochlorite to calcium-enriched mixture cement would affect its setting time or not. The setting time of calcium-enriched mixture cement was evaluated in three groups (n=9) as follows: group 1; calcium-enriched mixture cement, group 2; calcium-enriched mixture cement+2% chlorhexidine and group 3; calcium-enriched mixture cement+2.6% sodium hypochlorite. Then the mean values of setting time were calculated and the Kolmogorov-Smirnov test was used to evaluate the normal distribution of data. The Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis. Statistical significance was set at 0.05. The obtained data show that the mean setting time for groups 1, 2 and 3 were 105, 120 and 220 min, respectively. There was a significant increase in the duration of setting time in group 3 (sodium hypochlorite) in comparison with the two other groups ($P<0.05$). In conclusion, the researchers showed that the sodium hypochlorite significantly increase the setting time of calcium-enriched mixture cement, whereas chlorhexidine did not alter the setting time (Frough Reyhani M, Ghasemi N, Shakouie S, Rahimi S, Salem Milani A, Ranjbar B. Effects of Chlorhexidine and Sodium Hypochlorite on the Setting Time of Calcium-Enriched Mixture Cement. *Iran Endod J.* 2015;10(3):162-164).

Irina Grădinaru

AXIAL MODIFICATIONS OF PERMANENT LOWER MOLARS AFTER PREMATURE LOSSES OF TEMPORARY MOLARS

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AXIAL MODIFICATIONS OF PERMANENT LOWER MOLARS AFTER PREMATURE LOSSES OF TEMPORARY MOLARS (Abstract): **Aim**: The aim of our study was to determine the impact of premature loss of temporary lower molars upon the longitudinal axis of the first and second permanent molars. **Material and Methods:** The study groups included 61 patients, 6 -9 year olds with premature loss of primary molars and a control group of 24 patients with intact temporary teeth. We evaluated the angle between longitudinal axis of first and second lower permanent molars and occlusal plane. **Results.** It was observed that premature loss of lower second deciduous molar modifies more the vertical axis of first and second permanent molars than the premature loss of first lower primary molar. Reducing space occurs mainly through mesial inclination of molars that separates the edentulous breach. Temporary loss of both lower first molars on the same quadrant causes an accelerated eruption of both premolars increasing the prevalence of eruption sequence: "4-5-3-7".**Conclusions:** The preservation of the occlusal morpho-functional complex using space maintainers mainly when the premature loss of the second primary molars occurs is the best interceptive treatment option. **Keywords:** PREMATURE LOSS, AXIAL MODIFICATION, PERMANENT LOWER MOLARS, DENTAL CARIES, TEMPORARY TEETH.

The first one who debated upon the subject of premature loss of temporary teeth were *Hutchinson* and *Davenport* as early as the 1880s (1,2). The concept was defined as the exfoliation of deciduous teeth on the arch with more than 12 months prior to the normal eruption period of permanent teeth, exceeding the limits of normal variability of temporary teeth exfoliation sequences (3).

The most frequent causes that trigger the premature loss of temporary teeth are: untreated complicated dental cavities, traumatisms and premature root resorption.

The premature loss of temporary teeth, as a consequence of untreated dental cavity of temporary teeth may lead to serious tridimensional dental, dento-dental, dento-alveolar, occlusal and variably skeletal lesions. Thus, we control the migration of permanent teeth during intra-maxillary or oral eruption, the consequence being dento-alveolar incongruence. There may also appear super-eruptions of antagonist teeth, arch narrowing, reverse toothing, premature contacts and occlusal interferences (4).

Studies have shown that the premature

loss of temporary molars I mainly determines the distal migration of temporary canines. In this case, mesial displacement of permanent molars or their version cannot occur (5). Greater effects appear at the moment of the premature loss of second temporary molars, as they serve as a guide for the eruption of the permanent molar (6). The premature loss of second temporary molars – mainly mandibular - determines a shrinking of the arch length due to mesial inclination of permanent molars, compromising the „lee-way space" by affecting the posterior support area (7). The radiologic exam is mandatory for these patients. Although the cone beam computed tomography (CBCT) has progressively become a radiological method or reference in the oro-maxillo-facial sphere, the orthopantomography still represents a routine exam for children under 12 and not only. It thus represents a simple and economical way that uses a low radiation dose in comparison with the CBCT and allows a rapid quantitative and qualitative analysis both of the bone and the dental structures, allowing at the same time the quantification of the inclination degree of permanent molars in the context of the premature loss of temporary molars.

The main purpose of this study is to determine the impact of premature loss of mandibular temporary molars on the longitudinal axis of the permanent molars. A secondary objective is to verify whether there appear important modifications in the eruption sequence of the successors and the way the axis of the permanent molars is influenced.

MATERIAL AND METHODS

The study included 85 orthopantomographies of subjects aged 6 to 9 who reported to the Clinic of Pediatric Dentistry, Iasi. Out of these, 61 (71.8%) patients showed a premature loss of a lower temporary molar (study group) and 24 (28.2%) had intact dentition (control group). The study group was re-divided in all possible subgroups depending on the premature unilateral or bilateral loss of temporary molars. The precondition for the selection of subjects was that the incisors and permanent molars were intact. All patients received the required treatment and their written, informed and motivated approval was obtained.

All orthopantomographies were made at a single radiologic center by the same experienced technician in ideal conditions, using the radiologic device *PaX-Uni3D(Vatech)*®**.** At the moment of the orthopantomography the patients needed to have relaxed lips, a correct position of the head in relation to the chin support and the Frankfort plane needed to be parallel with the horizontal plane. Low quality, improper films or those with artifacts were excluded from the study from the beginning and the valid ones were saved digitally. Measurements were made by a single examiner using the *Easy Dent 4 Viewer*® software.

The orthopantomographies were processed by tracing the following structures (fig. 1):

-*the occlusion plane* – tangent line to vestibular cuspids of temporary molars/premolars and disto-vestibular cusp of lower right/left permanent molar;

-*the axis of primary permanent molar (M1)* – the line passing through the center of the crown and the center of radicular bifurcation;

-*the eruption axis of the second permanent molar (M2)* – the line passing through the middle of the crown and the center of the starting point of the radicular bifurcation.

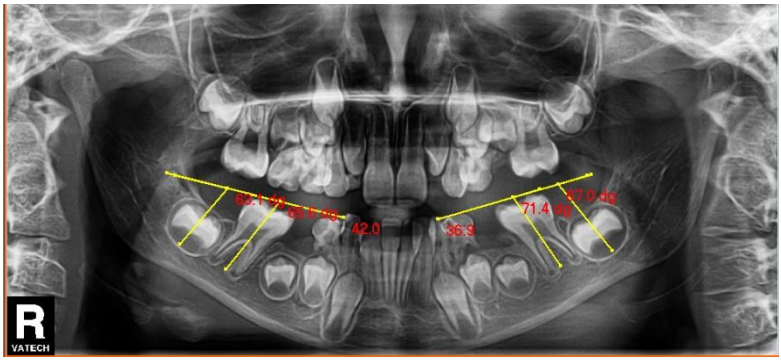


Fig. 1. Orthopantomographic aspect of premature loss of temporary molars

We thus evaluated the angle formed between the longitudinal axis of the first and second permanent molar and the occlusal plane in both lower quadrants. The software used for the data analysis was Statistical Package for the Social Sciences (version 20.0; SPSS, Chicago, III). We calculated the frequency and descriptive parameters (mean value, standard error, standard deviation, the minimum and maximum values) for each group. We used the *t-student test* to compare each group with the control group. The *t-pairs test* was used to compare the first permanent lower molars (M1) axes with second permanent lower molar (M2) axes of the two quadrants. The error limits used in the statistic interpretation are of 5% and the reliability range was of 95% .

In order to identify the possible errors during measurements we selected 25 radiographies. Measurements were repeated for the same radiographies and the *t-paired test* was used for the first and second measurement. No statistically significant difference was identified between the two measurements ($p>0.05$).

RESULTS

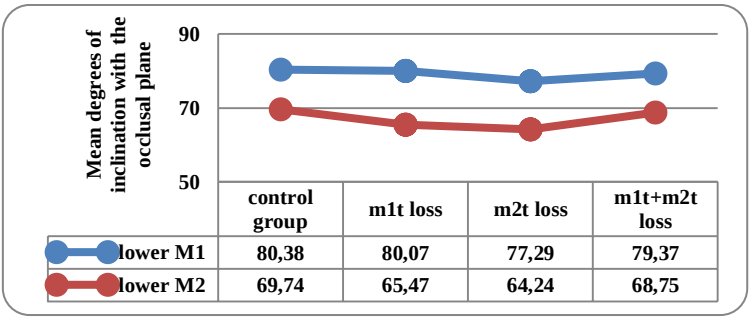
Out of the total 85 orthopantomographies analyzed, 45 (53%) patients were males (with an age average of 7.29) and 40 (47%) patients were females (average age 7.38). As far as the age distribution of subjects is concerned, there is a prevalence of patients aged 7 to 8 with an average age of 7.33.

In our study a total of 75 lower temporary molars were lost prematurely due to untreated dental cavities. The cumulated frequency of first temporary molar (m1t) and second temporary molar (m2t) losses shows a value of m1t close to m2t (37 (49.5%) for m1t and 38 (50.5%) of m2t).

From the point of view of angulation, the mesial-version mean for the control group of lower permanent M1 is of 80.38 ° (variation 69°-87°) and for lower permanent M2 of 69.74 ° (variation 50°-84°).

By analyzing the influence of *unilateral premature loss of lower temporary molars* on the inclination of permanent molar we may notice that the loss of lower m1t has very little influence on the axis of lower permanent molars (mean inclination of 80.078^{o}, with a variation between 73° and 85.4^{o} for M1 respectively a mean inclination of 65.47^{o} with a variation between 50^{0} and 81.50^{0} for M2). The loss of lower m2t determines an accentuated mesial-version of permanent molars (angular mean value of 77.29 ^{o} with a variation between 65.40^{o} and 85.50^{o} for permanent M1 and

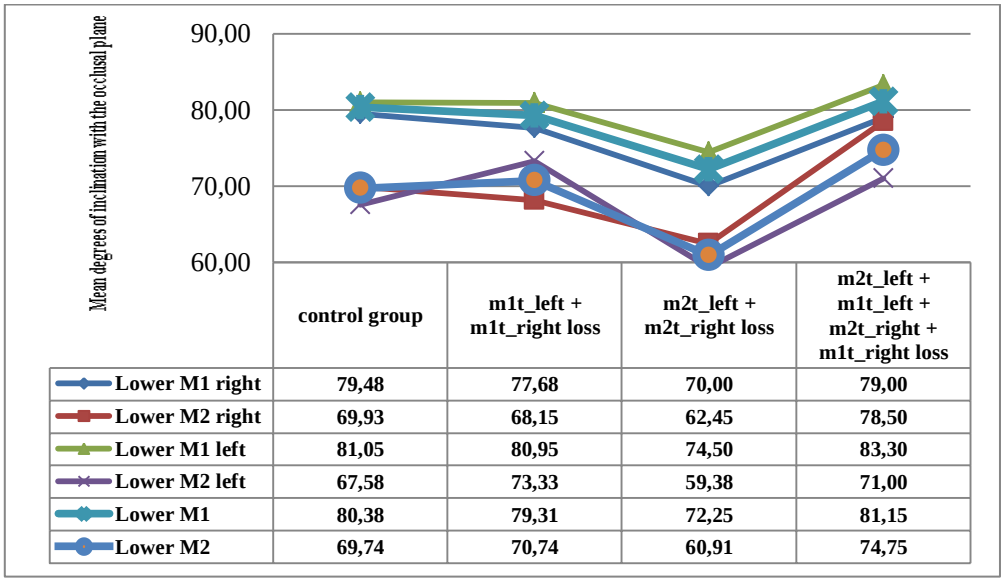
respectively a mean value of 64.24° with a variation between 30° and 83° for permanent M2) . In case of loss both of m1t and of m2t, the angle between the longitudinal axis and the occlusal plane will increase close to the values of the control group up to mean angular values of 79.275° for M1 and of 68. 75 ° for M2 (fig. 2).



	control group	m1t loss	m2t loss	m1t+m2t loss
lower M1	80,38	80,07	77,29	79,37
lower M2	69,74	65,47	64,24	68,75

Fig. 2. Influence of unilateral premature loss of lower temporary molars on the inclination of permanent molars

The influence of *bilateral premature loss of lower temporary molars* on the inclination of permanent molars determines a mesial version of permanent molars in cases of bilateral loss of m2t (mean angular values being of 72.25° for M1 and of 60.91° for M2). Bilateral loss of both m1t and m2t influences positively the axis of permanent molars, determining in these cases a slight overhang towards the distal (mean angular values being of 81.15° for M1 and of 74.75° for M2) (fig.3).



	control group	m1t_left + m1t_right loss	m2t_left + m2t_right loss	m2t_left + m1t_left + m2t_right + m1t_right loss
Lower M1 right	79,48	77,68	70,00	79,00
Lower M2 right	69,93	68,15	62,45	78,50
Lower M1 left	81,05	80,95	74,50	83,30
Lower M2 left	67,58	73,33	59,38	71,00
Lower M1	80,38	79,31	72,25	81,15
Lower M2	69,74	70,74	60,91	74,75

Fig. 3. Influence of bilateral premature loss of lower temporary molars on the inclination of permanent molars

Using the *t-test* we compared the axes of permanent molars, in the case of reunited global groups with premature loss of lower m1t, of lower m2t and of lower m1t+m2t, namely with the axes of permanent molars on the same side of the control group. The only statistically relevant situation is the loss of lower m2t which influences both permanent M1 axis and the permanent M2 axis (tab. I).

TABLE I

Results of the comparison between the group with premature loss of m2t and the control group

	Groups compared	N	Mean	Std. Deviation	Std. Error Mean	p –value
Lower M1	m2t global	36	76.189	6.5877	1.098	0.021*
	control	24	79.913	4.8021	0.9802	
Lower M2	m2t global	36	65.431	11.789	1.9648	0.04*
	control	24	71.354	8.8143	1.7992	
Lower M1	m1t global	33	81.424	4.7743	0.8311	0.643
	control	24	80.863	4.069	0.8306	
Lower M2	m1t global	33	70.933	9.0698	1.5788	0.247
	control	24	68.129	8.7279	1.7816	
Lower M1	m1t+m2t global	22	79.682	4.8291	1.0296	0.373
	control	24	80.863	4.069	0.8306	
Lower M2	m1t+m2t global	22	71.155	12.1297	2.5861	0.334
	control	24	68.129	8.7279	1.7816	
*Significant difference < 5.0%						

The correlative analysis between M1 axis and M2 axis in the two quadrants was made by comparison to the control group, using the t test for pairs (*t-pairs*). In both cases there is a moderate correlation, but statistically significant between the M1 and M2 axes (axis right M1 & axis right M2, r=0.321, p=0.01; axis left M1 & axis left M2, r=0.510, p=0.000).

DISCUSSIONS

The premature loss of temporary molars has as a main consequence the reduction of the space requires for the eruption of premolars and for the correction of small frontal dento-alveolar incongruence which is normally accomplished due to the leeway-space (8). The greatest amount of space lost is due to the inclination of permanent molars towards the mesial. This inclination depends mainly on the dental age at the moment of the extraction but also on the sequence/periodicity of eruption (9-11).

Based on the analysis using the software we could observe with higher accuracy the angle formed by the longitudinal axis of the permanent molars with the occlusal plane and we could thus estimate a mean value, a minimum, maximum value and a standard deviation for the control group. A similar study which was conducted using an older technique, namely by tracing the teeth images and structures on tracing paper using a black pencil and the measurement of angles with the protractor,

considers that, for instance, the correct position of lower molar 1 is at an angle that ranges from $+80^o$ -$+100^o$ (12). The values we obtained for the lower M1 are similar with those in the previous study: the mean value being of $+80.38^o$ with a variation between $+69^o$-$+87^o$. These aspects are relevant from the point of view of the inter-relation between the moment of premature loss of m2t and the moment of initiation of eruption of the first permanent molar. The context of premature loss of second temporary molar occurred after the initiation of clinical eruption of the first permanent molar.

Many longitudinal studies for a period from 2 to 8 months on children aged 6 to 9 with *premature loss of the first lower temporary molar* concluded that the shrinking of the mesial space from the second molar is due mainly to the distal displacement of the temporary canine and less to the migration or inclination of permanent molars (13-16). These results are also confirmed by us, since the axes of the lower permanent molars were very little influenced after the loss of first temporary molars, being comparative with the values obtained for the control group. Thus, the mean angular value for lower M1 at the control group was of 80.38^o while in case of m1t loss the mean value was of 80.07^o, and for lower M2 at the control group the mean angular value was of 69.74^o as compared to the mean angular value in case of m1t loss of 65.45^o**.**

Many studies (9-11,16) showed that the *premature loss of second temporary molars* determines a greater loss of leeway-space than in the case of first temporary molar, due both to the bigger difference in the mesio-distal diameter and to the fact that the first and second permanent molar lose the contact point and follows the trend of all teeth to lean mesially. This is also proved by our study by the much lower angular mean values for permanent mandibular molars in case of premature loss of the second mandibular temporary molar as compared to the mean angular values from the control group.

As a matter of fact, the only instance in our study which is statistically significant is the loss of lower m2t which influences the axis of both lower permanent molars, a logical consequence if we take into consideration the dynamic particularization of the alveolar and maxillary skeletal and mandibular growth and development on the age groups studied.

Another interesting aspect to analyze is the *loss of both lower temporary molars* on the same quadrant. A study conducted by Leroy R. *et al* (17) in 2009, showed that at the moment of cavity deterioration of both lower temporary molars and their loss subsequent to the cavity damage, the ideal eruption sequence „3-4-5-7" is reversed, increasing the prevalence of the eruption sequence „4-3-5-7", „4-3-7-5" and „4-5-3-7". Thus there occurs an accelerated eruption of both premolars. This is also confirmed by us in the present study through the increase of the mean angular values between the longitudinal axis of the permanent molars and the occlusal plane close to the mean angular values of the control group, leading to their distal overhang.

The moderate correlation between the axes of first and second molars in the same quadrant suggests the fact that any molar premature loss may have an impact on the entire dento-maxillary apparatus and may have later repercussions in the development of dento-maxillary anomalies.

The theme studied by us requires further complex clinico-radiological studies

with a powerful analytical-statistical approach with a higher number of subjects, ecological repartition on genders and age stages and by using the advanced measurement techniques of CBCT type.

CONCLUSIONS

Our study proves one more time the problematic of the premature loss of lower second temporary molar, leading to a necessity to conduct more studies in this field. Its pathology fails to be limited in an intra-arch context due to the extrusion potential of second maxillary temporary molar which may generate premature contacts, occlusal interferences and may lead to the Thielemann effect on the long term (diagonal syndrome) affecting the opposite articulation. The modification of the eruption sequence following the premature loss of temporary molars is a phenomenon which needs consideration, especially when an interceptive treatment is adopted, with the preservation of the occlusal morpho-functional complex using space maintainers.

Acknowledgments

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NEWS

LITERATURE REVIEW CONCERNING THE LOCAL DRUG DELIVERY SYSTEMS IN THE TREATMENT OF PERIODONTITIS

In periodontitis, in order to complement non-surgical therapy, there are multiple options of antimicrobials (metronidazole, chlorhexidine, minocycline, doxycycline, tetracycline), which can be locally delivered into the mucosa. These antimicrobials are used in periodontal pockets and can inhibit or eliminate periodonto-pathogenic micro-organisms as well as modulate the inflammatory response of tissues. Currently, limited data are available regarding the relationship between effect, efficacy and clinical status of the periodontium. A review realized by o group of researchers aimed to evaluate the effect and the efficacy of different types of local drug delivery systems in clinical parameters of periodontology. Researched papers using MEDLINE via PubMed, and LILACS databases related to five types of local drug delivery systems (chlorhexidine gluconate, doxycycline hyclate, metronidazole gel, minocycline ointment and tetracycline fibers) were reviewed aiming to address the mechanism of action and the evidence of clinical effectiveness of adjunctive use of these antimicrobials following surgical and/or non-surgical therapies. Inclusion criteria defined that articles must be randomized controlled trials performed in humans and published between 1996 and 2014. The adjunctive use of local drug delivery systems with controlled release properties may provide a defined, but limited, beneficial response on periodontal pockets. Local drug delivery as an active treatment or maintenance therapy depends on clinical findings, responses to treatment described in the literature, desired clinical outcomes, and patients' dental and medical histories, including their past usage of antimicrobials (Da Rocha HA, Silva CF, Santiago FL, Martins LG, Dias PC, De Magalhães D. Local Drug Delivery Systems in the Treatment of Periodontitis: A Literature Review. *J Int Acad Periodontol.* 2015; 17 (3) : 82-90).

Irina Grădinaru

OPTIMIZATION OF THE PREPARATION OF KOLLIDON® SR-BASED AMIODARONE HYDROCHLORIDE TABLETS WITH SUSTAINED RELEASE

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OPTIMIZATION OF THE PREPARATION OF KOLLIDON® SR-BASED AMIODARONE HYDROCHLORIDE TABLETS WITH SUSTAINED RELEASE (Abstract): **Aim:** The formulation of sustained release tablets of AMD·HCl using KOLLIDON® SR as matrix-forming agent. Chitosan, a natural polysaccharide with superior hydrating and absorbing properties was used in the formulation stage to optimize the release characteristics of those matrix tablets. **Material and methods**: Nine formulations of sustained release matrix tablets of AMD·HCl (200 mg/tablet) were prepared through direct compression. The concentrations of matrix forming agents were included as independent variables of a type 2^3 mixed factorial plan in order to develop formulations of AMD·HCl with optimal release characteristics. The dependent variables of that plan were the amount of AMD released from the tablets studied by using *in vitro* dissolution testing. The test was carried out in the paddle apparatus II for 12 hours in two pH media that were relevant to oral delivery: 2 hours at pH 1.2 and 10 hours at pH 6.8. The released AMD·HCl was quantitatively determined through a validated HPLC method. **Results:** The increase in KOL concentration leads to a decrease in AMD release rate at both pH values. The use of CHT resulted in a decrease of AMD release only at pH 6.8 in formulations with the lowest concentration of KOL. **Conclusions**: The retarding effect on the release of AMD·HCl in the tablets developed in this study was directly proportional to the KOL concentration in the formulation. **Keywords:** AMIODARONE HYDROCHLORIDE, TABLETS, EXTENDED RELEASE, KOLLIDON® SR, CHITOSAN.

Amiodarone hydrochloride (AMD·HCl) is one of the most prescribed therapeutic agents used in the treatment of cardiac tachyarrhythmia, due to its proven effectiveness. As AMD·HCl has limited oral bioavailability with many individual variations, the study on the inclusion of this active substance in oral delivery systems with sustained release characteristics might help optimize its biopharmaceutical characteristics.

Amiodarone (AMD) is an antiarrhythmic administered under the form of conventional release tablets (200 mg/tablet) for the treatment of ventricular and supraventricular rhythm disturbances, tachycardia in Wolff-

Parkinson-White syndrome, as well as in atrial fibrillation (1). AMD has a low oral bioavailability due primarily to its low solubility in water (0.2-0.7 mg/mL) (2).

MATERIAL AND METHODS

Materials: Amiodarone hydrochloride (Zhejiang Sanmen Hengkang Pharmaceutical Co. Ltd., China, purity 100.2 %), Kollidon® SR (KOL) and Chitosan practical grade (CHT) (BASF, Germany), Aerosil 200 (Degussa, Germany), and magnesium stearate (Union Derivan SA, Spain).

Preparation of matrix tablet: Nine AMD matrix tablets were formulated and prepared through direct compression method using a *Korsch EK0* tablet press (punch diameter of 9 mm, compression pressure of 8-10kN). To assess the influence of matrix-forming polymers on the release profile of AMD, a type 2^3 experimental plan (tab. I) was developed, in which the independent variables were marked as follows: X_1 - percentage of KOL (three values: 40, 50, 60), X_2 - percentage of CHT (three values: 3, 5, 7). The concentrations of these matrix forming agents were included as independent variables of a type 2^3 mixed factorial plan in order to investigate their influence on *in vitro* release of AMD.

TABLE I

The matrix of the experimental plan

Nº	X_1 (KOL)	X_2 (CHT)
1	40	3
2	40	5
3	40	7
4	50	3
5	50	5
6	50	7
7	60	3
8	60	5
9	60	7

In vitro dissolution studies: In vitro release studies were performed according to the specifications in the monograph "Dissolution test for solid dosage forms" European Pharmacopoeia 8th Edition (3), following this experimental protocol: *dissolution medium*: pH 1.2 solution (0.1N HCl) for the first 2 hours (as simulated gastric fluids) and pH 6.8 solution (phosphate buffer solution) for the next 10 hours (as simulated intestinal fluids); *Apparatus 2 (with paddles) SR 8 Plus Series* (AB & L Jasco); *bath temperature*: 37±0.5ºC; *rotation speed*: 50 rpm; the sampling interval was set at one hour during the 12 hours test (at each sampling hour, 7 mL of sample were replaced by the same volume of medium) (4-6).

AMD was quantitatively determined by a validated HPLC method. The dependent variables of the experimental plan were the amount of AMD released at each sampling hour, as follows: Y_1 (% of AMD released after 1 hour), Y_2 (% of AMD released after 2 hours), Y_3 (% of AMD released after 3 hours), Y_4 (% of AMD released after 4 hours), Y_5 (% of AMD released after 6 hours), Y_6 (% of AMD released after 8 hours) Y_7 (% of AMD released after 10 hours) Y_8 (% of AMD released after 12 hours). The data were statistically analysed and interpreted using the *Matlab 7.9.* software (7-10).

RESULTS AND DISCUSSION

These results show the major role played by KOL in the release of AMD from matrix tablet formulations. The amount of released AMD varied inversely with the percentage of KOL in the formulas for all formulations (regardless of the variable X_2). A particular behaviour was observed in F1 formula which released 63.63% of

the amount of AMD within the very first 2 hours of the dissolution test, in a medium of simulated gastric fluid. Another interesting feature of that formulation was that AMD was released almost entirely (released AMD = 99.77 %) at the end of the 12 hours test (fig. 1).

The influence of KOL was also confirmed by the results of quadratic regression analysis (tab. II, fig. 2,3.).

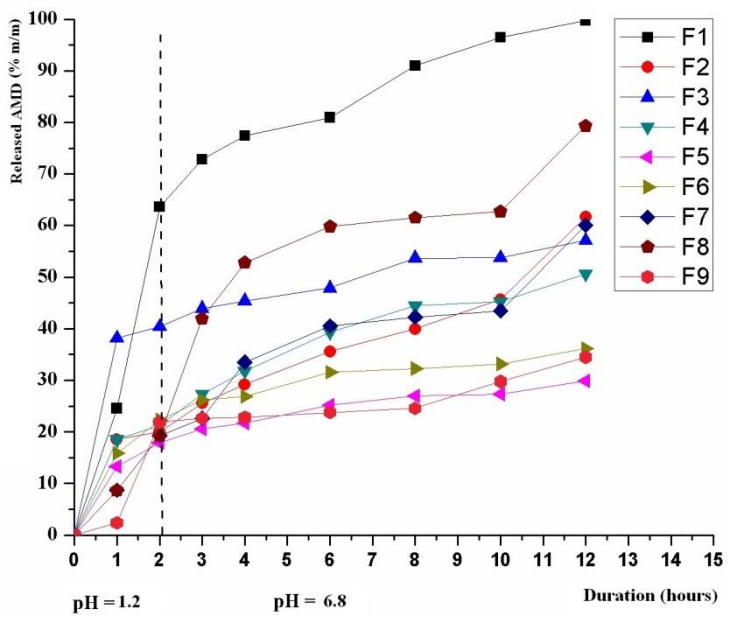

Fig. 1. The in vitro dissolution profile of AMD·HCl

TABLE II

Coefficients of the quadratic regression equations for predicting response variables

Independent variable	R^2	k	X_1	X_2	$X_1 \cdot X_2$	X_1^2	X_2^2
Y1	0.8866	51.5276	-0.7140	1.5436	-0.2498	0.0094	1.1337
Y2	0.8692	487.8390	-12.9188	-48.8073	0.3235	0.1024	3.0998
Y3	0.8779	549.8705	-16.3105	- 37.0329	0.3607	0.1359	1.6493
Y4	0.8462	596.2214	-18.7998	-30.0064	0.2664	0.1675	1.2707
Y5	0.8441	564.3599	-17.7954	-24.5035	0.2036	0.1611	0.9523
Y6	0.8054	640.2564	-19.7847	-30.8542	0.2447	0.1762	1.3021
Y7	0.8780	734.4493	-22.8863	-36.4873	0.3624	0.2008	1.2659
Y8	0.8249	824.4259	-28.3613	-16.1166	0.2131	0.2655	-0.1419

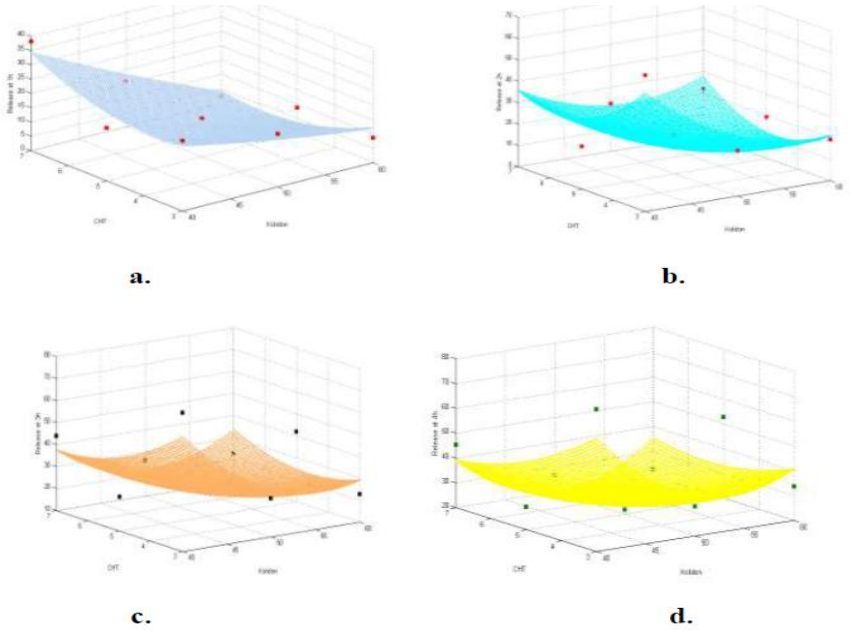

Fig. 2. The influence of formulation factors on the release of AMD - 1-4 hours
a. Y_1 (% of AMD released after 1 hour); b. Y_2 (% of AMD released after 2 hours); c. Y_3 (% of AMD released after 3 hours); d. Y_4 (% of AMD released after 4 hours).

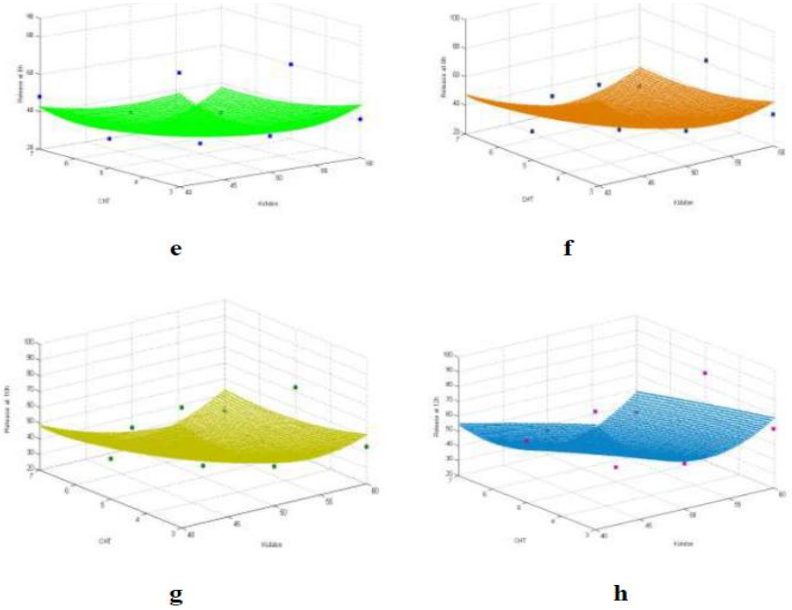

Fig. 3. The influence of formulation factors on the release of AMD - 6-12 hours
e. Y_5 (% of AMD released after 6 hours); f. Y_6 (% of AMD released after 8 hours); g. Y_7 (% of AMD released after 10 hours); h. Y_8 (% of AMD released after 12 hours).

CONCLUSIONS

During our study we observed that the X_2 variable (% CHT) initially had a positive effect on the release of AMD, but later it had a retarding effect. That influence of CHT at pH 1.2 could be explained by its solubility in that medium that promoted matrix hydration and consequently stimulated the release of the active ingredient. Following that moment (Y_1), the retarding effect on AMD release exerted by CHT was stronger than the effect produced by KOL (tab. II). The interactions between the two variables were positive, except for Y_1, but they could be considered negligible. We noticed a retarding effect on AMD release from the obtained formulations and that was directly proportional to the KOL concentration. Initially, CHT increased the release of AMD, but later we concluded that it inhibited the release process, and the interactions between the two polymers had a negligible effect on the release of the active ingredient.

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NR4A1-ACTIVATED RECEPTORS ASSOCIATED WITH Ca^{2+} CYTOSOLIC INCREASE IS NOT INDUCING AUGMENTED APOPTOSIS IN PRO-B CELLS

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NR4A1-ACTIVATED RECEPTORS ASSOCIATED WITH Ca^{2+} CYTOSOLIC INCREASE IS NOT INDUCING AUGMENTED APOPTOSIS OF PRO-B CELLS (Abstract) **Background**: The involvement of NR4A1 orphan receptors activation in pre-lymphocytes, pro-lymphocytes and lymphocytes apoptosis is controversial, also when related to calcium fluxes. **Aim**: The goal of our study was represented by the characterization of NR4A1 activation-induced effects on pro-B cells apoptosis in the presence of ionomycin, a calcium overloading inducer. **Methods**: All cells batches were evaluated under contrast-phase microscopy, using an Eclipse TE-300 Nikon microscope with a 40x objective. The apoptotic cells number was expressed as a percentage of the total number of cells, taking into account their morphological appearance. **Results**: The treatment of pro-B cells with 5 μM ionomycin and 1 mM Ca^{2+} in culture medium for 24 hours induced a modest, but significant apoptosis as compared to control series, evidenced by morphological appearance. On the other side, the pro-B cells treated with 50 μM Cytosporone B for 24 hours, together with 5 μM ionomycin and 1 mM Ca^{2+} show a reduced grade of apoptosis as compared to solely ionomycin and calcium series. **Conclusions**: Thus, NR4A1 orphan receptor activation might reduce the apoptotic effects of calcium overload in pro-B cells, an intriguing effect which might be further researched.
Keywords: PRO-B CELLS, NR4A1 RECEPTOR, APOPTOSIS, IONOMYCIN.

Nuclear receptors are members of a large super family of evolutionarily related transcription factors that control a plethora of biological processes. Nuclear receptors orchestrate complex events such as development, organ homeostasis, metabolism, immune function, and reproduction. Approximately one-half of the 48 human nuclear receptors have been shown to act as ligand-regulated transcription factors and respond directly to a large variety of endogenous hormones and metabolites that are generally hydrophobic and small in size (e.g., retinoic acid or estradiol). The second half of the nuclear receptors family comprises the so-called orphan receptors, for

which regulatory ligands are still unknown or may not exist despite the presence of a C-terminal ligand-binding domain, which is the hallmark of all nuclear receptors (1).

Three members of the NR4A orphan nuclear hormone receptor subfamily (NR4A1, NR4A2, and NR4A3) belong to the steroid nuclear hormone receptor super family. They share similar structural features and have no known natural ligand. They constitute immediate early genes that are induced by serum, growth factors and receptor engagement and are thus implicated in cell mitogenic responses. They have been shown to play a role in complex pathways of cell survival and apoptosis. Recent studies have also suggested an important role of these receptors in cell transformation and tumorigenicity via both their anti-apoptotic and pro-apoptotic functions (2).

Thus, the orphan nuclear receptor NR4A1/Nur77/TR3/NGFIB acts primarily as a transcription factor to regulate the expression of multiple genes. However, increasing research attention has recently been given to non-genomic activities of NR4A1. To be also mentioned, the role of NR4A1 in apoptosis is controversial (3).

The induction of thymocyte apoptosis through the NR4A1-mediated intrinsic pathway can be of physiological importance in the clonal deletion of autoreactive thymocytes during negative selection in the thymus and/or in thymocytes undergoing oncogenic transformation. Ionomycin treatment induces endogenous NR4A1 expression as well as apoptosis and cytochrome c release in thymocytes (4).

The goal of our study was represented by the characterization of NR4A1 activation-induced effects on pro-B cells apoptosis in the presence of ionomycin, a calcium overloading inducer.

MATERIALS AND METHODS

An IL-3-dependent mouse pro-B cell line was maintained in RPMI 1640 (Sigma-Aldrich) medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated fetal bovine serum and recombined murine IL-3 (Sigma-Aldrich), in an atmosphere with 5% CO_2 and at 37°C (5, 6).

Cells were grown at a density of around $5x10^5$ per ml before experiments, each one being performed using $1x10^6$ cells/ml. For some experiments (in triplicate) pro-B cells were treated with 5 μM ionomycin and 1 mM Ca^{2+} in culture medium for 24 hours. To test the NR4A1 receptor activation we administered 50 μM Cytosporone B for 24 hours, together with 5 μM ionomycin and 1 mM Ca^{2+}. The control Ba/F3 cells received no treatment for 24 hours (stated as normal around 12-15% apoptotic series). The second control series was represented by the administration for 24 hours of 1 μM Valinomycin (stated as almost 90% apoptotic series).

All batches were evaluated under contrast-phase microscopy, using an Eclipse TE-300 Nikon microscope with a 40x objective. The apoptotic cells number was expressed as a percentage of the total number of cells, taking into account their morphological appearance.

The statistical significance of test results was highlighted using the Variance One-Way ANOVA, All Pairwise Multiple Comparison Procedures (Holm-Sidak method) and the results were expressed as mean±S.E.M (n=3). Values of $p<0.05$ were considered statistically significant always.

RESULTS

For the pro-B cells, 87.53±3.65% are associating normal morphological appearance as shown in Fig. 1 (control series,

triplicate experiment).

The apoptosis was evident when we treated the cells with 1 μM Valinomycin for 24 hours (that means 9.64±1.31% normal morphological appearance, n=3), as Fig. 2 shows.

The treatment of pro-B cells with 5 μM ionomycin and 1 mM Ca^{2+} in culture medium for 24 hours (Fig. 3) induced a modest, but significant apoptosis as compared to control series (68.91±3.72% normal morphological appearance, n=3, $p<0.05$).

For some experiments (in triplicate) pro-B cells were treated with 50 μM Cytosporone B for 24 hours, together with 5 μM ionomycin and 1 mM Ca^{2+} (Fig. 4). In this particular case, 81.57±3.48% of the pro-B cells are associating normal morphological appearance, suggesting a reduced apoptosis (n=3, $p>0.05$ as compared to control series). Treatment with 50 μM Cytosporone B alone induced no significant apoptosis as compared to control series (personal observation, data not shown).

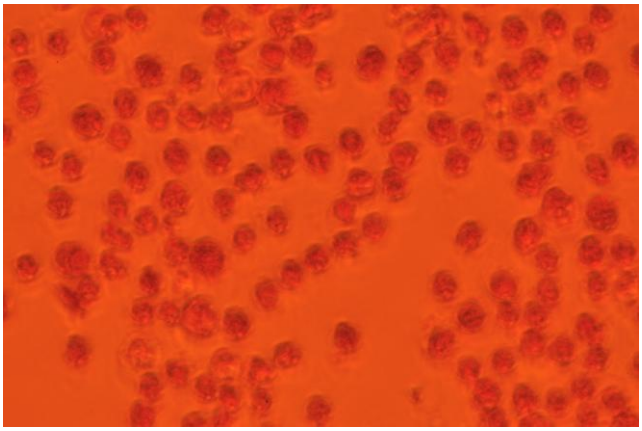

Fig. 1. Representative normal morphological appearance for murine pro-B cells (contrast phase, 40x).

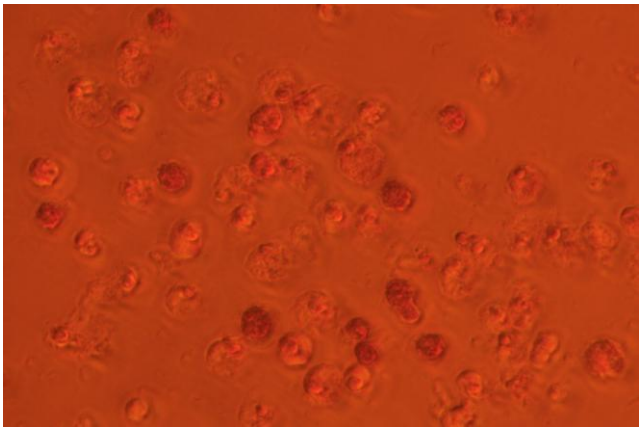

Fig. 2. Valinomycin (1 μM) is inducing massive apoptosis of pro-B cells after 24 hours treatment (contrast phase, 40x).

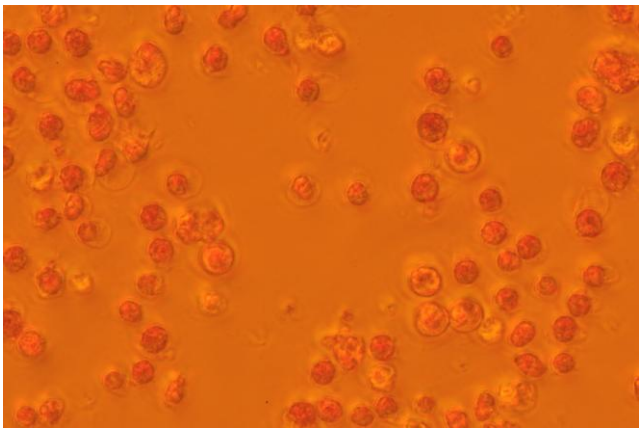

Fig. 3. The treatment of pro-B cells with 5 μM ionomycin and 1 mM Ca^{2+} in culture medium for 24 hours induced a modest, but significant apoptosis (contrast phase, 40x).

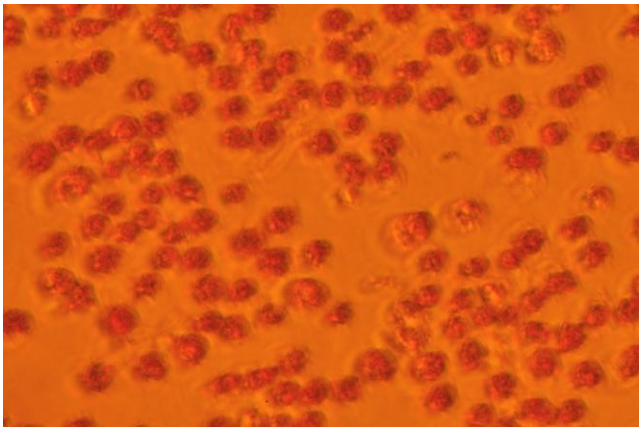

Fig. 4. For some experiments, the pro-B cells treated with 50 μM Cytosporone B for 24 hours, together with 5 μM ionomycin and 1 mM Ca^{2+} are associating reduced apoptosis (contrast phase, 40x).

DISCUSSION

Apoptosis, one of the best-studied forms of programmed cell death processes, plays an important role during the development and life-cycle of most multicellular organisms. This program might consist of the successive activation of unique apoptosis-specific genes, which are solely involved in the regulation of the programmed cell death. However, more and more evidence is accumulating that novel genes are not activated or induced during apoptosis, but rather many well-known genes previously described for their roles in processes such as proliferation and differentiation and belonging, for example, to the protein families of immediate-early genes and transcription factors become activated. The death-specific feature is achieved thereby by the extent, combination, and specific timing of gene expression (7).

The role of calcium elevations in apop-

totic and necrotic cell death has been a focus of research in recent years. Evidence has been presented that calcium oscillations can effectively trigger apoptosis under certain conditions and that dysregulation of calcium signaling is a common cause of cell death. These effects are regularly mediated through calcium signal propagation to the mitochondria and the ensuing mitochondrial membrane permeabilization and release of pro-apoptotic factors from mitochondria to the cytoplasm (8).

In HepG2 cells and primary hippocampal neurones in culture ionomycin, a well known neutral calcium ionophore, promoted the equilibration of Ca^{2+} gradients between cellular compartments, including endoplasmic reticulum, mitochondria and cytosol. Thus, $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ increased together and then recovered in parallel on removal of the ionophore (9).

Ionomycin caused some mitochondrial depolarization also in our previous experiments on pro-B cells, although this was low in degree (5).

Within the nuclear receptor family, Nur77 (also known as NGFI-B) distinguishes itself by its ability to bind a target sequence (the NBRE) as a monomer and by its role in T-cell receptor (TCR)-induced apoptosis in T cells (10).

Apoptosis accompanying negative selection is a central but poorly understood event in T cell development. The Nur77 nuclear steroid receptor and Bim, a proapoptotic BH3-only member of the Bcl-2 family, are two molecules implicated in this process. However, how they relate to each other and how Nur77 induces apoptosis remain unclear. In thymocytes, Nur77 has been shown to induce cell death through a transcriptional-dependent pathway, but in cancer cell lines, Nur77 was reported to induce apoptosis through conversion of Bcl-2 into a killer protein at the mitochondria. Whether this Nur77 transcriptional-independent pathway actually occurs in vivo remains controversial. Using an optimized fractionation protocol for thymocytes, here we report that stimulation of CD4(+)CD8(+) thymocytes results in translocation of Nur77 and its family member Nor-1 to the mitochondria, leading to their association with Bcl-2 and exposure of the Bcl-2 proapoptotic BH3 domain. In two T cell receptor transgenic models of negative selection, F5 and HY, a conformational change of the Bcl-2 molecule in the negatively selected T cell population was similarly observed. Thus, the Nur77 family and Bim pathways converge at mitochondria to mediate negative selection (11).

Defects in apoptosis mechanisms play important roles in malignancy and autoimmunity. Orphan nuclear receptor Nur77/TR3 has been demonstrated to bind antiapoptotic protein Bcl-2 and convert it from a cytoprotective to a cytodestructive protein, representing a phenotypic conversion mechanism. Of the 6 antiapoptotic human Bcl-2 family members, we found that Nur77/TR3 binds strongest to Bcl-B, showing selective reactivity with Bcl-B, Bcl-2, and Bfl-1 but not Bcl-X(L), Mcl-1, or Bcl-W. Nur77 converts the phenotype of Bcl-B from antiapoptotic to proapoptotic. Bcl-B is prominently expressed in plasma cells and multiple myeloma. Endogenous Bcl-B associates with endogenous Nur77 in RPMI 8226 myeloma cells, where RNA interference experiments demonstrated dependence on Bcl-B for Nur77-induced apoptosis. Furthermore, a Nur77-mimicking peptide killed RPMI 8226 myeloma cells through a Bcl-B-dependent mechanism. Because Bcl-B is abundantly expressed in plasma cells and some myelomas, these findings raise the possibility of

exploiting the Nur77/Bcl-B mechanism for apoptosis for eradication of autoimmune plasma cells or myeloma (12).

Human genes of polycomb group correlate with various hematological and epithelial cancers, identifying new mechanisms of malignant transformation and pave the wat for developing new cancer treatments and new diagnostic markers. B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) is a member of polycomb group, which participates in axial patterning, hematopoiesis, cell cycle regulation, and senescence, and represents a future target for treatment and diagnosis (13).

Nowadays cancer is still a major public health issue. Mortality by cancer is on the second place after the one caused by cardiovascular diseases. The high mortality and the increasing incidence of certain cancers justify a growing interest for the identification of new pharmacological agents efficient in cancer prevention and treatment (14).

CONCLUSIONS

In our study, the treatment of pro-B cells with ionomycin and Ca^{2+} in culture medium for 24 hours induced a modest, but significant apoptosis as compared to control series, evidenced by morphological appearance. On the other side, the pro-B cells treated with Cytosporone B for 24 hours, together with ionomycin and Ca^{2+}, show a reduced grade of apoptosis as compared to solely ionomycin and calcium series.

Thus, NR4A1 orphan receptor activation might reduce the apoptotic effects of calcium overload in pro-B cells, an intriguing effect.

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NEWS

RATES OF FORMAL DIAGNOSIS OF DEMENTIA IN PRIMARY CARE: THE EFFECT OF SCREENING

A timely diagnosis of dementia is considered of major importance to ensure adequate access to information, evidence-based treatment, care, and support for people with dementia. However, even in high-income countries with advanced medical care systems, 50% to 80% of all prevalent dementia cases are not formally diagnosed. The screening of older patients for cognitive impairment could be a method to improve the recognition of dementia. Previous studies have shown that screening for dementia in general practitioners' (GPs) practices did increase the diagnosis rates. Nevertheless, routine screening for dementia has been controversial and is not presently recommended in the dementia guidelines owing to the lack of evidence that patients benefit from it. In addition, patients with positive screening results often undergo no additional diagnostic assessment and do not receive specific treatment. For the benefit of patients, a positive screening outcome should initiate an adequate diagnostic assessment and, depending on the results, state of the art treatment and care. Furthermore, screening could result in potential harms. Older patients might avoid visiting their GP because they fear to be diagnosed with dementia. Also, false-positive screening results increase the probability for misdiagnosis, could result in unnecessary examinations and treatments, and might cause anxiety and depression in the affected subjects. Only 1.5% of the patients reported that they worried more about their health after the screening. Other surveys revealed that a clear majority of participants would prefer to be diagnosed early and expected to be properly informed about their diagnosis. Also, the proactive "dementia case finding scheme" was initiated by the government of the United Kingdom, aiming to increase the diagnosis rate of dementia and to improve dementia treatment and care on the population level. Thus, the present analysis revealed a strong effect of screening for dementia among primary care patients on the probability of receiving a formal diagnosis of dementia (Eichler T, Thyrian JR, Hertel J,l. et al. Rates of formal diagnosis of dementia in primary care: The effect of screening. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2015; 1: 87–93).

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BIOENERGETIC CHARACTERIZATION OF H9C2 CELLS USING THE EXTRACELLULAR FLUX ANALYZER

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BIOENERGETIC CHARACTERIZATION OF H9C2 CELLS USING THE EXTRACELLULAR FLUX ANALYZER (Abstract): **The aim** of the present work was to standardize the working methodology for assessing the bioenergetic profile of H9c2 cardiomyoblasts cells, with reference to the optimization of cell seeding number and the establishment of favorable concentrations for the classic modulators of mitochondrial respiratory function, in particular the one of a classical uncoupler, FCCP. **Material and methods:** The extracellular flux analyzer (XF, Seahorse Bioscience) is a novel high-throughput instrument able to monitor the metabolism of living cells by simultaneously measuring mitochondrial respiration and glycolysis. The *in vitro* platform will be further used to better understand the pathophysiology and the unrecognized side effects of drugs currently used in the therapy of major cardiovascular diseases. **Conclusions:** In the long run, characterization of novel pharmacological agents' effects on other cell lines, including tumoral ones, will be also considered. **Keywords:** EXTRACELLULAR FLUX ANALYZER, CARDIOMYOBLASTS, MITHOCONDRIAL RESPIRATION, GLYCOLYSIS

Assessing the bioenergetic profile of myocardium, the most metabolically active tissue, has become nowadays a highly investigated research field. Indeed, mitochondrial dysfunction is widely recognized as a central event responsible for the progression of the most non-communicable diseases.

In the past decade, a thorough insight into the subcellular pathophysiology of living cells and their changes in response to increased demands and environmental agents (1) with a particular interest for mitochondrial function/dysfunction has gained a lot of interest in the field of translational research (2-5). The Extracellular Flux Analyzer (XF, Seahorse Bioscience) represents a modern, automatic platform allowing the simultaneous measurement in real-time of the two major energy pathways: mitochondrial respiration and glycolysis (6). The 2 major parameters provided are: i) the Oxygen Consumption Rate (OCR) as indicator of mitochondrial respiration and the Extra-

cellular Acidification Rate (ECAR) as indicator of lactate release via glycolysis, respectively. By providing information regarding the oxygen-dependent and independent pathways for ATP generation, the XF analyzer allows a comprehensive characterization of cellular bioenergetic profile and, also interrogation of drug response and toxicity. With respect to the former issue, cells are metabolically perturbed via successive additions of compounds that modulate mitochondrial respiration (7). In order to perform a systematic characterization of the bioenergetic profile of H9c2 cell line, the present study was purported to (i) identify the effects of H9c2 cell seeding density on the basal and perturbed mitochondrial respiration and glycolysis; (ii) determine the concentration of a classical uncoupler, FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), that is responsible for the maximal bioenergetic response.

MATERIAL AND METHODS

Cell culture. H9c2 rat cardiomyoblast cell line (purchased from ECACC) was maintained in a humidified incubator gassed with 5% CO_2 at 37°C. Cells were grown in high-glucose (4.5g/l) Dulbecco's modified Eagle's Medium (DMEM) supplemented with 1 mM sodium pyruvate, 5 mM HEPES, 10% FBS, and penicillin-streptomycin (100 U/ml). Cells were seeded for 24 hours in Seahorse XF-24 cell culture plates at different densities: 10, 20, and 25 K cells/well, respectively.

Bioenergetic measurements. The experimental protocol for the characterization of the bioenergetic profile of H9c2 cells was adapted after Nicholls et al. (7). In the day of the experiment, DMEM was replaced by unbuffered XF Assay Media supplemented with 25 mM glucose. XF-24 cell plates were placed in XF Prep Station (37°C incubator without CO_2) for 60 minutes allowing pre-equilibration of the cells with the XF Assay Media. The oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) were simultaneously measured after the pneumatically sequential injections of 3 compounds that shift the energetic profile of the myoblasts: i) oligomycin (an ATP-synthase inhibitor, 1 μg/ml), ii) FCCP (a respiratory uncoupler, 1 and 3.0 μM), and iii) antimycin A (a complex III inhibitor, 5 μM) (7-9). Background correction wells (i.e., wells not seeded with cells) were used to normalize the data to background plate noise. Cells were randomized into 6 groups: 10 K/1 FCCP group (10 K cells/well injected with 1 μM FCCP), 10 K/3 FCCP group (10 K cells/well injected with 3 μM FCCP), 20 K/1 FCCP group (20 K cells/well injected with 1 μM FCCP), 20 K/3 FCCP group (20 K cells/well injected with 3 μM FCCP), 25 K/1 FCCP group (25 K cells/well injected with 1 μM FCCP), and 25 K/3 FCCP group (25 K cells/well injected with 3 μM FCCP). OCR was expressed in units of nmoles/minute/nr of cells and ECAR in mpH/minute/nr of cells.

The following OCR parameters were further calculated and analyzed (fig.1):

- Basal respiration: OCR measured before initializing the automated injections;
- ATP turnover: the amount of O_2 consumption used for ATP production;
- Proton leak: the amount O_2 used to maintain the proton gradient;
- Maximal respiration under conditions of uncoupled respiration;
- Reserve capacity: the difference between maximal and basal respiration (8, 10);
- Non-mitochondrial respiration: the

amount of O_2 consumption not due to mitochondrial processes.

OCR was corrected for oxygen flux due to instrumental background and non-mitochondrial respiration. The respiratory control ratio (RCR) was calculated as the ratio between the maximal respiration and the proton leak.

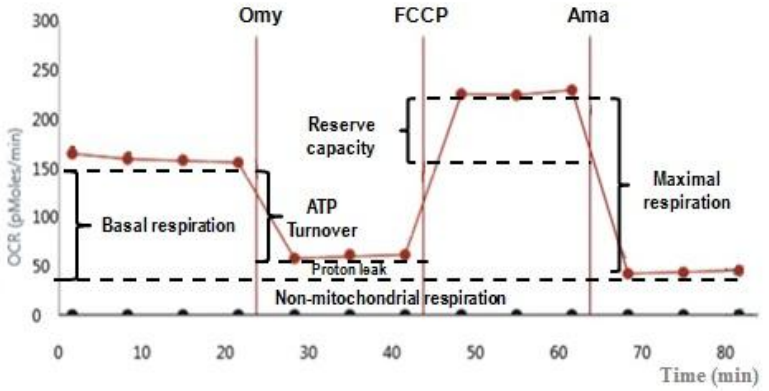

Fig. 1. OCR parameters in H9c2 cells: Basal respiration - OCR before injections; Proton leak - the difference in OCR after oligomycin (Omy) and antimycin A (Ama) injections; ATP turnover - the difference between basal respiration and proton leak; Maximal respiration - OCR after FCCP injection; Reserve capacity - the difference between maximal and basal respiration; Non-mitochondrial respiration - OCR after antimycin A injection. *Reagents.* All reagents and consumables were purchased from Sigma-Aldrich and Seahorse (Bioscience).

RESULTS AND DISCUSSION

We firstly characterized the cellular response to sequential injections. When oligomycin was injected, OCR suddenly decreased, because the proton channel of the Fo portion ATP synthase was blocked and the ATP synthesis via oxidative phosphorylation was inhibited; subsequently, the energy production shifted to glycolysis, which is reflected by an increase in ECAR. When FCCP was injected, OCR significantly increased as more O_2 was consumed to pump the excess protons back across the mitochondrial membrane, while ECAR increased due to cells attempt to maintain their energy balance by using glycolysis to generate ATP. Finally, when antimycin A was injected, OCR significantly decreased, since mitochondrial Complex III was inhibited and respiration was shut down; ECAR was maintained since cells shifted to a glycolytic state in order to maintain their energy balance (7, 8) (tab. I, fig. 2).

The first goal of this study was to identify the effects of H9c2 cell seeding density on the basal and perturbed OCR and ECAR. Firstly we observed that both OCR and ECAR values were sufficiently above the background noise and within the linear response range of the cells. In this experiment, we observed linear increases with OCR values as cell density increases, but when we reported OCR parameters to the number of cells, this observation was no longer valid. Still, the best RCR, the classical parameter for the mitochondrial qualita-

tive control (11), was obtained at 25 K cells/well. The ECAR data were less easily read, but still we noticed that ECAR also increased from 20 to 25 K cells/well.

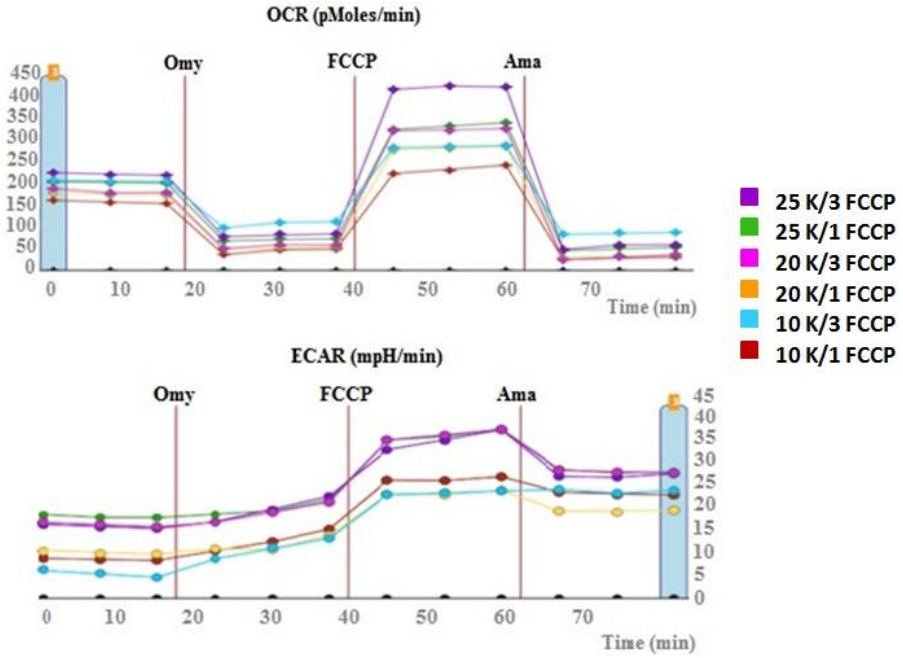

Fig. 2. OCR and ECAR evolution *vs.* time in the presence of pharmacological modulators.

TABLE I

OCR parameters and ECAR in H9c2 cardiomyoblasts

Cell groups	OCR (nmoles/min/nr cells)						ECAR (mpH/min/nr cells)
	Basal respiration	Proton leak	ATP turnover	Maximal respiration	Reserve capacity	RCR	
10K/1FCCP	12.13	5.44	6.7	20.42	8.3	3.7	1.3×10^{-3}
10K/3FCCP	13.53	3.8	9.72	18.83	5.3	4.9	1.6×10^{-3}
20K/1FCCP	6.7	1.7	5	8.94	2.3	5.3	0.8×10^{-3}
20K/3FCCP	7.2	1.8	5.38	11.52	4.34	6.4	1×10^{-3}
25K/1FCCP	4.8	1.33	3.47	9.91	5.12	7.5	0.85×10^{-3}
25K/3FCCP	6.25	1.51	4.73	12.56	6.31	8.3	1.3×10^{-3}

Data are expressed as mean values (n = 6-8/group)

Another goal of this experiment was to determine the concentration of FCCP responsible for the maximal OCR and ECAR responses, since high concentrations of FCCP may present an inhibitory effect (7-9). We noticed that OCR was maximal when FCCP was used in the higher concentration in groups 4 and 6, suggesting that cultured myoblasts present an increased reserve capacity, that is, an increased capacity to re-

spond to stress (8). Thus, after standardizing in a previous paper the methodology for high-resolution respirometry studies in permeabilized myocardial fibers we here provide for the first time in Romania a standardized protocol for the assessment of bioenergetic function of cardiomyoblasts (12).

CONCLUSIONS

We report here that the optimal bioenergetic profile of H9c2 cells was obtained at a density of 25,000 cells/well in the presence of 3 μM FCCP. These findings will be further used in experiments assessing the bioenergetic effects of different pharmacological agents on H9c2 cells, as well as on other cell lines (including tumoral ones), since this is one of the major applications of the XF analyzer besides the characterization of the changes in overall bioenergetics of the cells in response to stress and various insults.

ACKNOWLEDGEMENTS

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THE EFFECTS OF PREGABALIN ON PSYCHO-MOTOR ABILITIES AND COGNITIVE PROCESSES IN MICE

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THE EFFECTS OF PREGABALIN ON PSYCHO-MOTOR ABILITIES AND COGNITIVE PROCESSES IN MICE (Abstract): The purpose of our study was the experimental research on the effects of pregabalin in two behavioural models in mice. **Material and methods:** The experiment was carried out with white Swiss mice treated intraperitoneally as follows: Group I (Control): distilled water 0,1ml/10g body weight; Group II (PGB 10): 10mg/kbw pregabalin; Group III (PGB 20): 20mg/kbw pregabalin. The psychomotor abilities of pregabalin were tested on a LE-8811 Actimeter device (Panlab), in order to investigate both global motor behaviour and number of escape attempts during an eight-minute interval session. The exploration of memory processes performance was assessed using the Y-maze model, based on the natural tendency of mice to explore new environment. Data were analyzed using SPSS 13.0 for Windows software. The experimental protocols were implemented according the guidelines of "Grigore T. Popa" University Committee for Research and Ethical Issues. **Results:** The administration of pregabalin resulted in a dose-dependent reduction of mice horizontal and vertical movements, statistically significant compared to the Control group. The administration of both PGB10 and PGB20 induced no modifications of the spontaneous alternation percent; also, it did not influence the arm entries number compared to Control group in Y-maze test. **Conclusions:** The results reflect a significant dose-dependent diminution of number of escape attempts, exploratory and self-maintenance spontaneous behavior after pregabalin treatment, which could be correlated with an anxiolytic effect. Moreover, the study proved that pregabalin did not modify the animal cognitive processes performance or influence short-term memory of mice in the Y-maze test. **Keywords:** PREGABALIN, MICE, PSYCHO-MOTOR, Y-MAZE.

The (S-[+]-3-isobutylgaba) derivative pregabalin is a novel gamma-aminobutyric acid analogue, structurally related to the antiepileptic drug gabapentin, with a high efficacy as an adjunctive treatment for epilepsy and neuropathic pain (1).

It is a potent ligand for the alpha-2-delta subunit of P/Q type voltage-gated calcium channels that reduce synaptic release of neurotransmitters in the central nervous

system. Pregabalin-mediated reduction in calcium currents has also been proved to produce a significant inhibition of the release of glutamate and monoamine neurotransmitters involved in the modulation of anxiety pathways (2).

Pregabalin is structural related to the inhibitory neurotransmitter gamma aminobutyric acid (GABA), but lacks affinity on $GABA_A$, $GABA_B$, or benzodiazepine receptors. It does not influence the functionality of sodium channels or the activity of cyclooxygenase enzyme subtypes. It is also inactive on the opioid, serotonin and dopamine receptors (3).

This drug, with a high solubility and good permeability is rapidly and nearly completely absorbed after oral administration, without binding to serum proteins. The maximum plasma concentration is reached approximately after 1 hour. After single or multiple dose administration maximum plasma concentration and area under the curve concentration-time curve values are linearly augmented (4). The half-life time ranges from 5,5 to 6,7 hours depending on the administration route. Usually, the major way of excretion for pregabalin is urinary as unchanged drug, with a renal clearance of 73 ml/minute and an elimination half-life of about 6 hours. (5)

Some experimental researches are consistent with a mechanism that may entail reduction of abnormal neuronal excitability through reduced different neurotransmitters (glutamate, norepinephrine, substance P, calcitonin gene-related peptide) release (3, 4).

Pregabalin has been used to treat painful diabetic neuropathy, post-herpetic neuralgia, partial seizures, fibromyalgia, but also neuropathic cancer pain (6, 7, 8). It also has proven to be effective as a sleep modulating agent and an anxiolytic drug (9, 10).

The present study aims to investigate the effects of pregabalin on spontaneous behaviour and on spatial memory performances in mice.

MATERIAL AND METHODS

The experiment was carried out on white male Swiss mice (20-25g), distributed into 3 groups of 6 animals each treated intraperitoneally as follows: Group I (Control): distilled water 0,1ml /10 g body weight; Group II (PGB 10): pregabalin 10mg/kbw; Group III (PGB 20): pregabalin 20mg/kbw.

The animals were housed in groups of four in Plexiglas cages maintained on a 12 hours light/dark cycle in a temperature-controlled (22 ± 1°C) colony with access to water and food *ad libitum*, except during the experimental sessions. Before the beginning of the study, animals were allowed to adapt to the environment for one week. All experiments were carried out between 08:00 a.m. and 13:00 p.m.

Pregabalin (Mesochem Technology, China) was diluted in distilled water extemporaneously and injected intraperitoneally (i.p.) in a volume of 0,1ml /10 g body weight.

Spontaneous motor activity exploration. The psychomotor abilities induced by pregabalin were tested in the LE-8811 Actimeter device (Panlab) in order to investigate both global motor behavior and the number of escape attempts, during an eight-minute interval session. This device consists of three transparent cages each with eight infrared lights positioned in a frame around the cage and coupled to silent electronic counters. The apparatus is composed of a two-dimensional (X and Y axes) square frame, a frame support and a control unit.

Using this method spontaneous motor

activity was measured counting the number of times that an animal turns off a photocell beam. The system is completely modular: each frame may be used for evaluation of general activity: locomotors, stereotypic movements, rearing or exploration. Horizontal, vertical and stereotypic movements are registered simultaneously in the same experimental box.

Vertical movements were measured by number and length of bobbing. Horizontal movements indicate travel distance, and were measured based on time and count. In comparison, immobility was measured by the time that rats spent resting (11, 12).

Rats were placed individually in a cage and the spontaneous activity was measured by counting the number of light beam interruptions due to the animal's movement in the experimental area. The device is connected with a SeDaCom computer, which allows easy exportation of data in a format compatible with the Excel program for Windows.

Spatial working memory evaluation. The investigation of memory processes performance was assessed using the Y-maze model, based on the natural tendency of mice to explore novel environment (13, 14, 15).

This apparatus consists of 3 identical arms (40x9x16 cm, placed at 120 degrees from each other) and a central triangular zone. Each arm has walls with specific motifs all over the internal area allowing animals to differentiate it from the others. This experimental model was used to test if the rat could remember the arm it had just explored and would therefore enter one of the other arms of the maze (16, 17). Each animal was placed at the end of one arm and allowed to move unreservedly through the maze during an 8 min session. The short-term memory performance of mice was evaluated by counting the number of arm entries and determining the spontaneous alternation behaviour in the 8-minutes session. Alternation was defined as a consecutive entry in three different arms.

The alternation behaviour (%) was calculated using the following formula:

[number of alternations / (total number of arm visits – 2)] x 100 (18).

Data were analyzed using the ANOVA test implemented in SPSS 13.0 for Windows software. P-values less than 0.05 were considered statistically significant compared to those of the Control group.

The experimental protocols were approved by the University "Grigore T. Popa" Committee for Research and Ethical Issues. All procedures involving animals and their care were conducted in compliance with the international normatives and policies (19).

RESULTS

The results obtained in the Actimeter test were centralized, statistically interpreted and were represented in the following figures (fig. 1, 2).

The administration of pregabalin resulted in a dose-dependent reduction of mice horizontal movements (1313.45±1.21, respectively 1198.75±2.37), statistically significant ($p<0.05$) compared to the control group (1442.25±2.29) in the Actimeter test (fig. 1).

Intraperitoneal injection of pregabalin was associated with a dose-dependent diminution of vertical movements (372.25± 3.17, respectively 258.65±4.11), statistically significant ($p<0.05$) compared to the distilled water group (419.25±2.54), (fig. 2).

The treatment with two doses of pregabalin (PGB 10 and PGB 20) did not significantly modify the number of stereo-

typic movements (1511.25±2.32, respectively 1509.15±3.41) compared to the Control group (1514.25±1.53) in this behavioural experimental model in mice (fig. 3).

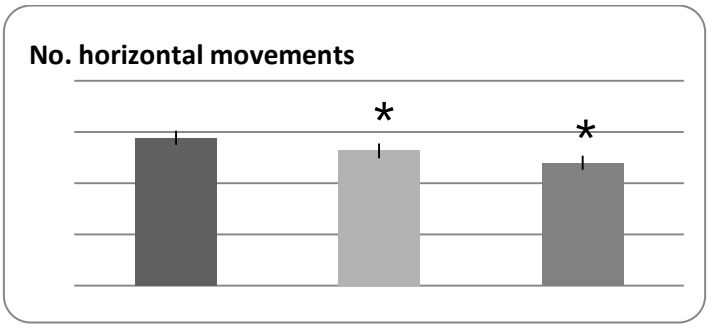

Fig. 1. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on horizontal movements in the Actimeter test (values expressed as mean ± SEM of horizontal movements for 6 animals; *p<0.05 vs Control).

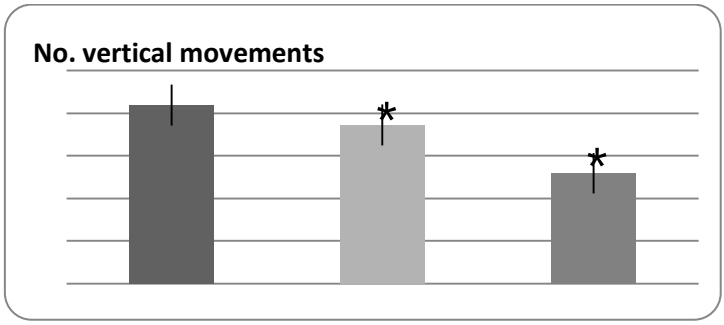

Fig. 2. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on vertical movements in the Actimeter test (values expressed as mean ± SEM of vertical movements for 6 animals; *p<0.05 vs Control).

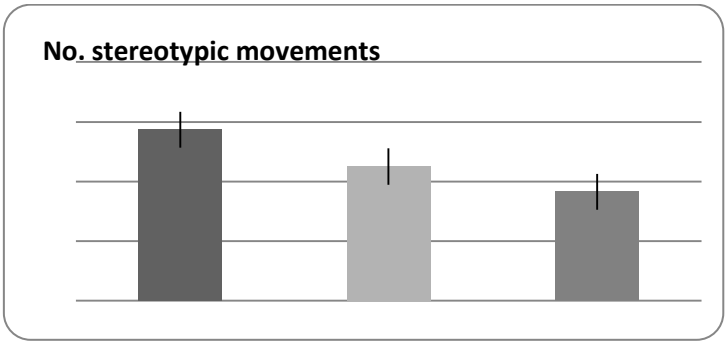

Fig. 3. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on stereotypic movements in Actimeter test (values expressed as mean ± SEM of vertical movements for 6 animals).

The results obtained in the Y-maze test were collected, statistically processed and were represented in the following figures (fig. 4, 5).

Intraperitoneal injection of the both doses (10 mg/kbw or 20 mg/kbw) of pregabalin did not significantly influence the number of arm entries compared to Control group in the same time interval, in the Y-maze assay (fig. 4). These results suggest that pregabalin administration did not modify the mice performances in this behavioural test (20).

The administration of PGB 10 did not induce modification of spontaneous alternation percent, compared to the Control group (fig. 5).

The use of PGB20 tended to increase the spontaneous alternation percent, but statistically non-significant compared to the group treated with distilled water in the Y-maze test (fig. 5). These results signify the lack of effect on the spatial memory performance of pregabalin administration in mice (13).

No significant changes were observed in the latency of the first arm visit, of the same arm return number and alternate arm return number between the groups treated with both doses of pregabalin and distilled water groups in this behavioural model in mice.

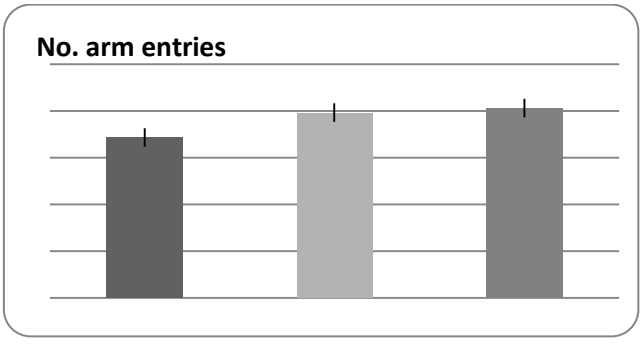

Fig. 4. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on arm entries number in Y-maze (values expressed as mean ± SEM of arm entries number for 6 animals).

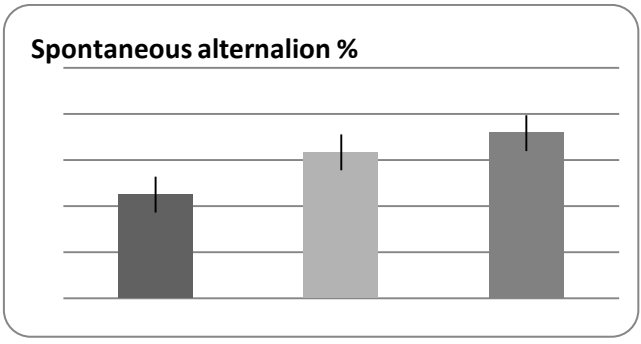

Fig. 5. Effects of of pregabalin (10 mg/kbw and 20 mg/kbw) administration on spontaneous alternation percent in Y-maze (values expressed as mean ± S.E.M of spontaneous alternation percent for 6 animals).

Moreover, the exploration capacity was not modified after the treatment with both doses of pregabalin in the same test in mice.

DISCUSSION

Limited literature data has been published regarding the involvement of pregabalin in spontaneous motor activity and cognitive processes of laboratory animals.

An experimental research conducted in the Elevated plus maze test revealed that pregabalin demonstrated evident short term anxiolytic effect, attenuating anxiety-like behaviours in mice exposed to traumatic stress. The authors of the study suggest that pregabalin may be an option for the treatment of anxious state after traumatic stress experience, lacking long-term protective or preventive actions (21).

Other experimental investigation proved that the administration of pregabalin attenuates ketamine-induced hyperlocomotor activity in an animal model of psychosis induced by ketamine, an NMDA receptor blocker that mimics the symptoms of schizophrenia (22).

Regarding the influence on cognitive functions it has been demonstrated that the treatment with pregabalin is associated with an impairment of the memory performance in step-down passive avoidance task in mice (23).

Using the behavioral experimental test, our research study has gathered information regarding three type of animal spontaneous motor activity: horizontal, vertical and stereotypic movements. The spontaneous motor activity is an elementary component of a normal animal behavior.

In our experimental condition the experiment proved that depending on the dose administered, pregabalin decreased mice horizontal movements, effect corresponding to a reduction of the general behavior exploration of the environment (24).

Intraperitoneal administration of pregabalin was associated with a diminution of vertical movements, parameter that reveals the animal trying to climb on the transparent walls of the registration cage, and is an indicative for attempt behavior, indirectly signifying fear and anxiety manifestations (11, 25).

In the Y-maze assay spontaneous alternation refers to the normal tendency of the animal to spontaneously choose alternate arms. Arm exploration was defined as entering at least the first third section of an arm (20).

In this behavioural model we noticed that neither 10 mg/kbw nor 20 mg/kbw pregabalin influenced the exploration capacity of animal placed in the device area.

Moreover, both doses of pregabalin did not modify the spontaneous alternation percent in the same behavioural experimental model in mice.

All these results suggest that pregabalin may be not involved in the mediation of cognitive function and learning capacity of laboratory animals.

CONCLUSIONS

The results reflect a significant dose-dependent diminution of the number of escape attempts, exploratory and self-maintenance spontaneous behaviour after pregabalin treatment, which could be correlated with an evident anxiolytic effect.

The study demonstrated that pregabalin did not modify the animal cognitive process performances or influence the short-term memory in the Y-maze test in mice.

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NEWS

LYMPHANGIOMA OF THE PALATINE TONSIL

Lymphangioma of the palatine tonsil is a rare, benign lesion that presents as a tonsillar outgrowth and causes symptoms related to irritation and airway obstruction. Histologically, the mass has abundant dilated lymphatic channels in a fibrous stroma with lymphoid and adipose elements. A lymphangioma is mostly a congenital lesion consisting of a benign proliferation of lymphatic channels.Approximately 50% of these lesions are apparent at birth, with 80% to 90% detected within the first 2 decades of life. In contrast to congenital hemangiomas, lymphangiomas do not involute over time. Although these lesions tend to surround and sometimes invade nearby anatomic structures, they have no malignant potential. There is also considerable debate over the etiology of lymphangiomas, which may represent true neoplasms, malformations, or hamartomas. Three morphologic types of lymphangiomas are distinguished. Capillary, or simple, lymphangiomas are usually found in the superficial skin and composed of capillarysized, thin-walled lymphatic vessels. Cavernous lymphangiomas are subcutaneous lesions composed of mildly dilated, large spaces that are larger than those seen in capillary lymphangiomas. Cystic hygromas, the third type, are most common in the neck and composed of large, dilated, cystic lymphatic spaces. Thus, these 3 types of lymphangiomas can be differentiated from one another based purely on the characteristics of the component lymphatic spaces. In addition, combinations of the 3 types can sometimes be found in a single lesion. Therefore, the 3 types of lymphangiomas represent the same pathologic process along a spectrum and should be considered as a unified concept. Moreover, differentiating lymphangiomas on the basis of histologic type appears without clinical implications. Lymphangiomas tend to occur in places where lymphatics are abundant, with more than 90% of all lymphangiomas arising in the head and neck region. Most are found in the skin and subcutaneous tissues, but they have also been described in the larynx, parotid gland, mouth, and tongue. The palatine tonsil is a far less common site for the development of lymphangioma and has rarely been reported in the literature. Regardless of the uncertain pathogenesis of tonsillar lymphangiomas, these lesions have histologic features that differ from their counterparts in other anatomic locations. Although the lymphatic spaces of tonsillar lymphangiomas are frequently dilated, they are generally not as large or as prominent as in the lymphangiomas found elsewhere. Furthermore, the lymphoid and fibrous stromal components in tonsillar lymphangiomas are frequently more abundant than the lymphatic vessels. Thus, tonsillar lymphangiomas may represent a somewhat unique lesion. Reports in the literature use different nomenclature when referring to these lesions, such as tonsillar lymphangiomatous polyp, angioma, angiofibroma, fibrolipoma, polypoid tumor containing fibroadipose tissue, hamartomatous tonsillar polyp, and lymphangiectatic fibrous polyp. Because a lymphangioma may resemble a true neoplasm of the palatine tonsil clinically, the lesion must be removed for accurate histologic diagnosis and to rule out malignancy. Lymphangioma of the palatine tonsil is treated with surgical excision and has no recurrence once completely resected (Mardekian S and Karp JK. Lymphangioma of the Palatine Tonsil. Arch Pathol Lab Med. 2013;137:1837–1842).

Doina Butcovan

TECHNOLOGICAL STEPS IN CLASS II EDENTATION PROSTHETIC REHABILITATION-CASE REPORT

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TECHNOLOGICAL STEPS IN CLASS II EDENTATION PROSTHETIC REHABILITATION-CASE REPORT We presented a case of a Class II Kennedy edentation treated by a cast removable partial denture (RPD). Removable partial dentures with attachments present clear advantages to both patient and clinician. However, partial denture designs that try to improve morphological and functional benefits also should attempt to minimize the potential for harm. The practitioner should be aware of the principles of partial denture design that aim to maximize the functional stability of the denture and, at the same time, reduce the risk of partial dentures to cause long-term harm to the remaining teeth and supporting soft tissues. **Keywords:** CAST RPD, ATTACHMENTS, METAL CERAMIC BRIDGE.

This case presentation outlines the clinical and technological stages involved in realization of a cast removable partial dentures (RPD) that represents the likely treatment option for a class II Kennedy edentation. Patient's complaints were related to poor appearance and loss of masticatory function. The clinical examination established the status of the remaining natural teeth, of the existing restorations, of the potential denture-bearing areas, and of the soft tissue. In addition to establishing the dental status of remaining teeth, the examination should include an assessment of occlusal relationships and the availability of space for the prosthesis. Additional investigations were required, including radiographs of all abutment teeth, being important to get as much information as possible to establish the treatment plan. The principal objectives when treating partially dentate patients are to preserve the remaining teeth in a stable and healthy environment, to prevent further pathological change and to restore function (1, 2, 3).

The preliminary casts were poured into preliminary impressions, with care taken not to omit detailed recordings of remaining teeth and potential denture-bearing area. The preliminary cast was surveyed to establish the design of the partial denture, essential step in getting a good clinical result**.** We decided the abutment teeth (1.6, 1.3, 2.3, 2.6, 2.8), the outline of the major connector (a palatal strap) and the saddle areas.

The fixed component of the partial denture was represented by three metallic crowns on 1.6, 2.6 and 2.8, and a metal-ceramic bridge with attachments on 1.3-2.3.

The combined thickness of metal and

porcelain of the facial surface of a metal-ceramic crowns should be at least 1,4 mm to meet minimum strength and shade standards. A thickness of 0,4 mm of metal over the facial surface of the die is needed to prevent the alloy from flexing under stress. If the metal substructure flexes, applied porcelain will fracture. Bond strength between porcelain and metal is best when the metal surfaces are gently rounded. Corners or angles must be avoided on the porcelain-bearing surfaces. All junctions between porcelain and metal on the external surface of a restoration should be as close to a 90 angle as possible (4).

The wax patterns were made to a thickness of 0,4 mm in the areas that received porcelain. The wax was uniform and wrinkle-free to avoid holes. To control thickness with a little more precision we adapted a calibrated sheet wax over the die's surfaces. A cervical collar of l mm wide was carved into the oral half of the pattern. This collar helps ensure complete casting of the facial margin area and its bulk resists distortion when the porcelain cools from its firing temperature. In this stage, on the distal abutment teeth of the bridge the attachments were applied, in the denture's path of insertion. The wax patterns were prepared for investment by spruing and the mold was obtained after burnout stage. The dental alloy Brealloy C+B 270 (Bredent, Germany) was melted and cast with an automatic casting machine (ORCACAST M). After divesting the crowns framework were finished and prepared for veneering.

To eliminate the possibility of an incorrect shade, the opaque porcelain was used as a foundation and built thick enough to blot out the metallic frame. The opaque porcelain was mixed with distilled water to a creamy consistency, applied with a sable brush and vibrated to expel water that may be trapped. When the desired thickness has been reached, the opaque was condensed and smoothed. The opaque layer needs to be 0,2 mm thick to perform its masking function.

Fig. 1. Metallic framework of the fixed appliances

The body porcelain should be 0,75 mm thick or thicker to reproduce the chosen shade. The porcelain powder was mixed with distilled water to a thick, creamy consistency. First the body mix was applied, starting from the labial surface and progressing around to the lingual surface. The porcelain elements were build larger one sixth than the finished elements so that to allow for shrinkage of the porcelain. After the body porcelain was correctly shaped, the incisal area was rehabilitated. The bridge was brushed to smooth and condense the porcelain. The incisal porcelain was added to the contacts' areas of the proximal sides to compensate for the shrinkage of porcelain when it is fired. After the bridge was fired and cooled, necessary adjustments were done to realize the appropriate shape (fig. 2). The final fire of the bridge obtains a glaze for a desirable glossy surface (5, 6).

Fig. 2. The incisal porcelain with glossy surface

The fixed appliances were checked on the cast and after into the oral cavity, surveying the cervical adaptation, the rehabilitation of the morphology, the esthetic parameters, the anterior guidance path and the relationship with the antagonists.

Into the dental laboratory a light curing custom impression tray was made over the fixed bridge, to obtain the final impression and further the master cast (fig.3).

Fig. 3. Functional impressions

The master cast was surveyed to determine the path of insertion, undercut areas and the design of the framework of the removable partial denture. Interferences can profoundly influence the choice of a suitable path of insertion and may prevent the use of a partial denture.

The preparation for duplication included the blocking out and relieving steps. On the duplicated cast, after the surveying, the wax up for the metallic frame was done from preformed wax profiles, sprued and invested. The dental alloy used for casting the framework was Brealloy F 400 (Bredent, Germany).

After divesting, finishing and polishing, the metallic framework fitting was checked on the master cast and into the oral cavity.

To obtain the acrylic component of the saddles, after the jaws relationship record, the master casts were mounted into an articulator, using baseplates and occlusion rims. It is the dentist's responsibility to get proper jaw relationship records and accurate measurements in the patient's mouth. The technician must be sure that the arrangement of the denture teeth harmonizes with the natural remaining teeth (7).

During the clinical try-in several aspects were checked: the dimension of vertical occlusion (DVO), the aspect and functionality of the artificial arches. There are at least three objectives in setting of the denture teeth. The first is to achieve maximum chewing function and stability, the second is to avoid interfering with the pa-

tient's speech, and the third is to restore natural appearances (8, 9).

The wax up of the acrylic saddles were transformed into the final components through the flasking, resin curing, deflasking, finishing and polishing stages (fig. 4).

Fig. 4. The final aspect of the removable prosthesis

CONCLUSIONS

It is necessary for both the dentist and the patient to be convinced that the replacement of missing teeth will produce significantly more benefit than harm.

For many patients with teeth missing in the anterior part of the mouth appearance is an overriding consideration. As with appearance, the patient's concept of the problem is as important in deciding on a replacement as the problem itself.

In many cases tilting and over-eruption eventually lead to an instable occlusal relationship. If the missing teeth can be replaced before severe tooth movements occur, many TMJ disorders and malocclusions can be prevented

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ETHICAL VIEWS, ATTITUDES AND REACTIONS OF ROMANIAN MEDICAL STUDENTS TO THE DISSECTING ROOM

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ETHICAL VIEWS, ATTITUDES AND REACTIONS OF ROMANIAN MEDICAL STUDENTS TO THE DISSECTING ROOM (Abstract) **Aim**: Our objective was to evaluate the attitudes and views of first year medical students towards cadaver dissection in anatomy learning and discuss various findings in relation with ethical problems). **Material and method**: The study was conducted at the"Iuliu Haţ ieganu" University of Medicine and Pharmacy, during the academic year 2012-2013 at the end of the second semester. There were 121 first year medical students included. We developed a questionnaire to asses among other, the degree of fear, anxiety and stress in the dissection room, methods of coping, ethical aspects of dissection and hand it to the students. **Results**: 34.7% of students experienced different levels of fear on exposure to the dissection room practical sessions. Many students experienced anxiety in reaction to dissection. In the first semester most students reported physical and behavioral reaction towards certain stimuli, with a decrease in the second semester. Recurring visual images of cadavers, reported by 57% of students in the first semester, dropped to 44.6% in the second semester. Students used most frequently the "rationalization and emotional detachment" as a coping method. **Conclusions**: Anatomists, most often the firsts who need to be aware of emotional and ethical issues, need to explain in detail the steps necessary for dissection and that dissection is performed with the respect of legislation, ethics and human rights. **Keywords:** ETHICAL ISSUES, DISSECTION, LEGISLATION, HUMAN RIGHTS, MEDICAL STUDENTS

Dissection of cadavers by the medical students is considered an essential requirement in learning anatomy and is the most universal and universally recognizable step in becoming a doctor (1).

Dissection of a human body during an anatomy course raises for first-year medical students questions about invasion of privacy, cadaver sources, dying and death. In anatomy, a student realizes that the first patient that he/she will care for is a dead one and experience considerable anxiety and stress (2).

Cadaver dissection has other learning outcomes besides anatomical learning, such as fostering teamwork and respect for the

human body, applying in practice the principles of bioethics and integration of knowledge from textbooks and didactic lectures with practice (3).

Anatomy can be defined etymologically using the term dissection (*Ana + tomein, Lat. cutting, incision, acting through dissection. Dissecare Lat., to cut apart*). The study of the human body without the use of dissection is a mere "lecture on the human form", which is the field of morphology, not anatomy (4).

The evolution of anatomy is, therefore, tightly bound to the history of dissection on human bodies. Ever since ancient times, corpses have been studied by scholars. In ancient Greece, Aristotle has practiced animal dissection, but in short time dissection was done on human corpses. Under the reign of king Ptolemeus, Herophilles (335 – 300 b.Chr.) was encouraged to practice vivisection on criminals. He was credited with no less than 600 such dissections (4). On the other hand, in ancient Rome human dissection was under religious and civic ban.

The precise descriptions from Galen's (129-216 A.D.) dissection textbook suggest that the author was no stranger to human corpse dissections, even though he admitted only to performing animal dissection.

The disappearance of the taboo on human corpse dissection occurred during the Renaissance period (XIVth–XVIth century) particularly due to medical scholars like Vesalius; the latter practiced systematic dissection for the first time (5). Hence, from the dissection method standpoint, the history of anatomy can be divided in prevesalist and postvesalist (4). The recognition of the utility of dissection and the subsequent shake-off of its licentious attribute has allowed the spectacular outspread of this scientific practice.

A parallel statistic on the number of hospital abandoned and donated corpses, respectively, at the Paris Faculty of Medicine between 1900 and 1999 has shown the progressive decrease of hospital abandons from nearly 2000 in the year 1900 to under 50 in 1980. The latter level has not changed ever since.

A study on records of the source of corpses used in the Cluj-Napoca anatomy department between 1987 and 2012 has found no donations to have taken place, the bodies for dissection being supplied by hospitals morgue services and forensic departments from Cluj-Napoca and nearby districts.

Ethical dilemmas concerning dissection. In the last decades, medicine is subject to probably the most extensive development from its long history. Terms such as biotechnologies or genetic engineering were not long ago, the privilege of science-fiction; nowadays, they stand as important fields of research, expanding the scientific knowledge. With all the progress of medicine, many domains maintain or rise new subjects of disputes, controversies or dilemmas. The role of the human tissue, broad sense, for the progress of medical sciences, either research or clinical practice, is not to be argued. Nonetheless new legal and ethical issues arise, reflected by the growing interest of public opinion in these matters (6, 7).

There is a special concern regarding the protection of individuals from whom the tissues come/are harvested and their proper use. The human body, even if dead, is referred to as tissue, broad sense. There is a special preoccupation for the respect to the person and the human body. Usually,

corpses and large body parts, that are easy to be recognized, are paid more respect than fragments of tissue, such as vials of urine or blood (8); it seems that the latter are perceived differently.

Respect for the human body, including corpses, was paid through all mankind history, starting from legal regulations and continuing with moral, social, ethical and religious regulations, which protect individuals, life, integrity and propriety, and regulates burial and incineration, and the respect paid to the dead (9).

Even so, these conditions are neither absurd nor arbitrary, corpses being used in the interest of the society, with the proper respect. For medical sciences, this contributes to the development of medical research, new means of diagnosis and therapy, or for medical education.

Cadaver dissection is performed by medical students during practical lessons/classes for the study of anatomy, by surgeons for studying new approach in surgical interventions. Dissection is performed for didactical or research purposes, obeying the law and the principles of medical ethics (10, 11, 12, 13).

Beneficence involves two fields - the individual and the society. Dissection involves applying the beneficence on the social level, through the public institutions and their benefits to society. Dissection performed during anatomy classes has didactic and scientific characteristics, contributing to professional formation and development of medical students, who in turn will become physicians, or of surgeons, pathologists or forensic specialists.

The principles of bioethics are stated in The Decalogue of Masci, The International Code of Medical Ethics and in The Deontological Code of The Romanian College of Physicians (14).

The Decalogue of Masci states the absolute respect of the patient, regardless of age. By extent, we can affirm the necessity of respect for the deceased human body, and in our case, for the corpse for dissection (15).

Perhaps the most important rule is the moral obligation of continuous professional self-improvement. Let there be the case of specialists dissecting corpses for professional development, such as achieving new techniques, surgical procedures or the study of anatomic position.

The International Code of Medical Ethics imposes to maintain high professional standards. These rules stress those mentioned by the Decalogue of Masci, mostly those concerning continuous self-improvement. In order to respect this rule, standard procedures in harvesting and fixing corpses and performing dissection are applied. Another rule is keeping the absolute secret concerning the patient, even after his/her death (16).

The Deontological Code of The Romanian College of Physicians states the principle of autonomy by saying that the physician is in the service of the human person, and the secret lays even after the treatment is over or the patient is dead (17).

Many medical schools from the US (18), the UK (19), Australia (20), and the Netherlands (21) have already quit dissection by various reasons (22). Out of many reasons, one has a certain importance for our case. The study conducted by Charlton and Smith (2000) reveals that the experience during an anatomy class can induce stress among some of the medical students (23). A small percentage of them may have feelings of horror, apprehension, guilt, wonder, gratitude, sadness and even over-

whelming physical reactions. Finkelstein and Mathers (1990) conducted a survey at Standford University Medical School and found out that five percent of the questioned students reported marked disturbance including nightmares, intrusive visual images, insomnia, depression, and learning impairments (24) According to Gustavson (1988), first year medical students have concerns about becoming "desensitized" to the human body, due to the emotional responses to the dissection process (25). Dinsmore et al (2001) found similar results (26). Penny (1985) found out that the strongest reactions were in anticipation of dissection (27).

Negative emotional experience among dissection also crosses cultural barriers. Nnodim (1990) found that about a quarter of the Nigerian students reported an increase in frequency of mental and physical symptoms suggestive of depression, and 5% remained highly upset at the halfway point of the course (28) A later study of the same author (1996) found no cultural differences between the student's attitudes (29).

If the principle of nonmaleficience is applied to patients, wouldn't it be right to apply the same principle to healthy individuals not suffering from a disease or an infirmity, as is the case of the medical students? Can the physical and psychological impairments inflicted by dissection be considered as violation of the principle of nonmaleficience?

Furthermore, we have to discuss about the right to intimacy and private life of the patient, which, during a dissection is exposed to looks and comments from the students.

A minimal distinction between dissection and desecration must be made. Dissection is performed in the spirit of respect towards the human body for precise scientific purposes, obeying the law and regulations, and by standard procedures. For example, the head and the face are kept covered until the study of that specific anatomic segment; the body parts that are not subject to current anatomy classes are kept covered. Dissection starts with skin dissection and continues with the other parts/organs.

We can state that dissection forges the spirit of the future physician, contributing to the understanding of scientific notions, but also to the forming and polishing of the character, by understanding the moral values, the gift of life, implications of the disease and lifestyle, respect towards death and cultivating empathy for the future patients (25).

This study was carried out to evaluate the attitudes and views of first year medical students towards cadaver dissection in anatomy learning and discuss various findings in relation with ethical problems of using human body for teaching and scientific research and with reports available in the scientific literature.

MATERIAL AND METHODS

The present study was conducted at the University of Medicine and Pharmacy "Iuliu Haţ ieganu", during academic year 2012-2013, at the end of the second semester. The subjects included in the study were 121 first year medical students in General Medicine who were willing to participate in the study. We explained the objective of the study and a questionnaire was given to the students. A psychologist, a psychiatrist and an anatomy instructor developed the questionnaire from interviews with medical students. Test-retest reliability of the ques-

tionnaire was conducted on 20 students with an interval of a week. The test preserved anonymity of the students and contained multiple choice questions about: degree of fear of the dissection room (DR), anxiety and stress in reaction to DR, sources and degree of disturbance in the DR, methods of coping, previous experience of a dead body before dissection, mental preparation for dissection, sympathy and respect for cadaver, ethical aspects of dissection, importance and indispensability of dissection for studying medicine.

RESULTS

121 students answered to the questionnaire 35 (28.9%) were males and 86 (71.1%) females. The mean age of the students was 19.79 (±0.717). Regarding religion, 75 (62%) were Orthodox, 21 (17.4%) Catholic, 12 (9.9%) Methodist, 11 (9.1%) Neo-protestant Cults and 2 (1.7%) without religion. 65 students (53.7%) were practicing their religions. For 22 (18.2%) of the students the religion is very important, for 53 (43.8) is moderately important, for 38 (31.4%) is slightly important and for 8 (6.6%) is not at all important.

A total of 65.3% of students reported no fear before they entered in the DR, and 34.7% reported varying degree of fear, out of which 9.9 were very scared.

First table shows the behavioral and physical reaction of students to the DR. Nausea; trembling and recurring visual images of cadavers were the most common reaction experienced by the students in the first semester.

TABLE I
Stress and anxiety in reaction to dissection room

Reaction	Percentage of students (Number of students) / First semester / Second semester									
	Never		Occasional		Often		Frequently		Always	
Fainting	71.9 (87)	81.0 (98)	20.7 (25)	19 (23)	2.5 (3)	0 (0)	5 (6)	0 (0)	0 (0)	0 (0)
Dizziness	65.3 (79)	76 (92)	20.7 (25)	6.6 (8)	4.1 (5)	6.6 (8)	13 (10.7)	13 (10.7)	5(6)	0 (0)
Palpitation	82.6 (100)	90.9 (110)	3.3 (4)	2.5 (3)	11.5 (14)	4.1 (5)	0(0)	2.5 (3)	2.5 (3)	0 (0)
Left de DR	72.7 (88)	79.3 (96)	6.6 (8)	5 (6)	7.4 (9)	2.5 (3)	10.7 (13)	10.7 (13)	2.5 (3)	2.5 (3)
Trembling	66.1 (80)	78.5 (95)	31.4 (38)	17.4 (21)	2.5 (3)	4.1 (5)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	46.3 (56)	68.6 (83)	47.1 (57)	28.9 (35)	6.6 (8)	2.5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Sweating	76.9 (93)	79.3 (96)	10.7 (13)	8.3 (10)	1.7 (2)	1.7 (2)	10.7 (13)	10.7 (13)	0 (0)	0 (0)
Loss of appetite	73.6 (89)	86.9 (105)	7.4 (9)	1.7 (2)	12.4 (15)	5 (6)	6.6 (8)	6.6 (8)	0 (0)	0
Insomnia	79.3 (96)	90.9 (110)	18.2 (22)	6.6 (8)	2.5 (3)	2.5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Recurring images of cadavers	43 (52)	55.4 (67)	50.4 (61)	40.5 (49)	6.6 (8)	4.1 (5)	0 (0)	0 (0)	0 (0)	0 (0)

TABLE II

Degree of disturbance related to certain stimuli in the dissection room

Stimuli in the DR	Percentage of students (Number of students) First semester / Second semester									
	None		Slight		Moderate		High		Very High	
Smell of DR	9.1 (11)	9.9 (12)	23.1 (28)	35.5 (43)	40.5 (49)	27.3 (33)	6.6 (8)	8.3 (10)	20.7 (25)	19 (23)
Sight of cadavers	28.1 (34)	38.8 (47)	40.5 (49)	33.9 (41)	30.6 (37)	26.4 (32)	0.8 (1)	0.8 (1)	0 (0)	0 (0)
Looking at the cadaver's face	32.2 (39)	38 (46)	19 (23)	17.4 (21)	38 (46)	40.5 (49)	5.8 (7)	4.1 (5)	5 (6)	0 (0)
Touching certain parts of cadavers	57.9 (70)	64.5 (78)	32.2 (39)	29.8 (36)	7.4 (9)	5.8 (7)	0 (0)	0 (0)	2.5 (3)	0 (0)
Fears of infection	62 (75)	65.5 (79)	19.8 (24)	17.4 (21)	6.6 (8)	6.6 (8)	11.6 (14)	10.7 (13)	0 (0)	0 (0)

In the second semester we noticed a decreased of physical reaction to the DR.

In the second table are gathered the reactions to the stimuli in the DR.

The most disturbing was the smell, only 9.1 % of the students don't have a problem with the smell and looking at the cadaver's face (67.8% of the students have some degree of apprehension).

In Table III are presented the coping methods with the DR. Most of the students (74.4%) used rationalization and emotional detachment as coping mechanism.

A percentage of 35.5% have already seen a dead body before entering the DR.

In our study all the students considered that cadaver dissection is ethically acceptable. An overwhelming majority of students (78.5%) considered cadaver dissection as important and indispensable in anatomy learning.

Only 46.3% of the students think that the cadaver they dissected was once a human being.

TABLE III

Methods of coping

Coping mechanism	Percentage of students				
	Never	Occasional	Often	Frequently	Always
Talking with another students	54.5(66)	21.5(26)	24(29)	0(0)	0(0)
Talking with anatomy instructors	90.1(109)	9.9(12)	0(0)	0(0)	0(0)
Rationalization, emotional detachment	25.6(31)	8.3(10)	20.7(25)	29.8(36)	15.7(19)
Humor	32.2(39)	23.1(28)	15.7(19)	18.2(22)	0(0)
Praying	72.7(88)	9.9(12)	15.7(19)	1.7(2)	0(0)

DISCUSSION

First year medical students experienced unpleasant or frightening feeling on their first exposure to dissection and dead bodies (30) as part of the curriculum.

To deal with difficulties that the stu-

dents might encounter, their reactions and feelings should be known (25).

This study indicates that 34.7% of students experienced different levels of fear on exposure to the DR practical sessions.

Other study has found percentage between 5% (31) and 50% (32).

In the first semester most of the students reported variable degrees of physical and behavioral reaction towards certain stimuli in the DR, the reaction decreased in the second semester. It seems that there is a consensus that all medical students may experience a variable degree of fear on exposure to DR (31, 32, 33)

Recurring visual images of the cadavers were reported by 57% of students in the first semester and by 44.6% of students in the second semester. This figure was much higher than (9.7%) reported in UK (34).

In this study, smell was the most disturbing stimulus, where 90% of students showed a variable degree of disturbance due to the smell, follow by looking at the cadaver face (67.8%).

Many students go through a process of psychological accommodation (35) due to rapid acquisition of coping mechanism to death (36).

The present study showed that the students have used different coping methods to overcome their fear in the DR. The most frequently used method by students was "rationalization and emotional detachment" (74.4%), followed by humor (67.8%).

CONCLUSIONS

Our study shows that many students experienced anxiety in reaction to dissection.

Anatomists are often the first teachers in the curriculum who need to be aware of emotional and ethical problems (2, 35). More attention should be paid to the first encounter with corpses, and students should be offered the opportunity to discuss their emotions.

Students should be make aware of the possibility that they will develop symptoms in reaction to dissection, that the symptoms are normal and they could talk about this with the anatomy teachers or in psychological counseling.

There is a need to explain in detail to students the steps necessary for the dissection and the fact that dissection is performed with the respect of legislation, ethics and human rights.

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HEPATORENAL DYSFUNCTION IN SEPSIS: EPIDEMIOLOGICAL, CLINICAL AND LABORATORY ASPECTS

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HEPATORENAL DYSFUNCTION IN SEPSIS, EPIDEMIOLOGICAL, CLINICAL AND LABORATORY ASPECTS (**Abstract**): The major impact of sepsis-induced multiple organ dysfunction on healthcare system in the European Union was estimated at 90.4 cases per 100000 inhabitants, compared to 58 per 100000 for breast cancer. The association of organ dysfunctions in terms of both the number of dysfunctions and the degree of organ dysfunction is the most powerful predictor of death in sepsis. **Aim:** To find medical and statistical correlations in hepatorenal dysfunction in sepsis patients. **Material and Methods:** This retrospective study included 117 patients diagnosed with sepsis at the Iasi Infectious Diseases Hospital, patients who presented liver/renal and other organ dysfunctions. The clinical, etiological, and laboratory data, and APACHE II prognostic scores were analyzed. The data were processed using *SPSS version 16.0*. **Results:** The etiological agents were Gram positive as well as Gram negative bacteria, and 40% of sepsis patients with hepatic/ kidney dysfunction presented hepatorenal syndrome. **Conclusions:** Over one-third of patients with sepsis-related hepatorenal dysfunction had a creatinine clearance of less than 30 ml/min, and we found statistical correlations between serum creatinine and APACHE II score. There were no statistically significant differences between the survival curves of patients with hepatorenal syndrome and those with sepsis-related hepatorenal dysfunction. **Keywords:** SEPSIS, HEPATORENAL DYSFUNCTION, KAPLAN MEIER CURVE

The major impact of sepsis-induced multiple organ dysfunction on healthcare system in the European Union was estimated at 90.4 cases per 100000 inhabitants, compared to 58 per 100000 for breast cancer (1). It is estimated that there are 1.8 million cases annually, but this figure reflects low recognition or diagnosis. Recent estimates show an incidence of sepsis in intensive care units of 0.25 to 0.38 per 1000 inhabitants, suggesting two million admissions to intensive care units (2, 3). Data are from intensive care units and research centers in UK Intensive Care National Audit and Research Centre (ICNARC) covering the last months of 2005 reported 8300 deaths from severe sepsis. Mortality rate from severe sepsis was estimated in many studies, ranging from 28% to 50% (4, 5, 6).

In the United States the incidence of severe sepsis is estimated to 300 cases per 100,000 inhabitants of which about half outside emergency units (7, 8). One in four patients who developed severe sepsis dies during hospitalization. Septic shock is as-

sociated with the highest mortality rate, approaching 50%. The association of organ dysfunctions in terms of both the number of dysfunctions and degree of dysfunction is the most powerful predictor of death in sepsis. Epidemiological controversy arises when discussing about treated cases, since eligibility for treatment differs according to time and country, with different cultural perspectives on end-of-life, differences in access to emergency departments; level of health insurance other economic and cultural factors (7, 9).

Sepsis encompasses a tremendously heterogeneous group of patients with a great variability of underlying conditions, infection sites, with a multitude of microbial agents with high virulence and variations in concentration, and with differences in inflammatory response and immunological context. Sepsis can be caused by a small inoculum of etiologic agent in conditions of high pathogenicity or reduced host defense capacity. The inflammatory response may be excessive when the microbial toxin or microorganism is very aggressive, or when genetic predisposition leads to important changes at the level of cellular effectors involved in the inflammatory response (10).

MATERIAL AND METHODS

The study was conducted in the Iasi Hospital of Infectious Diseases and included patients diagnosed with sepsis in the interval November 2012 - April 2014. Data in their medical records and annexed documents were analyzed. All patients signed upon admission an informed consent for the use of their data for research purposes. This study was performed in compliance with the Declaration of Helsinki, and was approved by the Ethics Committee of the University of Medicine and Pharmacy of Iasi.

A number of 117 sepsis patients presenting hepatorenal dysfunction, hepatorenal syndrome (HRS), and other organ dysfunctions were included in the study. The demographic data (age, gender, origin), length of hospital stay, laboratory data, etiology, baseline and post-baseline laboratory data, APACHE II prognostic scores, past history of liver/kidney dysfunctions, disease improvement or progression to death were analyzed. The diagnosis of sepsis was made according to the definition of suspected or documented infection and presence of inflammatory systemic response syndrome (at least two of the following criteria: fever/hypothermia, tachycardia, tachypnea, and leucocytosis/ leukopenia).

The diagnosis of severe sepsis referred to patients with the acute organ dysfunction: cardiac, renal, hepatic, respiratory and circulatory failure disseminated intravascular coagulation or shock. Biological samples were analyzed in the laboratory of bacteriology by direct examination, cultures, fixation method, and latex agglutination. We created an EXCEL database including the descriptive data and the qualitative and quantitative parameters, and used SPSS version 16.0 for data processing. The following tests were used: *t student test*, *chi-square test*, *Pearson correlation* for normally distributed variables, and *Spearman rank-order correlation* independent of data distribution types. When data were not normally distributed we used the *Mann-Whitney test* for comparing two sets of variables, the *Pairwise Comparison test* when sample sizes were small; *Kruskal-Wallis test* was used to compare multiple sets of data.

RESULTS

In age groups 50-70 and over 80 years there was a female prevalence; rural/urban distribution of the study patients was approximately equal.

The identified etiologic agents varied widely: methicillin-sensitive *Staphylococcus aureus (*MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus spp.*, *Enterococcus spp*. as well as beta-lactamase producing and not-producing Gram negative bacilli: nonfermenting Gram negative bacilli, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*.

Patients with hepatorenal dysfunction were predominantly referred from cardiology, diabetes, neurology and gastroenterology departments (fig. 1). At baseline the inflammatory syndrome was well represented, with an average of 13200 white blood cells/mm³ in the case of hepatorenal syndrome, average CRP level equal in patients with or without hepatorenal dysfunction, average fibrinogen level of 6.2 g/L in patients with hepatorenal syndrome compared to 5.8 g/L in those with hepatorenal dysfunction.

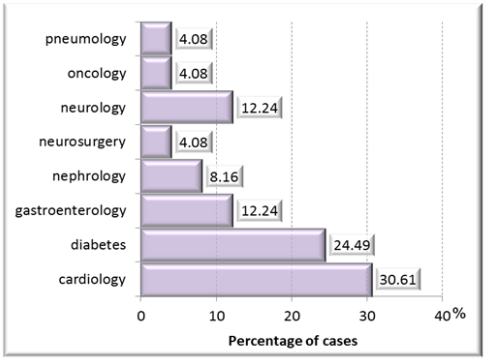

Fig. 1 Departments referring patients with sepsis and hepatorenal dysfunction

Only 40% of patients with hepatorenal dysfunction presented a hepatorenal syndrome (fig. 2), the average creatinine clearance rate in these patients as calculated by the Cockroft-Gault formula being 33 mil/min.

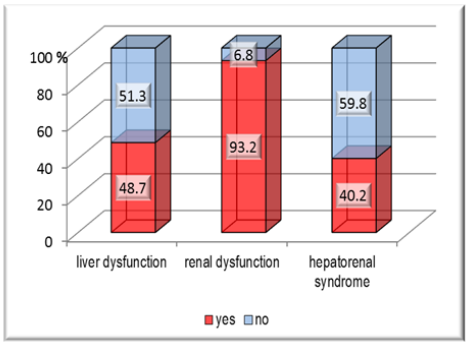

Fig. 2 Distribution of patients according to the presence of liver dysfunction, renal dysfunction and hepatorenal syndrome

Over 37% of the patients had a creatinine clearance below 30% (fig. 3). Using the Kruskal Wallis test (equivalent to ANOVA), we found a statistically significant relationship between creatinine clearance values and the severity of sepsis calculated by the APACHE score (*calculated* $p = 0.44$).

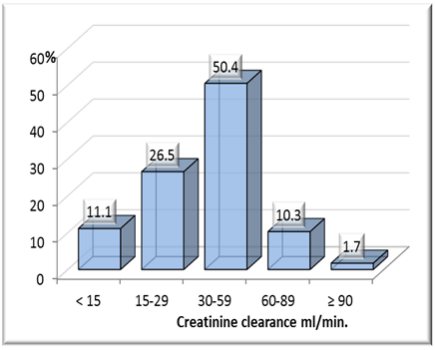

Fig. 3 Creatinine clearance in patients with sepsis-related hepatorenal dysfunction

The outcome of patients with hepatorenal dysfunction was favorable in most cases (69%), sometimes requiring referral to other departments (10.2%) and less than one-third (29%) died (fig. 4).

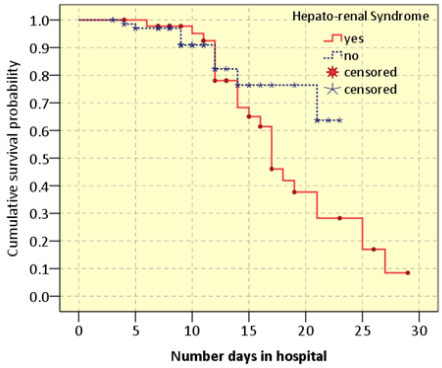

Fig. 4 Kaplan Meier survival curve in patients with hepatorenal syndrome and hepatorenal dysfunction in sepsis

DISCUSSION

Until 2006 definitions for HRS showed that it is a reversible functional kidney damage that occurs in patients with advanced liver cirrhosis or fulminant liver failure and is characterized by reduction in glomerular filtration rate and renal plasma flow in the absence of other causes of renal dysfunction (11). With the rise of knowledge, it was shown that the original definition lacked concision with respect to a large number of exclusion criteria, sepsis being one of them (12). Before revision, guidelines proposed, in 2007, the exclusion of bacterial infections for the HRS diagnosis (13).

The major mechanism of HRS consists of an extreme renal vasoconstriction caused by activation of Na retention and vasoconstrictor systems, resulting in a very low glomerular filtration rate (14). HRS type I occurs as a consequence of a severe reduction in the circulating volume caused on one hand by a major splanchnic vasodilatation and, on the other, by decreased cardiac output, this type of disorder being present in sepsis.

In our study, twenty-two percent of the patients with hepatorenal dysfunction received antibiotic therapy prior to admission, some of them having a change in their antibiotic regimen during hospital stay: most patients (73.9%) received a single antibiotic regimen, one third required only one change in their antibiotic regimen, while one patient received three antibiotic therapy schemes.

According to statistical analysis data, no statistically significant difference in the two functions defining the survival curve between the patients with hepatorenal dysfunction and hepatorenal syndrome was found.

CONCLUSIONS

Over one-third of the patients with sepsis-related hepatorenal dysfunction had a creatinine clearance below 30 ml/min, and we identified statistical correlations between serum creatinine and calculated APACHE II score.

No statistically significant differences were found between the survival curves Kaplan Meier of patients with hepatorenal syndrome and of those with sepsis-related hepatorenal dysfunction.

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NEWS

DISCOVERY OF CLOSTRUBIN, AN ANTIMICROBIAL AGENT ISOLATED FROM ANAEROBIC BACTERIA

Genome analysis of *Clostridium beijerinckii* revealed several cryptic gene clusters for secondary metabolite biosynthesis. When cultured under various conditions, *C. beijerinckii* produced a novel metabolite - a deep purple pigment, named clostrubin which was further isolated and its structure elucidated. The pentacyclic polyphenol folds in a manner that forms a perifused ring system (benzo[a]tetraphene), unusual for natural products. This is the first reported aromatic polyketide from an anaerobic bacterium. The study demonstrated that clostrubin is a potent antibiotic with activity against various pathogenic bacteria, such as MRSA, VRE, and mycobacteria (MICs of 0.12-0.97 μM) (Pidot S, Ishida K, Cyrulies M, Hertweck C. Discovery of Clostrubin, an Exceptional Polyphenolic Polyketide Antibiotic from a Strictly Anaerobic Bacterium. *Angew Chem Int Ed Engl*. 2014. doi: 10.1002/anie.201402632. [Epub ahead of print]).

Teodora Vremeră

CLINICAL AND EPIDEMIOLOGICAL ASSESSMENT OF THE INCIDENCE OF ARTERIAL HYPERTENSION IN A PERMANENT MEDICAL CENTER

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CLINICAL AND EPIDEMIOLOGICAL ASSESSMENT OF THE INCIDENCE OF ARTERIAL HYPERTENSION IN A PERMANENT MEDICAL CENTER (Abstract): The present study shows the results of monitoring, evaluation and prediction of health status in a patient group with hypertension (HTN) seeking care in a permanent medical center. *Materials and methods*: The study evaluated 144 patients registered at the Permanent Medical Center of the Plugari village, Iaşi County, who were diagnosed with HTN between 2011 and 2013. The study group included 80 male patients with an average age of 51.25 years with a diagnosis of essential hypertension (EHTN) stage I, II or III and 64 female patients (group II), with an average age of 48.4 years. **Results**: 44 patients (30.55 % of the total number of patients) were diagnosed with HTN stage I, of whom 27 were female (61.36 %) and 17 were male (38.64 %); 69 patients (47.93 %) had HTN stage II, of whom 21 were female (30.43 %) and 48 were male (69.57 %), and 31 patients (21.52 %) had HTN stage III, of whom 16 were female (51.61 %) and 15 were male (48.39 %). **Conclusions**: Patients at high risk for HTN and those with mild and moderate clinical forms were surveyed and were early treated. Economic and social benefits were achieved due to lower hospitalization costs, fewer days of temporary disability and reduced medical retirements. In our study groups, HTA had a higher prevalence, especially in men. **Keywords**: HYPERTENSION, CLINICAL EPIDEMIOLOGY, RISK FACTORS.

Clinical epidemiology of cardiovascular diseases (CVDs) has become increasingly important due to the fact that cardiovascular mortality ranks first nowadays in the world, especially in economically advanced countries, while there is also a trend of explosive growth in developing countries such as Romania (1, 2, 3).

Clinical research of CVDs puts the individual at the center of its development. Clinical epidemiology encompasses issues arising from the features of the larger population, from which these patients are drawn, aimed at identifying and managing risk factors that lead to disease and at finding effective means to prevent and control diseases.

The risk factors (RF) represent one of the major underlying elements of CVD

epidemiology, as they provide a rational and scientific basis for prevention and cure. Establishing the number of RF and their impact on CVD morbidity is a requirement of modern medicine, in which preventive measures gradually become just as important as treatment plans. The perfect case would be to succeed in replacing therapy with prevention (3,4,5).

The major aim of this study is to evaluate the clinical and epidemiological factors that play a role in the onset and progression of the disease, with a special interest in diagnostic stages and disease management in patients seeking care in a permanent medical center (6).

MATERIAL AND METHODS

The study included 144 HTN patients registered at the Permanent Medical Center of the Plugari village, Iaşi county between 2011 and 2013. They were divided into two groups: the first group included 80 male patients with an average age of 51.25 years with a diagnosis of essential hypertension (EHTN) stage I, II or III and 64 female patients (group II), with an average age of 48.4 years.

TABLE I

Age-related variability of blood pressure

Age groups (years)	Men		Women	
	SBP	DBP	SBP	DBP
20-29	126.1	75.8	114.5	77.0
30-39	125.5	80.8	117.2	70.8
40-49	133.8	84.2	124.6	82.3
50≥65	149.7	91.0	142.0	85.3
Group average	137.1	84.8	126.6	81.7

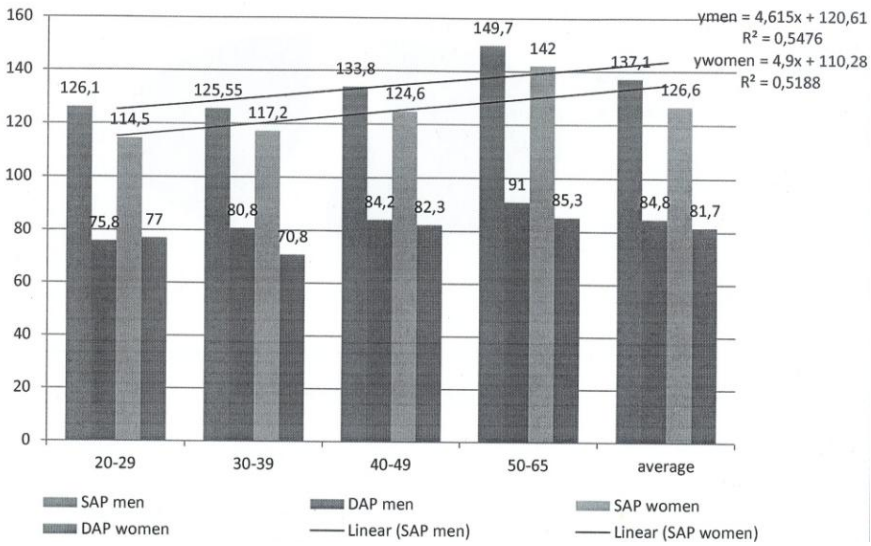

Fig. 1. Arterial pressure variability in relation with age group

Plugari village is divided into three rural areas with a plain landform, which are crossed by two rivers of local importance. Its population, mainly Orthodox, is undergoing a continuous demographic decrease, due both to migration to urban areas and especially to low birth rate and high mortality caused mainly by the aging trend in the community. Residents are employed in livestock farming and field cultivation (grain, fodder and vegetables).

The clinical and epidemiological investigation included history, objective clinical examination and recording blood pressure. Mathematical statistical evaluations were made using the *EpiInfo* 2000 program and X^2, DS, p, t-Student tests.

Mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) age groups based on decades showed a general tendency of progressive increase (tab. I, fig. 1).

The clinical and epidemiological sheet provided information on eating habits (lipid intake, daily consumption of cigarettes, smoking duration in years), lack of physical activity, physical effort, stress, behavioral types, dominant moods (aggressive, depressed, anxious, etc.)

RESULTS AND DISCUSSION

In population studies, the difference between "normal" and "pathological" is hard to demonstrate, due to variations depending on geographic area, lifestyle and socioeconomic characteristics. This is why our study centers upon the elderly (5).

As people grow old, they accumulate previously acquired und untreated diseases, so that at different ages an "inventory" of co morbidities at various stages can be found. Such associations express rather "the disease-related age" than "age-related diseases".

In the study group, the following conditions were diagnosed: hypertension (144: 3.84%), liver cirrhosis (152:4.06%); ulcer disease (129:3.44%); malignant diseases (109:2.91%); ischemic heart disease (102: 2.72%); chronic renal failure (14:0.37%); congenital anomalies of circulatory system (9:0.24%), chronic pulmonary heart disease and tuberculosis (5: 0.13%) (fig. 2).

There was a high ratio of CVDs, which are the leading cause of mortality, in all examinations of adults aged over 65 years made in the family doctor's practice. It is widely recognized that cardiovascular diseases have a chronic, disabling course and a poor prognosis, but early detection with screening tests is possible and required, especially in persons at high risk, leading to major medical and socioeconomic benefits.

HTN has an important place in the group of CVDs, as it affects about 10% of the general population, 25% of persons aged over 40 and 40% of those aged over 64. Although it is estimated that approximately 50% of HTN patients have mild forms of disease, the Framingham study has shown that even small increases in blood pressure are potentially dangerous, leading to shorter life expectancy (9, 10, 11).

In our study, group I included 80 male patients with an average age of 51.25 years with a diagnosis of essential hypertension stage I, II or III and group II included 64 female patients with an average age of 48.4 years (tab. II, fig. 3).

The most important mechanisms involved in the etiology and progression of HTN are as follows: increased sympathetic activity, altered transmembrane transport of ions (especially sodium and calcium ions), increased vascular reactivity due to the important role of endothelium in the regu-

lation of the vasomotor tone, stimulation of the renin-angiotensin-aldosterone system, dysfunction of local self-regulation mechanisms (prostaglandins and kinin-kallikrein system) metabolic factors, structural changes of blood vessels (8, 9, 10).

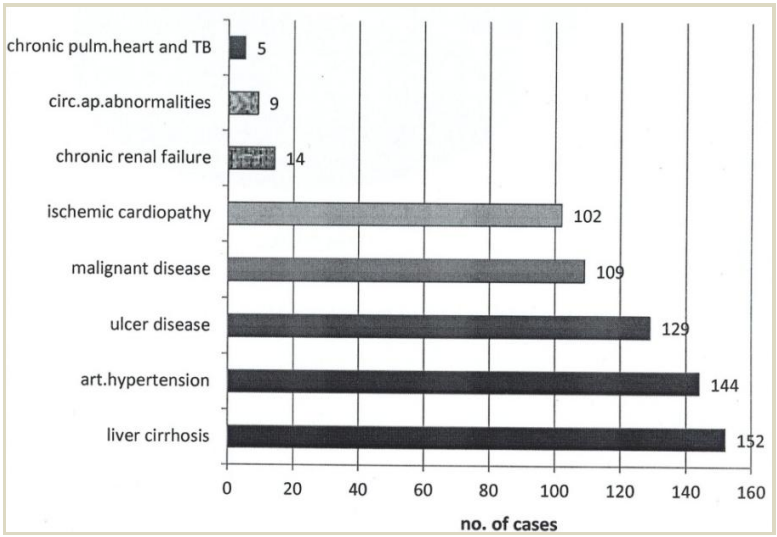

Fig. 2. Co morbidities registered in studied patients

TABLE II

Limits and the average age of the patients included in the study

Group	Gender	Number of patients	Age limits	Average age
I	M	80	42-64	51.25
II	F	64	40-65	48.4

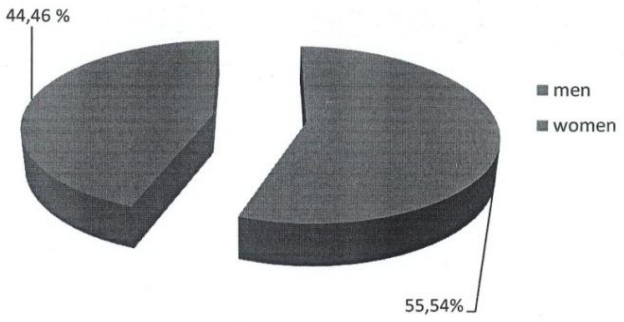

Fig. 3. Distribution of cases by sexes

Epidemiological surveys suggest that CVDs epidemic in Eastern Europe might be caused also by other risk factors, such as psychosocial stress, environmental pollu-

tion and nutritional deficiencies that might increase oxidative stress. This would be the result of an imbalance between different risk factors, as not only free radicals, but also protective antioxidants are produced, leading to an acceleration of atherogenesis. Recently, various infectious factors were also proposed as risk factors (10, 11).

Our study has rated the HTN risk factors, as follows: behavioral type (41.2%), a diet rich in fat and eggs (38.2%), stress (37.9%); sedentary lifestyle (25.8%), daily alcohol consumption (16.47%) excess salt intake (9.0%), passive smoking (4.7%) as compared to active smoking (1.94%). In women, endocrine disruption, especially during preclimacteric years, was also considered.

The analysis of the distribution of HTN staging showed that 44 patients (30.55%) were diagnosed with HTN stage I, of whom 27 were female (61.36 %) and 17 were male (38.64 %) ($p = \leq 0.05$); 69 patients (47.93%) had HTN stage II, of whom 21 were female (30.43 %) and 48 were male (69.57 %) ($p = <0.005$) and and 31 patients (21.52 %) had HTN stage III, of whom 16 were female (51.61 %) and 15 were male (48.39 %) ($p \geq 0.05$) (fig. 4).

The process of atherosclerosis caused stroke in some patients with HTN stage II (24 cases: 16.66%).

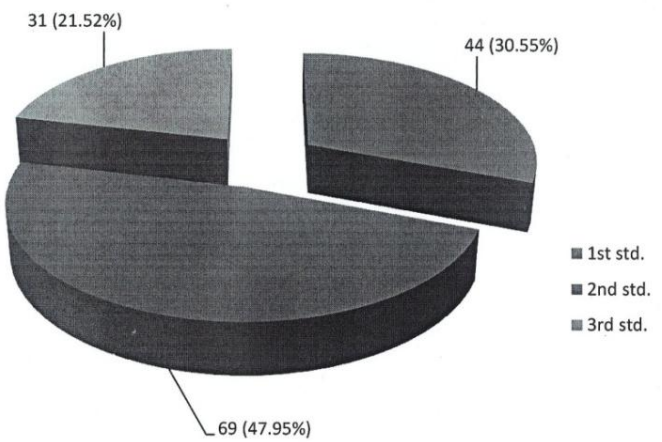

Fig. 4. Stadialisation of studied patients

Patients included in the study were on antihypertensive drug therapy (1, 2, 3 or more antihypertensive drugs) when blood pressure was measured, having a significant influence on results.

For instance, 42 patients (29.16%) have already received an antihypertensive drug; 59 patients (40.97%) two drugs and 43 patients (29.87%) over 3 antihypertensives.

The study of HTN values in groups based on age decades showed that 5 patients (3.47%) were aged less than 30 years, 51 patients (35.41%) in the 31-40 age group;

46 patients (31.94%) in the 41-50 age group; 34 patients (23.61%) in the 51-60 age group; 8 patients (5.55%) in the 61-70 age group (tab. III).

The study of the role of the genetic risk factor in HTN based on family history showed that 59 study group patients

(40.97%) had no parent with HTN; 62 patients (43.05%) had one parent with HTN and 23 patients (15.98%) had both parents with HTN.

TABLE III
Age distribution of HTN patients

Age groups (years)	No.	%
<30	5	3.47
31-40	51	35.41
41-50	46	31.94
51-60	34	23.61
61-70	8	5.55
Total	144	100.00

Analysis of the prevalence of some categories of risk factors

Smoking. In evaluating smoking patients, we have quantified both the number of cigarettes smoked per day and the smoking duration (in years). Women in the study group, 64 women (44.44%), were mainly non-smokers and of the total number of 80 male patients, only 43 (29.86%) were smokers.

Coffee intake. Coffee intake of one cup per day was seen in 58 patients (40.27%) and 2-3 cups per day in 20 patients (13.88%).

Sedentary lifestyle. It was evaluated based on questioning. The results show that 31 patients (21.52%), mainly women (24; 16.66%) are rather sedentary, as compared to 7 male patients (4.86%) ($p<0.005$). 113 patients (78.47%) were reported as active, but mainly men think of them as active 80 (55.55%), as compared to 33 women (22.92%) ($p\leq 0.05$).

Body mass index (BMI) was calculated by measuring patient's height and weight. By using the formula BMI = weight (kg) / height 2 (m^2), different levels of overweight and obesity, and also the relationship between obesity and HTN stages were assessed in the study group. In this respect, out of the patients with *normal weight* (BMI <25), 12 patients had HTN stage I (all male); 22 patients had HTN stage II (12 male and 10 female); 6 male patients had HTN stage III. Out of the *overweight patients* (BMI = 25-30), 11 patients had HTN stage I (7 male and 4 female), 25 patients had HTN stage II (16 male and 9 female) and 7 men had HTN stage III. In patients with *obesity class I* (BMI = 30-35), 14 patients had HTN stage I (equal male and female ratio), 28 patients had HTN stage II (12 male and 16 female) and 12 patients had HTN stage III. *Obesity class II* (BMI = 35-40) occurred mainly in patients with HTN stage II, while HTN stage I was diagnosed in only 1 woman and HTN stage III in 3 patients. *Obesity class III* (BMI > 40) was found only in 4 of the 144 HTN patients, of whom 3 patients with HTN stage II. Obesity class II and class III was more frequently associated with HTN stage II.

Abdominal perimeter, used as an index that quantifies the distribution of adipose tissue, and abdominal (android) obesity are widely recognized as the most common aspects associated with hypertension and vascular risk. In our study group, abdominal obesity was present in 37 men (25.69%) and 6 women (4.16%) ($p<0.005$). Median risk was correlated with values of

waist circumference ranging between 94 and 102 cm in men and of 80-88 cm in women and it was seen in 24 men (16.66%) and 19 women (13.19%). A high risk (> 102 cm in men and 88 cm in women) of 33 (23.61%) in men and of 22 (15.27%) in women ($p \leq 0.05$) was recorded.

The waist to hip ratio (abdominal-gluteal ratio) (WHR) was calculated by dividing the waist perimeter to the hip perimeter. Android obesity has WHR values higher than 0.8 in women and higher than 0.9 in men. Both men and women in the study group had a higher WHR than normal (43% of men and 29% of women) ($p \leq 0.05$). It was associated mainly with HTN stage II (26% of men and 19% of women) ($p \geq 0.05$).

Fasting blood glucose levels above 110 mg/dl were found in 49 patients (34.02%), of whom 23 (15.97%) were female and 26 (18.05%) were male ($p \geq 0.05$).

Cholesterol levels above 240 mg/dl were found in 83 patients (57.63%), of whom 36 (25.00%) were female and 47 (32.64%) were male ($p \leq 0.05$).

Clinical and epidemiological studies have also shown the presence of HTN risk factors, such as overwork, psychiatric treatments and negative emotions.

Studies of population groups similar to ours have shown risks over large temporal and spatial scales (3, 5, 8, 11).

CONCLUSIONS

Our clinical and epidemiological prospective study, conducted on a representative group of patients seeking care at the family medicine practice has made some contributions by showing the role of modifiable risk factors in the occurrence and outcome of cardiovascular diseases. Thus, it was shown that preventive measures taken to reduce cardiovascular risk under the supervision of the family doctor significantly decrease morbidity and mortality rates.

Clinical and epidemiological surveillance plays an important role in disease prevention, increases the use of ambulatory care and decreases the need for hospital care, with significant economic gains for the individual, the family and the community.

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NEWS

SLEEP APNEA AND EMERGENCE OF PNEUMONIA

Sleep apnea is a sleep disorder characterized by obstruction of upper airway which caused hypoxemia, hypercapnia and sleep fragmentation. This disorder is linked with cardiovascular diseases and cognitive impairment. Patients with sleep apnea have an increased risk of pulmonary aspiration and immune perturbations which could facilitate emergence of pneumonia. The link between sleep apnea and pneumonia was evaluated in a cohort study published in *Canadian Medical Association Journal*, conducted by Dr. Vincent Yi-Fong Su and colleagues. This study has a duration of 11 years and followed the emergence of pneumonia in a lot of the 34 100 patients (6816 with sleep apnea and 27 284 controls, with the same comorbidities). The results showed that the patients with sleep apnea had a higher risk to develop pneumonia than the control group (9.36% and 7.77% respectively). For the patients with severe sleep apnea, the risk of pneumonia was higher than the cases with milder severity. Also, the use of continuous positive airway pressure (CPAP) therapy has increased the emergence of pneumonia, the adjusted risk being 1.32 for the cases who received CPAP therapy. The authors showed that sleep apnea is a risk factor for pneumonia (Su VYF, Liu CJ, Wang HK et al. Sleep apnea and risk of pneumonia: a nationwide population-based study. *CMAJ*. 2014 DOI: 10.1503/cmaj.131547).

Cătălina Luncă

DENTAL ESTHETICS - INSTRUMENT FOR RECREATING A NEW FACIAL ESTHETIC TO THE ELDERLY PATIENT

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DENTAL ESTHETICS INSTRUMENT FOR RECREATING A NEW FACIAL AESTHETIC TO THE ELDERLY PATIENT (Abstract): **Aims:** The major objective of this paper consists in underlining the wide range of possibilities in assessing the elderly patient; it relies on clinical examination in order to exclusively improve the patient's physiognomic aspect. **Materials and methods:** In this context it has been organized a study which included 64 elderly patients aged between 60 and 90 years of age (median age 75), which presented themselves at the Clinical Service of Dental Semiology and Gerontostomatology between 2011-2012; they requested the restoration of the functions affected by odontal coronary lesions, periodontal disorders or edentations more or less expanded, malocclusions, malrelations of the mandible to the skull and unsuccessful or deteriorated dental or prosthetic treatments. **Results:** The restoration of dental arches has been performed relying on common sense and power of discernment, respecting both facial features and expression by redimensioning the lower part of the face, repositioning the mandible and using gnatoprosthetic devices. **Conclusions:** Solving the problems elderly patients confront themselves with and the success of the treatment has been possible only after a correct assessment of the involutive phenomena that influence the oral cavity, after understanding the local and general factors that predispose to oral disorders as well as the differences between various techniques and materials. **Keywords**: PHYSIOGNOMIC ASPECT, GNAT PROSTHETIC DEVICES, ORAL REHABILITATION, DENTAL ESTHETICS

Humans are social beings. Life within the society has helped them discover the physical, psychical and moral qualities of their fellow men, observe them and establish certain evaluation criteria about good and evil, what is pleasant and unpleasant in the way the people they gets in contact with present themselves. One of these criteria consists in the harmony of human features and their beauty.

Facial physiognomy which is a separate function of the stomatognat system, depends of the morphological configuration of the facial skeleton, the maxillaries, the dental arches and the soft parts which cover the osseous skeleton as well as of the position of the frontal teeth on the arch (1).

The facial aspect is not something static; it varies along with the action of the underlying muscles and the symmetry of their contractions. Under the influence of the orofacial muscles' contractions and of

the muscles that mobilize the mandible during mastication, speech and the other actions these muscles take part at, facial physiognomy experiences, with the passage of time, a continuous transformation (2). Consequently, facial physiognomy has a very well defined role of exteriorizing and communicating emotional conditions and so it represents one of the basic functions of the stomatognat system – the physiognomic function.

The harmony of facial features determined by the morphological configuration and the integrity of the skeleton and of the soft parts, is influenced by the aspect of the dental arches in the frontal region.

The pathological modifications, in such a visible area, can have essential implications on the individual's balance and harmony, leading to negative consequences on his integration in society and inter human relations (3).

Generalized aesthetic modifications are necessary when all anterior teeth are restored and occasionally, the first molars which become visible when the patient smiles. The abnormal morphologic or aesthetic facial balance requires the use of anatomic landmarks and in particular the interincisive papilla which can contribute to the initial assessment of the position of the median line (4).

The dental practitioner can intervene to a great extent in restoring the physiognomic aspect by improving the signs of ageing, providing a maximum support for the facial tissues at ease and while they perform their functions.

The major objective of this paper consists in underlining the wide range of possibilities in assessing the elderly patient and which relies on the clinical examination for exclusively improving the patient's physiognomic aspect.

MATERIAL AND METHODS

The present case study included 64 patients with the age between 60 and 90 years (mean age 75 years) assisted in the Clinical Service of Dental Semiology and Gerontostomatology between 2011-2012 and which required the restoration of the functions disturbed by odonto coronary dental lesions, periodontal affections or edentations, malocclusions, malrelations of the mandible to the skull and unsuccessful or deteriorated dental or prosthetic treatments.

The patients have been examined based on the results of an assessment chart correlating the reasons for which they presented themselves to the dentist with their desires; restoration of the altered physiognomic function requested only in the cases included in the treatments performed.

Facial examination was intended to evaluate the static and dynamic characteristics of the soft muscular-adipose tissues, the skeletal support, the dental arches as well as the relations these structures establish between them; it generates important data which will allow including the result of the treatment into a harmonious ensemble. The facial aspect is also determined by the amount and the quality of the muscular-adipose soft tissues and of the tegument, of their dynamics as well as of the bony structure consisting in the bones of the viscerocranium. The anatomic structures from the level of the face are analyzed as well as the aspect of the integuments, the facial grooves at ease and while smiling.

The relaxation of the muscle groups ensuring the mandible's mobility represents a reference position for facial examination.

The smile consists in the modification

of the physiognomy of the entire face. The wideness of the smile conditions the exposure degree of the dental arches and the area of esthetic interest which must be taken into consideration during the restoration treatment of the dental arches (5).

The smile represents the nonverbal type of communication most often associated to the exposure of dental arches. The position of the oral commissure while smiling determines the number of teeth visible during the action.

The evaluation of the dental arches considering only the esthetic aspect and the introduction of those specific types of treatment which focus only on the appearances can have severe consequences on the future evolution of the dento-maxillary system (6).

The intraoral examination focused on underlining the elements that contribute at altering the physiognomic function: teeth, periodontium, edentated crests, and occlusal relations. Special care has been given to bone reorganization, to the dimensions of the alveolar crests and to the relations between them and to the potentially prosthetic space.

Clinically, the purpose of oral rehabilitation in the case of elderly patients consists in making the dentures look as natural as possible. Consequently, both natural and artificial teeth must be in harmony with the patient's personality, age and sex (7).

RESULTS AND DISCUSSION

The distribution of the patients on age and gender groups indicated a higher frequency of the number of women (men 39-60.93%; women 25-39.07%) and of the age group 65-70 years (fig. 1).

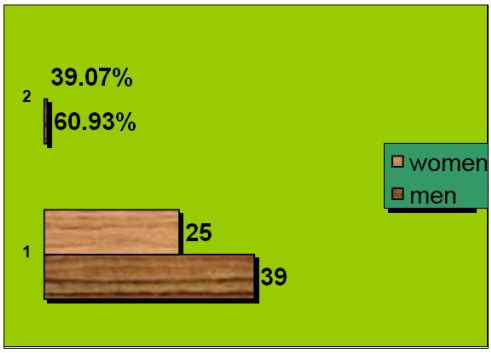


Fig.1. The gender distribution of patients

The distribution of the patients by age groups was as follows (tab. I).

TABLE I

Distribution by age groups

AGE GROUPS (years)	CASES	%
60-65	11	17.18
65-70	16	25.00
70-75	10	15.62
75-80	14	21.87
80-85	9	14.06
85-90	4	6.25

The syndromes recorded were: total edentation – 9 cases (14.06%); subtotal edentation – 10 cases (15.62%); partial edentation – 12 cases (18.75%); odontocoronary lesions – 13 cases (20.32%); parodontopathies – 11 cases (17.18%);

malocclusions – 9 cases (14.07%) (fig. 2).

The medical history indicated the motivation patients had for requesting dental treatment as well as their expectations; it was noticed that the number of women asking for the restoration of the physiognomic function was a lot higher than that of men.

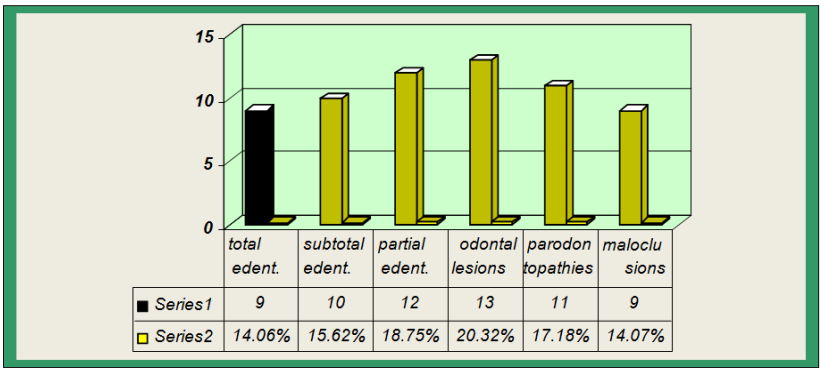


Fig. 2. Distribution of patients by syndromes

In the case of movable patients which benefited of old deteriorated devices or conservatory dental restorations, there have been a number of complaints related to functional unsatisfaction, the material used and the technological approach which lead to physiognomic disorders and needed to be optimized or redone.

The disorders of the stomatognat system with effects on the dental facial esthetics and the attempts of systematizing then according to the anatomical-clinical criteria are mentioned in the work of numerous authors. Thus, in a generic manner, we can say that the physiognomic aspect of the elderly is influenced by odontal and coronary lesions accompanied or not by substance losses, periodontal affections, integrity losses of the arch after edentation and dental migrations, occlusion disorders and by disturbance of the cranial-mandibular relation.

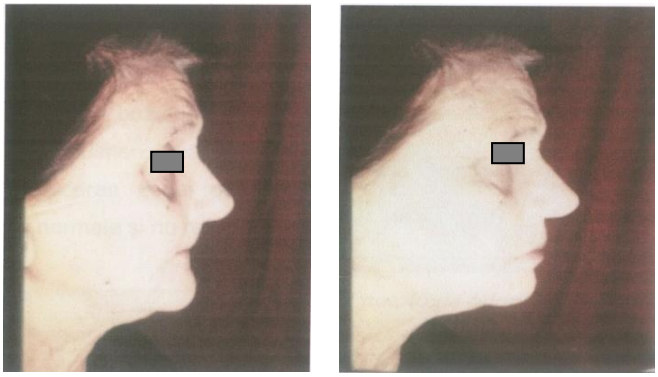
The local clinical examination often pointed out important clinical modifications of the stomatognat system and the prosthetic field, providing us useful information about how to chose and position the frontal teeth, the distribution of edentated spaces, the place of the occlusal plane, about how to model the vestibular crest or margins in removable prosthesis (8).

The low tonicity of the *orbicularis oris* is often noticed at the elderly patients and it is often a clue for the correct positioning of the frontal curvature (9) (fig. 3).

Fig. 3. Low tonicity level of the orbicularis

The facial examination indicates modifications of ageing associated to those due to total edentation at the level of the lips and

the oral cavity, commissures, facial grooves accompanied by asymmetries with under dimension of the lower part of the face and cranial-mandibular malpositions, due to either the loss of the support area or the incorrect prosthesis which does not ensure the normal relations and does not recreate the physiognomic aspect (10) (fig. 4).



a. b.

Fig. 4 a. Modifications characteristic to the ageing process; b. Defective prostheses which do not ensure the normal relations and do not recreate the physiognomic aspect

The intra-oral examination points out the existent oral pathology, in some situations with significant repercussions on the patient's physiognomy. The odontal and periodontal status at the elderly patient is quite heterogenous because of the involutive phenomena and the associated pathology; there are patients with a more or less complete dentition, with odontal coronary lesions, parodontopathies, and malocclusions, partial or total edentations (11).

The examination of the old dentures is also an important clue for the creation of the new dentures if we consider the technology they have been made with and the degree of functional satisfaction.

The restorative treatment focused on reestablishing the physiognomic aspect with composite resins correlating the color, the shape and the dimensions to those of the remaining teeth as an emergency solution and temporization.

In cases of dyschromia, enamel defects, dental usage, diastemata, dental malpossitions, development disorders of the lateral incisors, covering therapy with porcelain – metallic crowns which can combine aesthetic and mechanic qualities. The need of correct porcelain restorations at elderly patients confronts itself with two problems: first of all, each must serve at improving the resistance level and second, it must redo the initial physiognomy (12). The esthetic difficulties determined by the biological support can be corrected with the aid of porcelain bridges.

When talking about dental bridges, we have considered certain particularities in choosing the pillar teeth, the distribution of the potentially prosthetic space (modified because of the migration of the neighboring teeth at level 17 taking over space 16 and resorption of edentated crests) for intermediaries according to certain precise rules.

An excessive loss of the bone or of the frontal periodontal support on limited areas has lead to certain difficulties resulted from the exposure of the interdental spaces accompanied by the widening of the interdental papillary niches, the elongation of the clinical crown and sometimes visibility of the cemento-enamel junction with difficulties in ensuring the proper hygiene and which creates difficulties to the practitioner. The loss of parodontotic teeth with recurrent periapical cyst brings along advanced bony resorption associated to physiognomic disorders.

The use of acrylic appliances has been the temporary solution in these cases accompanied by the simulation of the loss of the gingival tissue, covering the exposed radicular surface, improving the esthetic aspect and preventing food impaction, which is a transitory situation until permanent prostheses.

In the case of the spaces too wide, it was proposed the intervention on the shape accompanied by motion towards the oral of the contact points and pronounced vestibular convexities and chromatic individualization using darker shades which make them narrower; in the case of narrower spaces, a shift of the contacts towards the incisal area and a more flattened morphology give the impression of normal proximal wideness. In case the teeth are much too short (frequent case due to dental usage) the esthetic aspect can be improved by flattening the vestibular side and cervix narrowing. Elongated teeth can be aesthetically corrected through the transformation of proximal contacts into contact surfaces, rounded shapes and false cervices.

When artificial teeth were put on, we have taken into consideration that the upper and lower incisors must not be in contact in the centric relation, without allowing the possibility for an anterior movement of at least 1 mm and an angle of at least 15° in protrusive occlusion which leads to the necessity of correlating overbite and over jet.

The restoration of dental arches has been performed with a lot of common sense and power of discernment, respecting both character and facial expression by redimensioning the lower part of the face, repositioning the mandible and using gnatoprosthetic appliances.

The aesthetic aspect has always been a matter of sensitivity regardless of the numerous norms and indications, hence, regardless of the patient's age; the purpose of restoration is that of obtaining aesthetically acceptable results (13). During the restoration process of the physiognomic function, the prosthetic variants took into consideration the type of edentation and the condition of the muco-osseous and dento-periodontal support.

It was noticed that the loss of teeth has affected the relative balance from the ageing process transforming ageing from a harmonious process into a disharmonious one. The multiple therapeutic possibilities for restoring the elder patient's physiognomy foreseen by modern dentistry are still limited by the patient's common sense and also by his financial possibilities (14).

Thus, it appears the problem of identifying a solution that is financially convenient for the patient and which ensures at the same time the complete functionality and esthetics specific to the restoration process.

Odontal and prosthetic treatments provide multiple possibilities for reestablishing the functions of the stomatognat system, of the physiognomic aspect which is influenced by both oral pathology and involutive process.

Nonetheless the elder patient can be helped in regaining his self confidence by reestablishing his physiognomic aspect, this must not be always more important than the other basic principles of dental practice. Regaining the physiognomic function of the stomatognat system by improving the gnatoprosthetic appliances represents an essential area of interest for the contemporary dentistry (15, 16) (fig. 5).

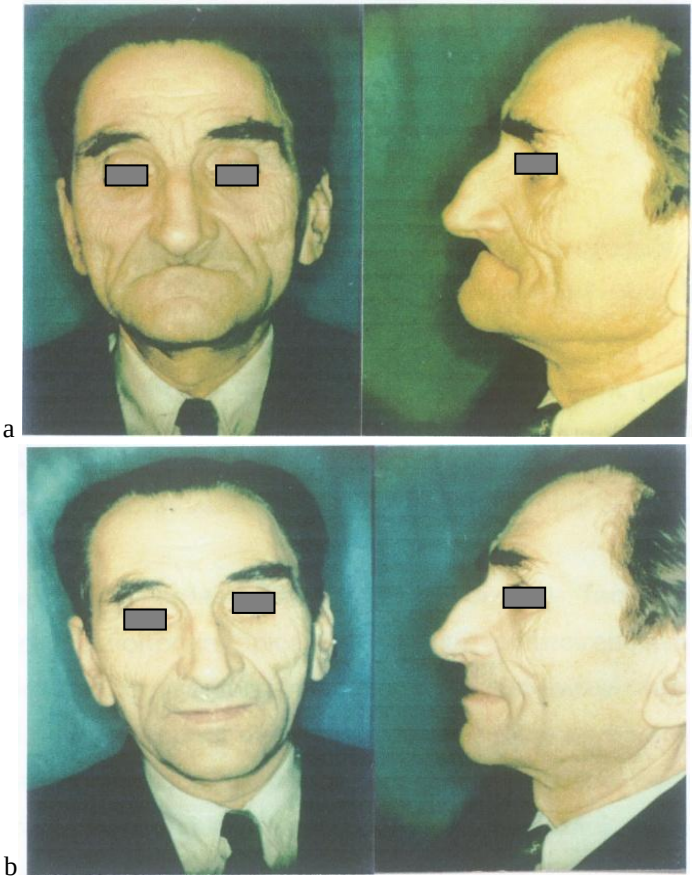


Fig. 5. a. Faces characteristic to a totally bimaxillary edentated person
b. Reestablishing the individualized physiognomic function

Considering all that was previously stated, as well as the high number of the elderly affected and which are every time more interested in their aspect, our main mission consisted in the possibility of improving their disharmonious ageing process by replacing their local infirmity and thus, improving the quality of their lives.

Esthetics at the elderly patient, in the contemporary dentistry, relies on an interdisciplinary collaboration based on a complex and complete medical analysis, subordinated at its turn to the concept of total oral rehabilitation. In this concept, we can reiterate what Goldstain stated, that "den-

tofacial esthetics represents the art of dentistry in its purest form".

CONCLUSIONS

Solving the problems of the elderly and the success of the treatments can only be reached after a correct assessment of the involutive phenomena which influence the oral environment, after understanding and knowing the local and general factors which predispose to oral disorders as well as the different techniques and materials.

The main objective that must guide our every therapeutic attempt must always consider the introduction of teeth into the general picture of facial structures, in a manner as efficient as possible so to not underline even more the infirmity that appeared due to an altered odontal, periodontal or arch integrity.

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OCCUPATIONAL HEALTH PROBLEMS AMONG DENTISTS IN MOLDAVIAN REGION OF ROMANIA

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OCCUPATIONAL HEALTH PROBLEMS AMONG DENTISTS IN MOLDAVIAN REGION OF ROMANIA (Abstract): **Aim**: The aim of this study was to evaluate the occupational health problems among dentists in the Moldavian Region of Romania**. Material and methods**: Questionnaire-based study was conducted on 152 dentists aged between 25-65 years practicing in 6 counties the Moldavian Region of Romania. The questionnaire included questions related to the dentists' occupational health pathology. Data were statistically analyzed using *SPSS 14.0* and chi-square test ($p<0.05$). **Results**: The majority of the dentists (91%) consider that they are exposed to an occupational risk and 41.8% of them experienced a percutaneus injury caused by sharp instruments in the last year.74.6% of the dentists are protected by vaccination against hepatitis B and 76.1% against influenza. 49.3% of the dentists reported eye injuries caused by solid particles (13.2%), blood splashes (14.7%) and/or chemicals (20,5%). Allergies associated to professional activity were reported by 76.1% of the dentists. **Conclusions**: The dental staff must be informed in order to recognize, control and prevent the potential occupational hazards in the workplace. **Keywords**: OCCUPATIONAL HEALTH, DENTISTS, PREVENTION

Occupational health of the medical staff in the dental office is of particular concern in order to reduce exposures to risk factors as percutaneous injures, exposures to airborne pathogens, contact with materials and chemicals, ergonomic hazards, noise, vibration, radiations, eye injuries, allergens, stress (1). Most of the occupational exposures can be prevented by applying the concept of Standard Precautions during the clinical activity in the dental office (2).

MATERIAL AND METHODS

In order to assess dentists'occupational health problems in Moldavian Region of Romania a questionnaire-based study was conducted involving 268 dentists, 72.7% women and 27.3% men, aged between 25 and 65 years, working at private dental offices in 6 counties of Moldova: Iasi, Botosani, Neamt, Suceava, Bacau and Vaslui.

The questionnaire included 21 questions on the occupational health problems reported by the dentists related to exposures by sharp instruments, musculoskeletal disorders, and ergonomic position during clinical activity, vision and hearing problems, exposure to vibration, allergies, exposure to stressful situations.

Data were processed using *SPSS 14.0* system and compared using Chi-square test

($p<0.05$). The approval of the Ethics Committee of the "Gr.T.Popa" University of Medicine and Pharmacy Iasi was obtained.

RESULTS

91% of the dentists consider that they are exposed to an occupational risk during the clinical activity. From those 22.2% mentioned the risk of contamination with pathogenic agents.

During the professional activity 41.8% of the subjects experienced a percutaneus injury caused by sharp instruments in the last year (fig.1).

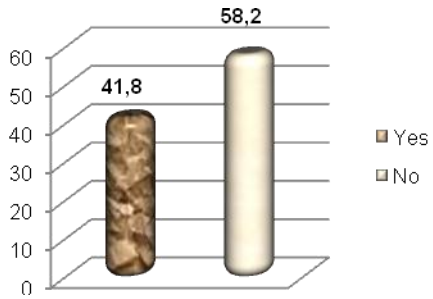

Fig. 1. Prevalence of dentists' percutaneus exposures during the last year

From the exposed dentists 10.4% reported the incident to a specialist in occupational health in order to follow an appropriate post-exposure protocol. 74.6% of the dentists are protected by vaccination against hepatitis B and 76.1% against influenza.

The number of hours spent daily by the dentists at work ranges between 1-4 hours (34.9%), 4-7 hours (51.2%) and over 7 hours (34.9%).

49.3% of the dentists reported eye injuries caused by solid particles (13.2%), blood splashes (14.7%) and/or chemicals (20.5%) (fig.2). Protection goggles are used by 72.4% of the respondents.

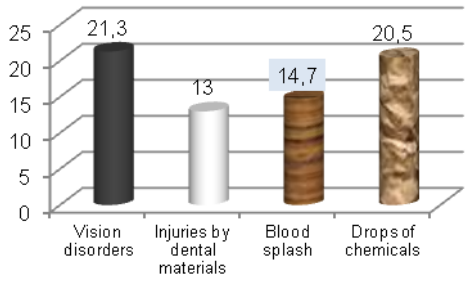

Fig. 2. Factors related to dentists' ocular health

The musculoskeletal disorders were reported by 70.1% of the subjects. The most frequent symptom was the pain located in the cervical spine (47.3%), lumbar spine (36.7%), dominant arm (43.1%) and hip (10.4%) (fig.3). Other mentioned musculoskeletal symptoms were: muscular spasm (28.3%), paresthesia (15.8%) and functional limitation (27.1%).

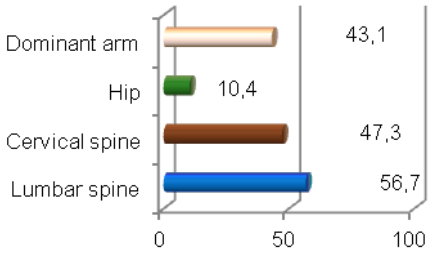

Fig.3. Dentists' occupational musculoskeletal pain localization.

Allergies associated to professional activity were reported by 76.1% of the dentists. The most incriminated allergens were the latex protective gloves (13.4%) and the impression materials (8.8%) (fig. 4).

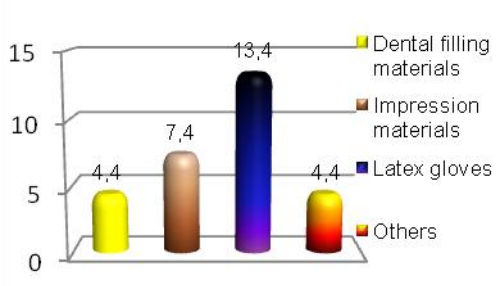

Fig.4. Allergens associated to dentists' occupational pathology

The mental illnesses associated to the professional activity reported by the subjects were: depression (5.9%), alimentation problems (5.8%) and anxiety (1.4%).

The self- reported professional stress is rated by most dentists in the 5 (22.4%) and 7 (20.9%) level on a scale between 1 and 10.

29.9% of the dentists are smokers but this habit is not considered as a work-related one.

DISCUSSION

The modern dentistry has advanced with giant steps but it is still associated with important occupational risks for the dental team. Extensive literature reviews reveal the hazards represented by percutaneous exposures, eye injuries, noise, vibration, ergonomic factors, contact with allergens and toxic chemicals, stress.

Dental personnel can be exposed to pathogenic microorganisms including staphylococci, streptococci, cytomegalovirus, HBV, HCV, herpes simplex virus, HIV, *Mycobacterium tuberculosis* and other viruses and bacteria from the oral cavity and respiratory tract. These pathogens can be transmitted through direct contact with blood or other contaminated fluids, indirect contact with contaminated instruments, equipment or environmental surfaces, contact of nasal, conjunctive or oral mucosa with droplets and inhalation of airborne microorganisms (3).

In our study most dentists consider that they are exposed to occupational risk factors from which the most important is represented by exposure to blood through incidents caused by needle stick and sharps instruments. About half of all dentists reported such an exposure during the last year. The prevalence of sharps injuries reported in literature reviews is about 50% in United Kingdom and Thailand, 28% in Queensland Australia, 138% in Durban South Africa (4, 5, 6, 7).

From the exposed dentists only ten percent reported the incident to a specialist in occupational health demonstrating a poor knowledge regarding the post-exposure protocol which must be followed in order to decrease the risk of diseases transmission. Prevention of percutaneous exposures must be achieved by dental team education, immunization, and use of protective equipment and safety devices. Even although the dentists are concerned about the possible transmission of blood borne pathogens like hepatitis B virus, hepatitis C virus or HIV only 74.6% of them are vaccinated against hepatitis B.

The eye problems associated to the dentists' professional activity refer to injures caused by fragments of dental filling materials, dental tissues, calculus or metal, blood splashes or chemical drops. The prevalence of the ocular problems reported in the literature range between 10% and 73% (8, 9). The eye injuries have a high risk of infection and can affect the professional performance of the dentist. Even though dentists' eye protection is highly recommended only 72.4% of the respondents in our study use goggles

or face shields.

Another important risk factor is represented by the contact with allergens like latex gloves, dental filling materials, impression materials, chemicals, anesthetic gasses, disinfectants. In our study three quarters of the respondents reported allergic reactions, especially in the form of dermatitis caused by latex gloves.

Musculoskeletal disorders represent one of the most serious occupational pathology hazards. Three quarters of the dentists in our study declare to suffer symptoms, especially pain, located in the spine and dominant arm. Our results on lumbar pain are comparable with those, reported by other authors: 50.0% Al Wazzan, 55.2% Ratzon, 54.3% Legat, 52.2% Barlean, 54.2% Gibels (10, 11, 12, 13, 14).

Dentistry is considered one of the more stressful medical specialties due to specific factors like physical strain, patients' pain and anxiety, treatment compliance, competition, isolation, monotony, lack of appreciation, economic pressures or seeking ideal results. Our results illustrate a low prevalence of the mental disorders compared with that reported by other researchers. In Lituania 95.7% of the dentists suffered psychological problems associated to professional stress, sleep disorders -78.8%, tension- 80.5% and anxiety- 96.4% (15). In Great Britain 60% per cent of dentists reported being nervy, tense or depressed, 58.3% reported headache, 60% reported difficulty in sleeping and 48.2% reported feeling tired for no apparent reason related to work stress (16). In order to decrease those harmful effects dentists must identify the stressors, apply appropriate stress management strategies and to adopt healthy habits and attitudes**.**

CONCLUSIONS

Exposure to numerous biological, chemical, environmental, physical, and psychological risk factors is highly associated with the clinical activity in the dental office. The dental staff must be informed and educated in order to recognize, control and prevent the potential occupational hazards in the workplace.

Acknowledgements: This research was supported by the European Project „ Ergonomics, prevention and performant management in dental medicine by adopting European standards "Contract: POSDRU/ 81/3.2/S/55651, 2010- 2013 –Project funded by European Social Fond "Invest in People''

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NEWS

CELL THERAPY IN BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW

Bisphosphonate-related osteonecrosis of the jaw is a clinical condition found in patients who have received intravenous or oral bisphosphonate therapy for various diseases related to bone. This report describes an innovative aproach for the treatment of this condition using autologous bone marrow stem cells, platelet-rich plasma, beta tricalcium phosphate, and demineralized bone matrix. The patient was a 71-year-old woman with history of multiple myeloma treated with intravenous zoledronic acid during 4 years. After a tooth extraction, the patient presented with a painful bisphosphonate-related osteonecrosis lesion with no healing wound and cortical bone exposure. The patient was surgically managed with a standardized protocol of autologous stem cell therapy combining bone marrow harvest, cell concentration procedures, and intraoral surgery. CT scan performed 6 months later showed improvement of bone and concentric ossification. The authors concluded that cellular therapy might be considered a new strategy to heal bisphosphonate-related osteonecrosis of the jaw lesions [Gonzálvez-García M, Rodríguez-Lozano FJ, Villanueva V, Segarra-Fenoll D, Rodríguez-González MA, Oñate-Sánchez R, Blanquer M,Moraleda JM. Cell therapy in bisphosphonate-related osteonecrosis of the jaw. *J Craniofac Surg* 2013; 24(3): 226-228].

Gianina Iovan

THE THIRD AGE – A CURRENT ISSUE FOR THE FAMILY PHYSICIAN

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THE THIRD AGE–A CURRENT ISSUE FOR THE FAMILY PHYSICIAN (Abstract): Since our birth, we are programmed to experience predetermined stages of life whose passage is inevitable as the passage of time (childhood, youth, adulthood, old age). Although each of these stages of development differs in its specific tasks, the old age seems to be the most burdensome, resulting in significant changes: physical and intellectual decline, major changes in the importance of the professional, marital, parental and social role. One of the main indicators of the quality of life, the average life expectancy increased significantly in the last 3-4 decades and the mankind gained decades of active life in a relatively short period of time. Therefore, one of the goals of medicine has become the prolongation of life, the pursuit of old age, the wish to remain healthy, to be able of physical and intellectual performance and useful in the private and social life. The old age is conditioned by a complex combination of factors: the individual genetic predisposition, the lifestyle of society and the environment, all of which affect the opinions regarding health and subsequently the attitude towards health. The family physician has the social obligation to research and promote health, to fight diseases. The family physician achieves this by researching the aging field and by trying to find solutions to improve the quality of life in the elderly, which is a prerequisite of maintaining the integration of the elderly into family life and society, of preserving the biological and social independence of aged persons. The maintenance of an active private and social life is a method of fighting premature aging associated with infirmity and disease. This action cannot be successful without the active involvement of the elderly and without their acceptance in the society. Aging is a biological process that affects the entire body, following the period of development and it is considered the final stage of biomorphosis. **Keywords**: AGING-ASSOCIATED DISEASES, AGING MECHANISMS, VULNERABILITY, DEPENDENCY, QUALITY OF LIFE.

Many centuries ago, it was considered that medicine should accomplish the following duties: to preserve health, to cure diseases and to prolong life. This latter objective was achieved not only through the use of biomedical sciences, but also due to better socioeconomic conditions.

It is believed that the successful eradication of mortality from cardiovascular disease and cancer would lead to an increased life expectancy (1, 2). Even so, the maximum limits of human survival phenomenon would be not influenced. To achieve this goal it would be necessary to

"break" certain specific genetic barriers.

The efforts to extend the natural limits of life cover the following targets: the deciphering the human genetic code, the discovery of the mechanism of transmission of hereditary characteristics, genetic engineering and so on. This could reveal a deeper understanding of the phenomenon of life. The scientific progress in this field brings an exaggerated enthusiasm, manifested through extremely optimistic assumptions that are often unjustified. The exact mechanism by which the human body is aging is not understood (2). Some theories suggest the damage caused by ultraviolet radiation and the body wear induced by end products of metabolism. Other theories claim that aging is a predetermined genetically controlled process.

Gerontology defines aging as a cumulative effect of body interactions with various factors throughout life (3, 6). These factors are represented by environment, cultural influences, dietary behaviors, physical exercise, leisure, diseases, etc.

It has been shown that aging is not caused by the action of a hormone or of a "suicide gene", which at some point may become active. Aging is rather a very complex phenomenon. Life span is "rigid", as it varies within small limits in the same species. Human life expectancy is substantially reduced compared to the biological, genetically determined life expectancy which is characteristic for the human species, but not due to the evolution of the physiological aging process carried to the last vital human resources, but due to aging-associated diseases, chronic and degenerative pathology. The prevention and treatment of cardiovascular diseases and cancer are still not possible without the progress in understanding the mechanisms of aging and the correlations between aging and associated pathology (1,2).

The contribution itself of elderly persons is not of less importance. The main issue is to "be able to grow old" and "to learn to grow old", because nowadays the term geragogy is used to denominate an appropriate pedagogy of this stage of life. Aging means first of all the edification of a way of thinking. Nowadays aging is seen as a universal biological phenomenon, which is basically a natural physiological process and not a morbid one. The idea of old age as a disease belongs to the history of concepts about aging (4, 9). This should be seen not as a drama, as a catastrophe, but as the last stage of life with its advantages and disadvantages, as any other stage of life. The changes seen in the aging process affect the clinical picture of some diseases and represent sometimes an impediment to gathering data, in making a diagnosis, a prognosis and a treatment schema. Aging is the progressive and irreversible alteration of body functions which cannot be eliminated and causes a greater vulnerability of the individual. The term “vulnerability” or “fragility” was introduced in the medical language in the last decade of the last century (5, 14).

With the passage of time, biological aging occurs and changes in all organs are seen. The damage to a body system brings damage to another and consequently vulnerability. An autonomous person may become deeply dependent and may evolve to a syndrome of renunciation leading to a fatal end.

Aging should not be necessarily identified with diseases, even if during old age illness may be more common. Aging should also not be identified with decline, because even if some functions and skills may regress, other can be maintained until old age, and some can even improve. Phys-

ical abilities decrease progressively. The adaptability to changes diminishes, but it can be enhanced through self-education. However, intellectual functions are not affected by aging and their results are similar to those in young people and adults. The elderly maintain their ability to learn (2).Therefore human beings can change their life expectancy, by increasing it or, unfortunately, often by shortening it.

SIGNIFICANCE OF GERIATRICS AND GERONTOLOGY

Geriatrics is a branch of medical science dealing with prevention and treatment of diseases in the elderly (6, 7) and gerontology studies the human aging process from the point of view of physiological doctrines and, later, in the context of biology and medicine (6, 8).

The long-term observations provided information about the social life of elderly, about the old age as the age of wisdom, as the age of outcomes, which brings peace with others and a gradual detachment from the hot matters of the world.

Gerontology includes four aspects, as follows: the social, economic, psychological, physiological and pathological aspect. They affect the aging process and the research studies each of these aspects in order to prolong the life and to revive the years (8).

Ageing has always fascinated mankind. The increasing average age of the population is the result of socio-economic development: the improvement of nutrition, housing, education and hygiene of the population, especially in developed countries (9). In developing countries, there is a high mortality, resulting in a lower average age.

It is generally admitted that a mature person is likely to reach an old age, according to his genetic heritage. A distinction is made between the primary aging, which is determined by genes, and secondary aging, which depends on environmental factors (10).

Most of the elderly do not realize that they will have the opportunity to practice the activities that they have dreamed of during the retirement period, a period which in some cases has a negative impact on the life of the aged persons. The mood of the elderly is an important aspect of the quality of life. The aged persons with poor health are more vulnerable, more dependent, and show a much stronger need to stay with their families. Although family support is considered the most important and most appropriate "form of care" for the elderly, for independent reasons, the family is put in the situation to dedicate less and less time and to provide insufficient support to grandparents / parents. In such cases, the elderly become in permanent or temporary need of a personal assistant. However, it is not uncommon for a family with different generations to encounter difficulties in adapting to the new roles imposed by growing old (10, 11).

Cessation of employment, the decreased freedom of movement, diseases and even death affecting people in the circle of friends lead to a major decrease in the social status of the aged persons. This brings a sense of loneliness and isolation.

There are cases when the family helps older people to achieve a better life and to maintain their autonomy for as long as possible. Disturbed families condemn elderly persons to dependence for social reasons, but also by use of violence and abuse, which result in disabling medical conditions. On the other hand, the lack of any family is a precipitating factor of vulnerability and dependence (12).

The aged patients often experience con-

fusing and difficult situations, resulting in a conflict between the desire to be useful to the family and the need for support, care and attention from the loved ones. Some old persons feel like they are a burden to the family and prefer to live alone or in nursing homes and to suffer all the consequences that these choices bring in their lives. Factors leading to loss of autonomy and hence to a higher level of dependence are mainly medical and social. Medical factors are represented by diseases of various body systems that lead to a certain degree of disability. Social factors include the lack of housing and proper financial assistance. Housing is an important indicator of the comfort of human life and it is of major importance for the elderly, who are vulnerable and have special needs.

The elderly experience many difficulties and aging itself is not a disease, but the clinician takes into account the physiological performance of the patient by correlating them with age, in order to develop post-disease rehabilitation programs (13,14). Optimal medical and social care of the elderly brings high costs for families and communities.

Mood is an important indicator of the quality of life. Sadness is common in elderly and it is sometimes associated with depression. Loneliness is sad reality of most aged people and may be associated with vulnerability (15, 16).

MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES OF AGING

Old age is a period of profound deterioration and involution of the main functions of the human body. The normal signs of aging are generally the same for all people, although they do not necessarily occur at a certain age. Each person has its own pace of aging, dependent on genetic factors and lifestyle.

One of the most significant indicators of the quality of life in the elderly is the health status.

A poor health status leads to a higher risk of autonomy loss. Some persons can grow very old and maintain a high degree of autonomy just because they have succeeded to keep healthy. But health status is influenced by many factors, so that the individual, the family and the society have to ensure optimum conditions since young age (14, 15).

Aging is a continuous gradual process that begins at birth and continues throughout all stages of life, until old age, when most body functions decline. An autonomous person can become deeply dependent and evolve to a fatal outcome. In order to describe this chain of events, the domino theory was used, a metaphor which describes the propagation of diseases as a succession of falling domino pieces (14, 16).

The aged person represents a "deposit of morbidity" which was accumulated throughout life. Therefore the elderly need diversified and specialized care that combines medical and social aspects. Over the years, the physical decline is felt more intensely. The general process of aging is unique to each person and diseases occur depending on individual sensitivity. Among the most common aging-associated diseases are: cardiovascular diseases (hypertension, coronary heart disease, and stroke), diseases of the musculoskeletal system (arthritis, osteoporosis), sensorial changes (hearing and visual disabilities), and dementias.

Changes occur gradually in all parts and organs of the body (8). The biological aging is caused by changes in various organs and systems, but, in the elderly, some events occurring in life accelerates the

aging. Therefore the aging can often be accompanied by an increased vulnerability to psychological disorders, which is called psychological aging (15). According to E. Erikson's theory, (cited by Bocsa), the last period of life is a continuing effort to preserve the integrity, but each aged person experiences differently life events, according to his previous life experience, economic status and social interactions (11).

Physical decline increases in the course of time, the overall appearance of the skin changes, the percentage of body water decreases, muscle atrophies occur, the physical capacity decreases, the number of neurons in the central nervous system decreases, various dysmetabolic conditions exacerbate (diabetes, liver diseases, kidney diseases or pancreatic diseases), hypoxia may occur due to the cardiovascular pathology or to the use of toxic substances (alcohol, drugs), leading to the insufficient oxygenation of the brain. The aging process varies with each person, depending on the individual sensitivity to certain diseases.

The psychological aging, caused by disturbing events, represents an increased vulnerability to psychological mood shifts (15, 16).

Mental decline manifests itself at a cognitive, affective and behavioral level. Cognitive changes are among the most important medical conditions of aging, with a great impact on the quality of life and entourage. The elderly experience quite often negative feelings. Sadness, anxiety, concerns are considered normal in any effort to adapt. Elderly limit their activities because of physical impairment, which reduces physical capacity and mobility, their field of interest and hobbies narrows, focusing mainly on certain areas, which are improved and deepened. Changes are either rejected or accepted with high anxiety (17,18).

Retirement often coincides with the end of working life. People who work in areas that allow activity after official retirement are the exception to the rule. The abandonment of the ritual of work is sometimes seen as a positive experience, as an opportunity for fulfillment of desires for which there was never enough time, as well deserved moment of rest after long years of work. There may be also feelings of uselessness, the need to rethink the meaning of one's own existence and to discovery of new sources of satisfaction. A negative factor is the decrease of income, especially in the present socioeconomic situation, when retired people represent a socially disadvantaged class. The decreased freedom of movement, diseases and even death affecting people in the circle of friends, the sense of loneliness and isolation are also a negative aspect of the old age.

The *physiological aging* affects differently the organs and the aging process itself may have develop differently, depending on the correlation between biological and chronological age (10). The physiological aging can be gradually seen in the osteomuscular system (decreased muscle strength and lower physical capacity, slower and shorter walking, reduced hand movements, stooped position and height reduction, the reduction of the articular cartilages, bone rarefaction and reduced bone mass, limitation of joint movements), in the cardio respiratory system (reduced vital capacity; lower thoracic movements, slow heart rate, increased systolic blood pressure and decreased diastolic blood pressure, poor response to increased heart activity), in the gastrointestinal and genitourinary system (delayed bowel function, decreased secretion of digestive enzymes, gastric hypoacidity, slow filling of the stomach; reduced filtering capacity of the kidneys, increased

prostate volume), in the nervous system (brain cell atrophy, slowing reflexes).

The *psycho-physiological aging* refers to physiological aging associated with pathological processes, as a result of the action upon the body of external and internal environmental factors that change the normal aging process in a biostructural context, such as: internal factors (heredity, wear, the mental life), external factors (ecological, socio professional, socio cultural, relational factors).

The *sensory senescence* affects the sight (loss of elasticity of the lens, significantly reducing visual field, that leads to deterioration of image quality, of visual habituation and to decreased lacrimation), the hearing (decreased auditory sensitivity, decreased phonemic awareness), the touch (decreased tactile sensitivity), the smell and the taste (are less affected, but the nose becomes prominent; dry mouth, reduced saliva and reducing taste buds can be seen), the attention (voluntary attention and focus decrease mostly, leading to decreased memnotic and cognitive capacity), the memory (low short term memory and preservation of the long-term memory, leading to difficulties in assessing chronological events and to impaired temporal-spatial orientation), the thinking (decrease of ideation, spontaneity, flexibility of thought, favoring rigidity and inflexibility, increased frequency of stereotypes, difficulty in rapidly adapting, a greater conservation or development of synthesis, generalization and schematization capacities, which leads to a greater reflexivity, circumspection, temperance, contemplation), the language (decreased verbal flow, slow rhythm of speech and vocabulary achievement, weakening voice -phonasthenia), the affectivity (is predominantly negative and manifests itself as depression, apathy, reduced emotional resonance), the emotional behavior (irritability, emotional lability), the instinctual behavior (dominated by the changes in sexuality, with reduced sexual activity, reduced endocrine support, but not accompanied by a proportional decrease in eroticism and libidinal tension) (9).

All these changes require network of social support as extended as possible, made up of family members and of former colleagues, friends and acquaintances, as a result of recognition of self-worth.

Besides biological and psychological aging there is a social aging because unfortunately old age is associated by most people with disease, weakness, conservatism, lack of judgment, irritability and dependence on others. The elderly are often treated with contempt and the fact that they valuable to the society is ignored (17).

Ageing beautifully is conditioned by a good biological and psychological state and is the result of an accumulation of genetic factors, of character traits, of autobiographical data. An active attitude towards life, adopting and maintaining a healthy lifestyle, with conscious involvement in well organized activities of interest are essential for a happy old age.

SOME RECOMMENDATIONS FOR AGING BEAUTIFULLY

The secret of healthy aging and physical independence is the regular physical activity. The body remains strong through physical exercise, which protects and improves the emotional and cognitive state. In aged persons suffering from a chronic disease, physical activity can reduce the need for medication used to control or treat the disease in question.

Mental and emotional vitality are closely related to the physical one. The thought has a powerful effect on the body and the

physical well-being affects the emotion and thought. Social relations play also a beneficial role. The emotional and cognitive health status can improve through regular physical activity, which produces certain substances that generate well-being. On the other hand, inactivity can exacerbate depression, anxiety and stress.

Emotional health can be protected or improved by maintaining the interaction with friends, family and community (1, 9, 15). Memory and intelligence can improve or maintain by means of daily intellectual stimulation, but also using repetition and routine. It is necessary to avoid stress, that has possible adverse effects on mental state and positive thinking can prolong life. A proper diet maintains the good functioning of the body. In older ages, the balance of the body can deteriorate and lead to diseases that make the elderly even more fragile.

It is necessary for every individual to be actively involved in decision making on his own health, to learn more about themselves and to properly manage the personal medical information, that includes treatment schemes, medical care, physical exams, screening tests and recommended vaccinations.

CONCLUSIONS

Harmonious aging depends strongly on the opinion and also on the relationship between elderly and younger people.

The most important thing to do for the elderly, as a reward for what they have succeeded to pass on to the younger generations is to show understanding, patience and civility, to overlook their impulsive behavior and to give them confidence and the feeling of usefulness.

Their experience and wisdom can always help and it represents a resource to be exploited.

Healthy individuals with a healthy lifestyle can delay or prevent many aging-related diseases.

In people with chronic diseases or disabilities, the rehabilitation exercises may have a positive effect on physical and mental health. Regardless of the moment when a healthy behavior was adopted, this behavior improves the quality of life and may increase life expectancy.

Ageing beautifully, in a good biological and psychological state, may be the result of an accumulation of genetic factors, of character traits and of autobiographical data, but an active attitude towards life, adopting and maintaining a healthy lifestyle, with conscious involvement in well organized activities of interest at an individual, family and community level are also important.

Understanding the needs of the elderly, both healthy and sick, is one of the most important ethical issues. It concerns the society, the family and especially the family physician.

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NEWS

EFFECT OF PERIODONTAL DISEASE ON DIABETS

Periodontal diseases, including the reversible form gingivitis, affect up to 90% of the world's population. Periodontitis is reported to affect half of adults or more with the severe form periodontitis affecting around 10% (range: 5–20%) of adults and moderate periodontitis affecting around one-third. In aged populations the prevalence of periodontitis is even higher: It is reported that 70–90% of individuals aged 60–74 years suffer from periodontitis. It is estimated that 346 million people have diabetes worldwide with the prediction of 439 million by the year 2030, representing an increase of 54% in 20 years to encompass almost one tenth of adults 20 years and older. Currently, there is increasing interest in links between periodontal disease and non-oral, systemic, inflammatory-related diseases and conditions, such as atherosclerotic cerebro-cardiovascular conditions and events. The biologic plausibility supporting such connection is based on the fact that inflammatory periodontal disease due to reaction to pathogenic biofilm stimulates a chronic systemic inflammation and thus contributes to the cumulative inflammatory burden in the host. Inflammation precedes diabetes onset and is linked to insulin resistance and development of diabetes as well as its complications. Since effective therapy and management of periodontal disease are well established, it is important to know for future prevention and control of diabetes whether periodontitis indeed plays a role in the development and control of diabetes and its potentially fatal complications.If such a causal relation indeed exists, a new paradigm in dental and medical standard of care for screening, prevention and management of diabetes could be developed in the future. Close collaboration among each patient's health care professionals would be warranted, especially among medical and dental care providers (Effect of periodontal disease on diabetes: systematic review of epidemiologic observational evidence. *Periodontol 2013;84(4 Suppl.):S135-S152).*

Doina Butcovan

CURRENT ISSUES IN GASTRIC CANCER EPIDEMIOLOGY

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CURRENT ISSUES IN GASTRIC CANCER EPIDEMIOLOGY (Abstract): Gastric cancer, one of the most common malignant tumors of digestive tract continues to be a major health problem by frequency, aggressiveness and low rate of cure in symptomatic stage. Although its incidence is decreasing (especially in the West), globally the gastric cancer is ranked fourth in incidence among cancers at various sites. Despite these developments, the gastric cancer mortality, overall declining globally, is high, especially in the West where even if diagnosed fewer cases of gastric cancer, TNM stages are advanced and have a poor prognosis. In contrast, in Japan, where the incidence is still high, the percentage of cases diagnosed at the stage of "early gastric cancer" has greatly increased, thus improving prognosis. Gastric neoplasia affects more men, age range 50-70 years, disadvantaged social classes and black race. In Romania the gastric cancer incidence is increasing over recent years, presenting variations across the country being more common in men compared with women, reaching a peak of incidence around age 60. Gastric cancer mortality in the world places Romania among the countries with average mortality. Gastric cancer prognosis remains extremely reserved, in close correlation with tumor stage at diagnosis, surgical treatment being the only possibility to provide improved survival, especially in the early stages. Improvement of survival rate in recent years is due to increased gastric resectability result of an earlier diagnosis, a more complex treatment and a closer monitoring of the population at risk. **Keywords**: GASTRIC CANCER, INCIDENCE, MORTALITY

Gastric cancer still remains one of the most common malignant tumors of digestive tract responsible for a mortality which remains high (1). Adeno-carcinoma accounts for over 95% of malignant gastric tumors tract, that is why the generic name of gastric cancer refers, in most cases, to adenocarcinoma, other gastric tumors (lymphoma, stromal tumors, sarcoma, carcinoid) being very rare (2, 3). Both main locations of gastric adenocarcinoma are proximal (cardial) and distal (noncardial).

Despite the decline of distal gastric cancer, the incidence of proximal tumors increased continuously since 1970 (4). These types of gastric tumors are prevalent in populations of different geographical regions, different races and from different socio-economic groups. There may also be differences in the genetic susceptibility, disease profile, clinicalmanifestations and prognosis, the differences related to differ-

ent locations of gastric cancer suggesting the hypothesis that they are distinct diseases with different pathologies (5).

Epidemiologically gastric cancer has two distinguishing features: variability of incidence and mortality rates in different countries and geographical areas and the downward trend of these indicators.

GEOGRAPHICAL VARIATIONSOF GASTRIC CANCER

In 2008, 988,000 people worldwide were diagnosed with gastric cancer, international incidence having wide variations. The highest incidence rate is recorded in East Asia (China, Japan, Korea, Mongolia), the rate being estimated at 42 cases per 100,000 masculine gender persons and 18 new cases per 100,000 women. A high incidence is recorded in Eastern Europe and South America, with more than 20 new cases per 100,000 men and 9 cases per 100,000 women. The lowest incidence is estimated in North and South Africa (6).

Difference in gastric cancer incidence and mortality is not only recorded in an international context, but also within the same country. In Japan, the gastric cancer remains the most common type of cancer in both men and women, the incidence of gastric cancer being the highest (100 cases per 100,000 inhabitants), here this neoplasia being the first cause of death (6). The high incidence of this disease in Japan has led to the development of efficient programs for early detection of gastric cancer. In Japan, gastric cancer is more common in northern areas (Miyagi) than the south (Fu Knoka). Distal tumors predominate in Japan contrasting with an increased incidence of proximal tumors in Western countries, although here in recent years, there has been an increase in the proportion of proximal gastric cancers. In the U.S. the decline of incidence was dramatic, gastric cancer becoming the 14th leading cause of death each year being estimated at 21,900 new cases per year and 13,500 deaths per year (7). Also, the same unevenness in the distribution of the disease is reported by American authors, the highest rates of mortality from gastric cancer being recorded in the northern states: Iowa, Michigan, New York, Connecticut compared to Georgia, Louisiana in the southern of United States (8). In 2008, the European Union has reported 83,000 new cases of gastric cancer, the incidence being of 18.9 new cases at 100,000 inhabitants per year, with rates 1.5 times higher in males with the top of incidence in the seventh decade of life. The highest rate of incidence was registered at Lithuanian men and Estonian women and the lowest in Sweden, an average incidence being registered in the UK (9).

The multicentre study conducted in Romania in 2003 by the Romanian Society of Digestive Endoscopy, which evaluated the prevalence of gastric cancer among adults reporting to specialized medical services has shown that the national prevalence of gastric cancer was 2.9 to illnesses at 100,000 people aged over 18, with high variations by region: $2.8\%_{000}$ inhabitants in Moldova, $6.6\%_{000}$ in Transylvania, $3.45\%_{000}$ in the western region and $1.9\%_{000}$ in Oltenia and Wallachia. Demographic peculiarities of gastric cancer patients were as follows: 66.4% were males compared with 33.6% women, 51.7% were from urban areas compared with 48.3% in rural areas, and the average age of patients was 63.07 ± 12.10 years. 95.5% of cases had at the time of diagnosis, advanced gastric cancer most commonly located in the gastric body

(40.1%) and antrum (33.8%). According to Borrmann classification, 31.4% had type I polypoid, 32.1% type III ulcero-infiltrative. Histological, according to Lauren classification, the most common histological type was intestinal type (63.8%). Early gastric cancer was diagnosed in 4.4% of patients (10).

TEMPORALTRENDS OF GASTRIC CANCER

In 1930, gastric cancer was the leading cause of cancer mortality in Europe and the U.S. In the past 70 years the death rate declined dramatically in developed countries, especially due to the decrease of prevalence 11). However in the last 30 years the incidence of gastric adenocarcinoma at cardial level increased 5-6 times in developed countries.

Tumors in the cardial area are now nearly half of gastric cancers among men in the U.S. and UK. Also increased the incidence of esophageal adenocarcinoma whose main etiologic factors are represented by obesity, gastroesophageal reflux disease and Barrett's esophagus.Cardial adenocarcinomas share certain epidemiological characteristics of oesophageal adenocarcinoma (13).

In England the standardized incidence rate was reduced to more than half, from 30 cases per 100,000 male populations in the years 1975-1977 to 13 cases per 100,000 men in 2006-2008. The downward trend is similar for women too, being estimated at 14 cases per 100,000 women to 5 new cases per 100,000 women (14).

Gastric cancer was the first cause of cancer death in the world until 1980, when it was surpassed by lung cancer.

DISTRIBUTION OF GASTRIC CANCER BY GENDER, ENVIRONMENT, AGE AND RACE

Gastric neoplasm affects both sexes, but unevenly, the male / female ratio being of 2-3/1, with no significant difference between urban and rural areas, the incidence being certainly higher for vulnerable social categories.

Disease frequency increases significantly with age, the elderly being the most affected groups. Gastric cancer is an exception under age 30, after 40 years the frequency of disease increases reaching the maximum level after the age of 60.

In Romania, the average age of patients with gastric cancer is 68 years. Male / female ratio, regardless of histological form remains around 2.41 / 2.5 (15). By frequency indices, in our country gastric cancer ranks second among men neoplasia and third at women. In the world, Romania ranks seventh of gastric cancer in men and eleven among women (15).

Black race seems to be more frequently affected by the disease, recent statistics suggesting a ratio of 60/36 in favor of black population. Immigrants initially keep the risks of disease similar to those in origin countries, but these risks are modified in descendants, becoming similar to those from the area where they migrated, demonstrating the important role of environmental factors in the occurrence and progression of the disease.

Cardial carcinoma affects men 5 times more than women and 2 times the whites more frequently than the black race. More, proximal carcinoma incidence rate is relatively high in certain professional groups (14). An upward trend recorded the esogastric region carcinoma (6.18%) with reserved prognosis, decreasing age of onset,

predominantly diffuse infiltrative type, predominantly in men.

PATHOLOGICAL CONSIDERATIONS ON GASTRIC CANCER

Approximately 90% of gastric cancers are adenocarcinomas, divided into two categories: well differentiated or intestinal type and undifferentiated or diffuse type. Intestinal type correlates with gastric body gastritis, gastric atrophy and intestinal metaplasia, whereas the diffuse type is related to pangastritis without atrophy (16).Intestinal type is more common in men, black race and older age groups, while the diffuse type is about equally distributed by gender and is more common in young people. Intestinal type is predominant in high risk areas such as East Asia, Eastern Europe, South America and Central America (17). Diffuse type has aa more uniform geographical spread. Reducing the incidence of intestinal type is based on the general decline of gastric cancer. In contrast, the incidence of diffuse type, especially the cell "in signet ring" increased.

For Romania, the report of histopathological types is represented by the intestinal carcinoma / diffuse carcinoma of approximately 1.7 / 1.8, i.e. 46-48% of intestinal type carcinoma and 27% diffuse type carcinoma, unclassified forms being of 25-27% (15).

After WHO histological types for Romania are: papillary carcinoma 4.6%, tubular carcinoma 26.1%, mucipar carcinoma 17.2%, carcinoma with "signet ring" cell 22% and undifferentiated carcinoma 29.9% (9).

By mode of histological differentiation, well-differentiated carcinoma accounts for about 23%, average differentiated carcinoma 18.6%, poorly differentiated carcinoma 28% and undifferentiated carcinoma 30%. The most striking upward trend is manifested by poorly differentiated carcinoma. A retrospective study conducted in our country by the Ministry of Health in 2000 showed an increased incidence of poorly differentiated carcinomas in the "signet ring" as other forms with poor histological differentiation which recorded 57.9%.

An upward trend recorded the esogastric region carcinoma (6.18%) with reserved prognosis, decreasing age of onset, predominantly diffuse infiltrative type and predominantly in men.

In patients younger than 40 years, gastric carcinoma is most often diffuse, with high localization and low histological differentiation. The incidence of this form is currently about 1.92% (18).

An improvement in the prognosis of gastric cancer could be obtained by the discovery of neoplastic process in the "early carcinoma" stage due to endoscopic diagnostic techniques with histological confirmation. Thus, of all gastric cancers included in the study of authors from Cluj-Napoca, early carcinoma accounted for 5.1% of the total (11).

Intestinal metaplasia of different types is associated with gastric cancer in percentage of 11.5% and with duodenal ulcer in percentage of 4.8%. Intestinal metaplasia type III, colonic, represents between 8-12% of the total intestinal neoplasia, being found in approximately 3% of all gastric biopsies from patients with various symptoms, which were subjected to endoscopic biopsies.

Regarding the correlations between different types of dysplasia and gastric cancer types, was revealed that non metaplastic-dysplasia is found in 46.2% of diffuse-type gastric cancer, 7.7% of intestinal type cancers and 53.8% of intermediate type cancers.

Dysplasia of mucosa with intestinal metaplasia was found in 23.1% of diffuse-type cancers, 84.6% of intestinal type cancers and 30.6% of intermediate type cancers.

The causes which determine gastric cancer include genetic and environmental factors. Genetically are described the p53genetic mutation, loss of heterozygosity (LOH) at APC locus, deletion or suppression of FHIT gene. Environmental factors include rich diet in salt, nitrates, chronic alcohol consumption, and previous gastric interventions.

Helicobacter pylori was considered by WHO as class I carcinogenic agent, the risk of detecting a malignant tumor of the distal portion of the stomach is 2-3 times higher in patients with Helicobacter pylori infection than in the control group.

CONCLUSIONS

Since 1980 the prevalence and mortality due to gastric cancer (particularly the distal localization) decreased significantly in all countries and in all ages of 2-7% per year and instead increased the number of patients with adenocarcinoma located at the esogastric junction and at the gastric initial portion.

Gastric cancer still remains a disease with extremely poor prognosis and increased mortality which is exceeded only by lung cancer mortality.

In general, the countries with high incidence of gastric cancer have a survival rate higher than the countries with low incidence. This association is based mainly on the difference in survival due to tumor localization in the stomach. Thus, tumors located in the cardial area have a worse prognosis than those in the pyloric antrum with a lower 5-year survival and increased perioperative mortality.

When the tumor is localized to the mucosa, 5-year survival is approximately 95%. In the U.S. few cancers are discovered at an early stage, which leads to decreased 5 years survival at fewer than 20%. Similarly, in European countries the survival varies between 10-20%.

Possibility to perform screening for early detection of cancer resulted in a significant decrease in mortality in high-risk areas. In Japan, where are used screening procedures very frequently, gastric cancer mortality has halved from 1970.

In Romania, gastric carcinoma does not tend to decrease the incidence seen in developed countries. Moreover, there are growing higher forms of aggression.

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NEWS

NEW INSIGHT INTO MOLECULAR EPIDEMIOLOGY OF MRSA STRAINS IN ROMANIA

A recent study by Székely and co. brings new information regarding methicillin-resistant *Staphylococcus aureus* strains (MRSA) circulating in Romania. The authors used pulsed-field gel electrophoresis (PFGE), *spa* typing and SCC*mec* typing to describe the clonal relations and virulence profiles of MRSA isolates from patients admitted to intensive care and surgical units, during 2010, in Mureş County Emergency Clinical Hospital. The study identified 25 pulsotypes clustering into 4 major clonal groups, named from A to D. The majority of MRSA isolates, included into clonal group A (82%), were associated with the successful HA-MRSA clone, spa type t030, which carried SCC*mec* type III and enterotoxin A genes. Group B was represented by 15 % of the strains, which were assigned to spa types t127, t015 and t321. These strains harbored SCC*mec* type IV and enterotoxin genes. Two strains that belonged to spa types t044 and t582 were included into clonal groups C and D, respectively. They were PVL-producing strains (Panton-Valentine leukocidin) and had genes encoding for enterotoxin G and SST-1 (toxic shock syndrome toxin). The remaining two strains were non-typeable by PFGE and belonged to spa type t034, which is characteristic for livestock-associated MRSA (LA-MRSA). These strains were isolated from community onset infections, harbored SCC*mec* type V and were negative for the searched exotoxins. The study concluded that most MRSA strains were clonally related and suggested an intrahospital origin, but also pointed out the danger of new emerging LA-MRSA strains. (Székely E, Man A, Mare A, Vas KE, Molnár S, Bilca D, *et al*. Molecular epidemiology and virulence factors of methicillin-resistant *Staphylococcus aureus* strains in a Romanian university hospital. *Rev Rom Med Lab*. 2012; 20: 370-382).

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INVOLVEMENT OF IMIDAZOLINE SYSTEM IN DRUG ADDICTION

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INVOLVEMENT OF IMIDAZOLINE SYSTEM IN DRUG ADDICTION (Abstract): The imidazoline system (a concept which is barely over decades old) consists in imidazoline receptors I_1, I_2 and I_3 and their active endogenous ligands, the most important of which is agmatine. Brain contains both I_1 and I_2 of imidazoline receptors. One of the most important functions of this system is modulation of different behaviour components, such as suicidal behaviour, stress, anxiety, food intake etc. Own data preliminary data revealed different effects of α_2 and imidazoline-receptors antagonists on morphine conditioned place preference (efaroxan reduces its intensity, while idazoxan has no influence); this reveals a possible different role of I_1 and I_2 receptors in reward system. **Keywords**: IMIDAZOLINE RECEPTORS, REWARD SYSTEM, DRUG ADDICTION

The concept of imidazoline brain system is only a few decades old. The imidazoline system consists in imidazoline receptors I_1, I_2 and I_3 and their active endogenous ligands. I_1 and I_2 imidazoline receptors are membrane located non-adrenergic sites. Brain contains both I_1 and I_2 imidazoline receptors.

One of the most important natural agonists is agmatine (decarboxylation product of arginine). Identification of agmatine as an endogenous ligand for imidazoline receptor leads to important knowledge over imidazolinic system roles in the body (1). There is a particularly of the connections between imidazoline and adrenergic brain system, as certain imidazoline agonists and antagonists also act on α_1 and $\alpha_2$adrenergic receptors.

Existing evidence show the importance of imidazoline in modulating central nervous system functions. Existent data show that I_1 and I_2 receptors have different effects on some behavior components, such as suicidal behavior, stress, anxiety, food intake etc.

Imidazoline system is important for both normal and pathological behavior. Thus, changes in the imidazoline system balance have been detected certain neurological and especially psychiatric diseases.

REWARD SYSTEM

The biological mechanism and neural pathways mediating behavior motivated by events commonly associated with pleasure have been identified and described. These events are termed "rewards" and are viewed as primary factors governing normal behavior. The neural pathways involved in pleasure-mediated behavior are referred to as "brain reward system". Main

rewards may be classified as natural (such as food, liquid intake, sex) and environmental (drugs of abuse, brain electrical stimulation of certain areas). The neuroanatomical elements of rewarding stimulation have been identified and ventral tegmental area, nucleus accumbens, ascending mesolimbic dopamine system, middle forebrain bundle are among the most important structures mediating reward. Dopamine is considered the most important neurotransmitter responsible for reward, but glutamate, serotonin, substance P, norepinephrine, endogenous opioids, GABA are also critically involved (2).

SUBSTANCES ACTING ON IMIDAZOLINE RECEPTORS AND THEIR INFLUENCE ON REWARD- RELATED BEHAVIOR

The imidazoline system has at least two important characteristics: some agonist and antagonists also act on the α-adrenergic system, and agmatine is synthesized from arginine, which is also a source of nitric oxide. There are I_2 binding sites on monoamine oxidase (MAO) molecule, an essential enzyme for catecholamine metabolism. The fact that some agonists or antagonists of imidazoline receptors have also effect on α-adrenergic receptors or MAO also suggests certain interactions between imidazoline and adrenergic systems.

Imidazoline receptors have been identified in all brain areas; as to imidazoline system involvement in addictions, it is of special importance that this type of receptors is present in brain reward system cells (neurons and neuroglia). Experimental research on laboratory animals shows that agmatine influences some types of drugs addiction, most of them referring to opioid addiction. Clonidine (mainly known as α_2-receptors agonist, but also I_1-receptors agonist) is a very important agent used for withdrawal treatment.

Agmatine itself has no reinforcing effect, but it determines a reduction in opioid dependence either systemically (intraperitoneally or subcutaneously) or centrally (intrathecally or intracerebroventricularly) (3). This effect is believed to be mediated by imidazoline I_1-receptors (4).

Pretreatment with agmatine (40 or 80 mg/kg, intraperitoneally) significantly inhibited the acquisition of intravenous morphine self-administration as assessed by the nose-poke response and morphine intake. The mean number of days required to meet the acquisition criteria for intravenous morphine self-administration was significantly prolonged. After acquisition of intravenous morphine self-administration, chronic administration of agmatine (40 or 80 mg/kg x 30 days, bid, intraperitoneally) during the extinction period significantly prevents the re-acquisition of intravenous morphine self-administration. The ability of agmatine to inhibit the acquisition and re-acquisition of intravenous morphine self-administration suggests a possible use of agmatine in the treatment of opioid dependence (5).

Agmatine prevents tolerance to opioids analgesia either systemically (intra-peritoneally or subcutaneously) or centrally (intrathecally or intracerebro-ventricularly) (6) and reduces fentanyl self-administration (7). These facts can be of real therapeutic benefit, especially as numerous recent studies proved that I2-agonists, including agmatine, potentiate opioid- induced analgesia (8, 9, 10) Also, a very beneficial fact is reversion of morphine-induced memory impairment by agmatine, effect which is antagonized by idazoxan (I2 antagonist) (11).

Also, agmatine reduces the intensity of

morphine-induced conditioned place preference in mice (12) in a dose-dependent manner. Conditioned place preference is an experimental model investigating the rewarding effects of different stimuli, where the animal develops an association between the subjective states produced by the stimuli and the environmental factors associated to these stimuli.

There are also other addictive substances than opioids the behavioral effects of which are lowered by agmatine.

Repeated agmatine treatment altered nicotine sensitization in mice (13).

Regarding the effects of psychostimulants on brain reward, several studies have also lead to relevant conclusions. Also, agmatine, at doses that did not produce place preference, aversion or altered motor activity significantly decreased the development of methamphetamine-induced place preference when agmatine was administered in combination with methamphetamine during place conditioning (14).

The specific I_1 receptor agonist moxonidine reduces cue-induced reinstatement of cocaine-seeking behavior, suggesting that moxonidine might have substantial potential for treating addictive disorders (15).

Agmatine (5-20 mg/kg) significantly blocked ethanol (0.5 g/kg)-induced locomotor hyperactivity. These doses of agmatine did not affect the locomotor activity in naive mice when they were administered alone. Other results suggest that agmatine has an important role in ethanol-induced locomotor hyperactivity in mice. There may be a relationship between the addictive psychostimulant effects of ethanol and central agmatinergic system (16).

A theoretical rationale supports the fact that tizanidine (α_2-adrenoreceptor agonist that inhibits noradrenaline release and binds to imidazoline receptors) can be an effective treatment for alcohol withdrawal syndrome as well as for the prevention of relapses (17).

Our preliminary data revealed different effects of α_2 and imidazoline-receptors antagonists on morphine conditioned place preference (efaroxan, 1mg/kg reduces its intensity, while idazoxan, 0.25mg/kg has no influence), while neither of these substances significantly modifies the intensity of opioid dependence in rats.

DISCUSSION

Based on the presented data referring to substances acting on imidazoline receptors and the way they modulate the reinforcing effects of some addictive substances, several hypotheses referring to the mechanisms by which imidazoline system modulates reward pathways have been formulated.

There are important functional connections between imidazoline system and other neuromediator systems involved in drug dependence. The most important functional connections in case of imidazolinic system are those with catecholaminergic system.

A first very important element of these connections is that some I_1-receptor agonists (such as clonidine) are also α_2-receptors agonists. Other substances which act as competitive I_1-receptor antagonists (such as efaroxan) also act on adrenergic receptors.

The problem of imidazoline system involvement in addiction might also be connected with nitric oxide involvement in the mechanism of production of those dependences. Arginine (the amino acid which also serves for arginine precursor) is also the main source of nitric oxide. Among others, a possible involvement of inducible nitric oxide system has also been incriminated for

agmatine effect of reducing morphine-induced conditioned place preference, as minoguanidine, a specific inducible NOS (nitric oxide synthase) inhibitor, significantly reduced the effect of agmatine (18). On the other hand, agmatine is a weak competitive inhibitor of neuronal NOS (9). It is known that morphine administration is followed by increased NO released, due to increased activity of constitutive NOS, but it decreases the activity of inducible NOS (via constitutive NOS-derived NO) (20, 21).

Agmatine, administered before morphine withdrawal determined a decrease of glutamate level in nucleus accumbens (a key element in reward mesolimbic dopaminergic pathways) in rats; also, agmatine acts as a NMDA-receptor antagonist (22, 23). Given the essential role of glutamate neurotransmission in brain reward system, these might serve as a reasonable explanation for the above-presented data related to agmatine capacity of reducing some aspects related to drug intake.

However, many aspects related to imidazoline-reward system interaction mechanisms are yet to be studied.

CONCLUSIONS

Imidazoline agonists determine inhibitory effects on several aspects related to the action of some drugs of abuse on reward-modulation pathways. Considering their potentiating effect on opioid analgesia and reversal of morphine-induced memory impairment, imidazoline agonists might represent valuable pharmacological agents for addiction treatment. Also, substances such as clonidine, which has been long used in withdrawal treatment, might due their beneficial effects in patients with opioid or alcohol withdrawal not only to imidazoline receptors blockade but also to adrenergic receptors blockade. Existent data are also opening the perspective of therapeutic use of other imidazoline system modulators.

Regunathan, 2006 (24) showed that agmatinase inhibitors (substances which blocks the enzyme which brakes-down agmatine) will offer a way to enhance agmatine brain concentration. This may be a useful in therapy to reduce the intensity of opioid addiction or even opioid withdrawal syndrome.

We believe that both imidazoline I_1 and I_2 agonists and antagonists might modulate reward system activity, this being of particular relevance for therapy.

Involvement of imidazoline system in behavior is surely a very fruitful study domain.

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THERAPY WITH CLIMARA IN SURGICAL MENOPAUSE

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THERAPY WITH CLIMARA IN SURGICAL MENOPAUSE (Abstract): Hormone replacement therapy in surgical menopause is a prophylactic measure that is used for preventing the short and long term effects of the lack of ovarian hormones. **Material and methods:** This is a retrospective study conducted between 2004 and 2006 at the Iasi "Elena-Doamna" Hospital of Obstetrics and Gynecology on two series of patients: 46 patients with surgical menopause who received treatment with transdermal estradiol (Climara), and 20 surgical menopause patients not receiving this treatment who served as controls. **Results:** A decreases in the average levels of total cholesterol, triglycerides and LDL cholesterol and an increase in the HDL cholesterol level were identified in the series receiving Climara compared to the controls. The climacteric symptoms improved in the patients receiving treatment. **Conclusions:** Transdermal therapy with estradiol (Climara) is an effective method of treatment in surgically induced menopause**. Key words:** SURGICAL MENOPAUSE, CLIMARA, CARDIOVASCULAR DISEASE

Surgically induced menopause by bilateral oophorectomy is followed by short and long term effects: cardiovascular disease and osteoporosis. Cardiovascular diseases are one of the main causes of illness and death for women in industrialized countries (1,2).

Epidemiological studies (3) show that the incidence of cardiovascular diseases increases in postmenopausal women The risk for ischemic heart diseases is higher in women with ovariectomy, and hormone replacement therapy reduces this risk (4, 5). Studies show that a decrease in ovary function from bilateral ovariectomy increases the risk of myocardial infarction and the use of estrogens reduces this risk (6,7,8). The positive effect of estrogenic therapy with estradiol on cardiovascular diseases was proved ever since many years.

The favorable effect of estrogenic therapy in women with surgical menopause is due to the action on plasma lipoproteins and endothelial function (9, 10, 11).

MATERIAL AND METHODS

Our study is a retrospective study carried out at the Iasi "Elena-Doamna" Hospital of Obstetrics and Gynecology during 2004-2006. 46 patients with bilateral oophorectomy received transdermal therapy with 3.9 mg estradiol (Climara) (series I) and 20 patients with surgical menopause

not receiving this treatment served as control group (series II).

The following data were recorded: age, socio-economic status, personal history, type of intervention, symptoms, and biological blood levels during the research. A standard file was used to record all changes noticed during therapy.

The collected data were transferred to EXCEL and SPSS 17.0 databases and processed with the predefined statistical functions. For the statistical analysis (ANOVA) both descriptive and analytical methods were used. The statistical indicators used were:

- Mean value: simple arithmetic mean, median, module;
- Dispersion: standard deviation, variance.

Data were presented with 95% confidence intervals, and for testing the differences the χ^2 and t-Student's t- tests were used.

The correlation between parameters was analyzed by using the Pearson's correlation coefficient (r), which ranges between $-1 < r < +1$, indirect correlation if the values are negative, and direct correlation if the values are positive, and its square form (R^2).

RESULTS

*Age-distribution***.** After comparatively analyzing patients' age in the two study series and applying the ANOVA test, the following were noticed:

- In the series of women with surgical menopause treated with Climara (series I), the age varied between 35 and 60 years, with a moderate variance in the range of values (11.41%); the mean age of this series was 49.22;
- In the series of women with surgical menopause without treatment (series II), the age ranged between 40 and 60, with a slightly reduced variance in the range of values compared to series I (10.50%); the mean age of this series was 50.40.

Rural-urban distribution was homogenous for both study series – the patients came mainly from urban areas: series I – 89.1%, series II – 70% (χ^2=2.42; GL=1; p=0.119).

Distribution of patients according to marital status showed a higher percentage of married women: series I (95.7%) and series II (100%), no other significant differences between the two series being recorded (χ^2=0.03; GL=1; p=0.868).

Distribution of patient according to educational level showed statistically significant differences between the two series (χ^2=3.95; GL=1; p=0.047), in series I the percentage of women with high school education being significantly higher (89.1% *vs.* 65%).

Case history

The distribution of patients according to body mass index showed that there were more overweight women in the series receiving Climara (45.7% *vs* 35%), and no obese women in series II (19.5% *vs* 0%), this distribution being statistically significant (χ^2=7,18; GL=2; p=0.028).

The average BMI values showed significant differences (t=4.15; GL=64; p<0.001) and according to that series I was classified as being made of overweight women while series II was generally made of normal weight women.

The average blood pressure was normal for both series I (13.1/7.98 mmHg) and series II (13/8 mmHg).

Case history data revealed:

- Series I: hemorrhagic uterine fibro-

ma (71.7%); obesity (15.2%); dysfunctional hemorrhage (10.9%);

- Series II: hemorrhagic uterine fibroma (50%); varices (45.0%); uterine fibroma (25%); AHT (25%); chronic cervicitis (20%).

Most women were menopausal for 1-4 years, 50% in both study series, followed by those menopausal for less than 1 year (32.6% in series I and 45% in series II), distribution of frequency which is not statistically significant (χ^2=2.16; GL=2; p=0.340).

Symptoms

Hot Flushes: -in series I, when the study was initiated 73.9% of the patients were experiencing hot flushes, its frequency decreasing to 41.3% after 12 months, with alternating periods of hot flushes, and at the end of the study this symptom was present in only 8,7% of the cases (χ^2=40.53; GL=3; p<0.001);

- at the beginning of the study hot flushes were experienced by 80% of the women in series II, its incidence significantly decreasing with every subsequent medical check up, being present in about 30% of the women when last monitored (χ^2=10.56; GL=3; p=0.014).

Migraine:-in series I, at the beginning of the study, 28.3% of the patient complained of migraine, the same percentage being recorded after 6 months of therapy but with migraine-free intervals in 19,6% of the women; at the end of the study this symptom was reported by 4,3% of the patients (χ^2=11.33; GL=3; p=0.010);

- in series II, at the beginning of the study in 50% of the women had migraines their incidence decreasing after 2 years below 10% (χ^2=13.37; GL=3; p=0.004).

Instability /agitation: - in series I, instability/agitation was rarely found at the initial examination investigation, after one year it being still present in only one patient (χ^2=2.23; GL=3; p=0.525);

- in series II, at the beginning of the study, instability/agitation was found in 10% of the women; after 6 months 50% of the patients in this series presented this symptom in 40% of them alternating with symptom-free interval; at the end of the study instability/agitation was found in 25% of the patients in this series (χ^2=7.99; GL=3; p=0.046).

Insomnia: -in series I insomnia significantly decreased in frequency during the study interval, and varied in intensity from 43.5% to 6.5% (χ^2=19.20; GL=3; p=0.0002);

- in series II, insomnia was present in about 30% of the cases throughout the study (χ^2=0.17; GL=3; p=0.982).

Libido: - in series I, after 6 months monitoring, 35 % of the patients had normal libido, and at the end of the study 37% of the patients (χ^2=20.05; GL=3; p=0.0002);

- in series II, libido was a very rare symptom (χ^2=1.04; GL=3; p=0.792).

Pollakiuria: -series I: pollakiuria was met in 8.7% of the cases at the beginning of the study, but its frequency diminished until it disappeared at the end of the study (χ^2=4.16; GL=3; p=0.245);

- series II: pollakiuria was present every now and then in about 20% of the cases throughout the study (χ^2=0.28; GL=3; p=0.965).

Exercise urinary incontinence: -this symptom was met in 4.3% of series I patients at the beginning of the study and its frequency diminished until it disappeared at the end of the study (χ^2=2.26; GL=3; p=0.520);

- in series II, IUE was a symptom reported intermittently by 1-2 patients

(χ^2=0.64; GL=3; p=0.887).

Biochemical parameters

Blood glucose level: The average blood glucose levels were initially significantly higher in series I (p<0.05). During their monitoring, a significant decrease was recorded in series I, while in series II patents the levels kept increasing so that at the end of the study significantly higher blood glucose levels were recorded in series II (p<0.05).

Triglycerides: During the monitoring, the beneficial effect of the treatment with Climara was evident, the average triglyceride levels being significantly lower when compared with those in series II patients (p<0.001) in which triglyceride level significantly exceeding the reference range (figure 1).

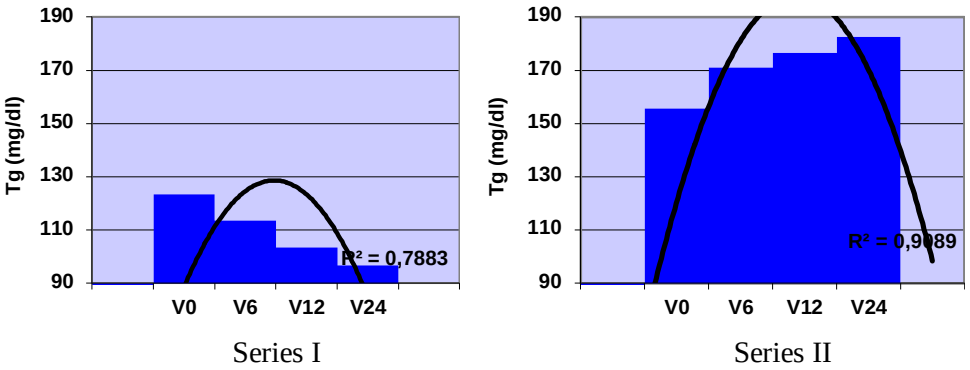

Fig. 1. The evolution of average triglycerides levels in the study series

Total cholesterol: All along the monitoring, the average total cholesterol levels in series I women decreased significantly below the reference range, while in series II they kept increasing slightly exceeding the upper reference range. The average total cholesterol levels showed significant differences between the two series (p<0.05), emphasizing the beneficial effect of the treatment with Climara (figure 2).

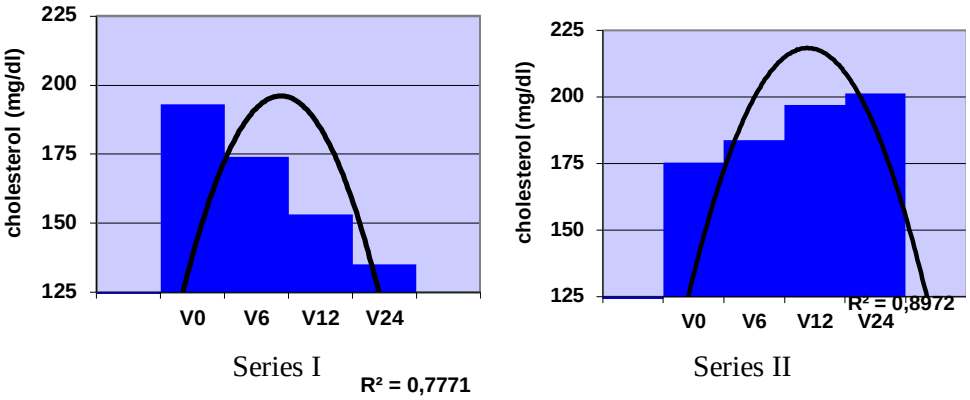

Fig. 2. The evolution of average total cholesterol levels in the study series

LDL cholesterol: The average LDL cholesterol levels were significantly higher at the beginning of the study in series I when compared with those recorded in series II ($p<0.05$), but during the monitoring we noticed the beneficial effect of the treatment with Climara expressed in significant decreases in LDL-cholesterol levels, while in the series without treatment its average levels increased in time ($p<0.001$) (figure 3).

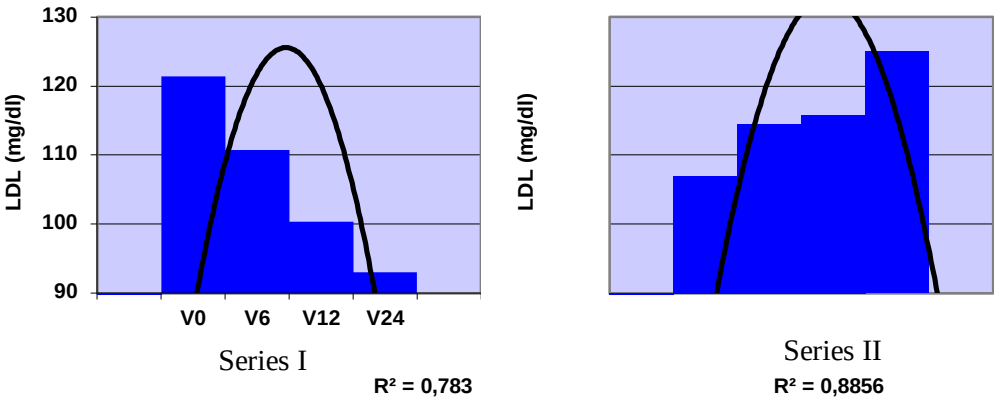

Fig. 3. The evolution of the average LDL cholesterol levels in the study series

HDL cholesterol: The average HDL cholesterol levels have increased in both study series, but in the patients treated with Climara, all along the monitoring the average values were significantly higher ($p<0.001$).

DISCUSSION

One of the most important roles of hormone replacement therapy is that it eliminates the symptoms of menopause, especially of the vasomotor and urogenital symptoms that appear immediately after oophorectomy (12).

The long term benefit is that it lowers the risk of developing cardiovascular diseases. The protective cardiovascular effect of estrogen therapy manifests through favorable effects on lipid metabolism (13).

In our study comparing the menopause symptoms in the two patient series the following are evident:

- at baseline:
  - no significant differences in the the investigated symptoms ($p>0,05$);
- at 24 months:
  - series I: libido was significantly more frequent ($p=0,017$) and hot flushess disappeared ($p<0.001$).
  - series II: significantly more hot fluses ($p=0,065$), associated with irritability/aggitation ($p=0,012$), insomnia ($p=0,030$) and polakiuria ($p=0,041$).

As to biochemical parameters at 24 months: - triglycerides, total cholesterol and LDL cholesterol levels decreased significantly ($p<0.001$) in the patients on Climara treatment ;

- HDL cholesterol levels have increased in both study series. In series I patients the levels were significantly higher ($p<0.001$), demonstrating the cardiovascular protective effect of Climara treatment.

No patient discontinued therapy at two years.

CONCLUSIONS

The clinical benefits of hormone replacement therapy in the elimination of

menopause symptoms are widely accepted, as proved by our study in which the patients receiving Climara became menopause-symptom free.

Long term prevention of cardiovascular diseases is an important benefit. In series I patients significant decreased in triglycerides, total cholesterol and LDL cholesterol levels, and increases in HDL cholesterol levels, with protective cardiovascular role, were found.

The treatment must be initiated as soon as possible.

Climara administrated in surgical menopause women resulted in a short and long term improvement in their quality of life. Climara is well tolerated by women in surgical menopause.

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HIBRIDIZAREA GENOMICĂ COMPARATIVĂ PE MICROREȚELE: CONSIDERAȚII TEHNICE ŞI APLICAȚII

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ARRAY CGH: TECHNICAL CONSIDERATIONS AND APPLICATIONS (Abstract): During the past ten years a particular molecular technology - array comparative genomic hybridization (aCGH) - has received a great deal of attention. Array CGH can detect simultaneously sub-microscopic copy number changes across the whole genome, thus overcoming the limitations of karyotyping or locus-specific techniques. Array CGH has become an important tool for clinical diagnostics and gene-identification studies and is having a great impact on the understanding of pathologies, the counselling of families and patient management. Different types of array CGH platforms at increasingly higher resolution have been developed, differing mainly in the type of the interrogating probes and in their coverage of the genome. Here, we review the array CGH methodology and its various applications in clinical diagnostics and research. Although it's an expensive technology and differentiating between pathogenic and benign copy number variations is a challenging task, array CGH is an efficient and robust method for assesing disease-causing genomic imbalances and will probably replace karyotype as the primary cytogenetic test. **Key words:** ARRAY COMPARATIVE GENOMIC HYBRIDIZATION, MOLECULAR KARYOTYPING, COPY NUMBER VARIATION, MENTAL RETARDATION

Explorările citogenetice reprezintă o metodă importantă de diagnostic în clinică, anomaliile cromozomiale determinând: sindroame plurimalformative, retard mental, tulburări de comportament, tulburări de sexualizare şi reproducere, cancere. Începând cu anii '70, tehnica de marcaj cromozomial în benzi G, efectuată pe celule aflate în diviziune şi folosind o mărire de 10.000 ori, a fost principalul instrument folosit în diagnosticul anomaliilor cromozomiale echilibrate şi neechilibrate. Cariotipul evaluează întregului genom, evidenţiind pierderile sau câştigul de material genetic într-un singur test, dar nu poate detecta anomalii mai mici de 5-10 Mb şi nu poate fi decât parţial automatizat (1).

Prin introducerea tehnicii FISH (*Fluorescent In Situ Hybridization* – Hibridizare fluorescentă in situ) a devenit posibilă detecţia anomaliilor cromozomiale submicroscopice. FISH poate detecta un locus (evidenţiind microdeleţiile specifice anumitor sindroame plurimalformative) sau mai mulţi loci (evidenţiind regiunile subtelomerice) într-un singur test, dar este o metodă laborioasă, care necesită o evaluare clinică minuţioasă, importantă pentru alegerea

adecvată a sondei(lor) (1).

Tehnologiile bazate pe microrețele a schimbat major analiza genomului uman, combinând rezoluția înaltă a tehnicii FISH cu posibilitatea de a scana întregul genom oferită de cariotip. Hibridizarea genomică comparativă pe microrețele (aCGH) permite detecția precisă şi specifică a anomaliilor cromozomiale neechilibrate din întreg genomul, la o rezoluție mai mică de 10 kb (1).

METODOLOGIE

Principiul aCGH este asemănător cu cel al CGH convențional. Microrețelele sunt alcătuite din câteva mii de sonde diferite (fiecare specifică unei secvențe de ADN), nemarcate, fixate pe o lamă de sticlă sau pe un *chip* de silicon, în mici puncte („spots") cu coordonate precise, dispuse în rânduri ordonate, sub forma unei rețele cu densitate înaltă (*chip* ADN). Pentru realizarea analizei este extras ADN genomic atât de la pacient (din limfocite, fibroblaste sau alte țesuturi) cât şi dintr-o probă de referință (aceeaşi linie celulară ca a pacientului dar provenită de la unul sau mai mulți indivizi normali). Cele două probe de ADN sunt fragmentate (cu ultrasunete sau prin digestie cu o enzimă de restricție), marcate cu fluorocromi diferiți (Cy3 - verde pentru proba test, Cy5 - roşu pentru proba martor), amestecate în cantități egale, denaturate şi co-hibridizate pe microrețea (2,3). Secvențele de ADN repetitiv sunt blocate prin adăugarea de Cot-1 ADN. După spălare, microrețelele sunt scanate cu un *microarray scanner*, iar imaginile obținute sunt analizate folosind un program ce cuantifică intensitățile de emisie ale fluorocromilor roşu şi verde pentru fiecare spot din rețea şi calculează raportul lor. Acest raport este proporțional cu raportul numărului de copii ADN din genomul martor şi cel de referință. Dacă intensitățile celor doi fluorocromi sunt egale într-un spot, (raport Cy3/Cy5=1, sau $\log_2$=0) această regiune din ADN-ul pacientului este interpretată ca fiind normală/echilibrată. Dacă este prezentă o deleție, ADN-ul martor hibridizează preferențial, iar raportul Cy3/Cy5 va fi subunitar (ex. 1:2, $\log_2$=-1). În schimb, dacă există o duplicație, ADN-ul pacientului va hibridiza preferențial, iar raportul Cy3/Cy5 va fi supraunitar (ex. 3:2, $\log_2$=0,58.) (fig. 1) (2).

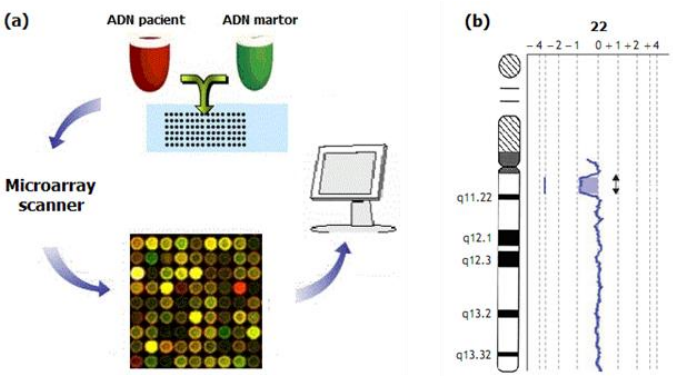

Fig. 1. Principiul tehnicii aCGH.

(a) Proba ADN de la pacient şi cea de referință sunt marcate cu fluorocromi diferiți şi cohibridizate pe o microrețea ce conține fragmente de ADN genomic fixate pe o lamă de sticlă. După scanare, semnalele fluorescente sunt captate şi analizate digital, în funcție de intensitate, rezultând o reprezentare grafică.
(b) Exemplu de aCGH: deleție 22q11.21. Axa orizontală indică logaritmul raportului de fluorescență (valori negative – deleții; valori pozitive - duplicații). Axa verticală indică locusul pe cromozom.

TIPURI DE REȚELE

În practică pot fi folosite diferite tipuri de rețele: rețele „țintite", rețele ce acoperă întregul genom, sau o combinație a lor.

- ***Rețelele „țintite"*** (*targeted*) conțin fragmente specifice de genom - precum cele din regiunile subtelomerice sau cele în care se produc frecvent microdelеții/ microduplicații implicate în producerea unor sindroame plurimalformative -, dar nu acoperă întregul genom. Acest tip de rețea este folosit în scop diagnostic (postnatal sau prenatal) sau de screening (4,5).
- ***Rețelele pan-genomice*** („*whole genome*" sau „*tiling path*" - cu clone suprapuse) permit acoperirea întregului genom cu o rezoluție care depinde de distanța dintre sonde; acest tip de rețele identifică cu 5% mai multe anomalii decât o rețea „țintită" (6). Rețelele „*whole genome*" cu densități foarte mari sunt folosite în cercetare şi au dus la definirea a noi sindroame şi la caracterizarea punctelor de ruptură în diverse anomalii cromozomiale sau mutații genice, dar nu au aplicabilitate clinică datorită identificării a numeroase CNV (*Copy Number Variations* - variații ale numărului de copii), dificil de interpretat (7).

TIPURI DE SONDE

Sondele pot fi de tip BAC (*Bacterial Artificial Chromosome* - cromozomi artificiali bacterieni), ADN complementar, oligonucleotide sau SNP (*Single Nucleotide Polymorphism* – polimorfism al unui singur nucleotid). Inițial au fost folosite sonde de tip BAC ce conțin ADN izolat din clone de dimensiuni mari (150–200 kb) dar treptat s-a renunțat la ele deoarece fabricarea lor este laborioasă, iar rezoluția este limitată. Sondele oligonucleotidice, obținute prin sinteză chimică, asigură o rezoluție mare datorită dimensiunilor reduse. Sondele oligonucleotidice care conțin SNP-uri permit atât identificarea dezechilibrelor genomice de tip deleție/duplicație, cât şi a anomaliilor fără modificarea numărului de copii, cum sunt pierderea heterozigozității şi disomia uniparentală (8).

INTERPRETARE

Aplicarea tehnicii aCGH la indivizi normali a identificat numeroase rearanjări genomice, imposibil de detectat prin alte metode. Aceste segmente de ADN, cu lungimi de peste 1 kb, care sunt prezente în genomul persoanelor diferite într-un număr variabil de copii şi nu provin din inserția sau deleția unor elemente transpozabile, au fost numite variații ale numărului de copii (CNV) (9). Termenul este neutru în ceea ce priveşte patogenitatea şi frecvența de apariție, dar în situațiile în care frecvența în populație este de peste 1%, se foloseşte termenul de polimorfism al numărului de copii (10). Relevanța clinică a CNV este incertă, deoarece identificarea unei astfel de variații nu se asociază obligatoriu cu apariția unei boli.

Testarea clinică prin aCGH, indiferent de tipul de sonde sau de rețea utilizată impune următorul algoritm. Dacă este detectată o anomalie patogenică cunoscută, ea va fi validată prin altă metodă (FISH sau Q-PCR) şi raportată. În continuare, vor fi testați părinții cu scopul de a identifica genitorul de la care a fost moştenit CNV. Metoda va fi aplicată şi la viitoarele sarcini pentru a stabili prezența/absența modificării patologice la produsul de concepție. Dacă este detectat un CNV neasociat cu o anumită afecțiune, rezultatul obținut este comparat cu informațiile din *Database of Genomic Variants* (DGV – Baza de date a variantelor genomice), cu scopul de a vedea dacă variația a mai fost întâlnită la indivizi sănătoşi. Dacă anomalia nu este găsită în DGV sau numărul de cazuri rapor-

tate este mic vor fi testați și părinții. Dacă unul din aceştia prezintă CNV-ul, cel mai probabil el reprezintă o variantă benignă. Dacă variația a apărut *de novo*, este aprofundată cercetarea regiunii în care a fost identificat CNV-ul pentru a identifica genele prezente şi posibila lor implicare patologică. În ambele situații CNV este raportat la DGV, astfel încât să poată fi comparat cu rezultatele altor cercetători (7).

Cu timpul, completarea bazelor de date internaționale (DGV, Decipher, Ecaruca), ca urmare a eforturilor colective, va permite detectarea CNV similare şi determinarea patogenității acestora. Deocamdată, în absența eşantioanelor de ADN de la părinți, utilizarea în scop diagnostic a metodei aCGH la rezoluție foarte mare nu este posibilă, datorită sutelor de polimorfisme dificil de interpretat (8).

AVANTAJELE ŞI DEZAVANTAJELE aCGH

Metoda aCGH prezintă numeroase avantaje:

- permite detecția globală, cu rezoluție înaltă a deleţiilor/duplicațiilor genomice, ceea ce constituie un avantaj raportat la metodele țintite (FISH sau MLPA) care oferă informații referitoare la locii a căror secvență de ADN se regăseşte în sondele folosite.
- în raport cu analiza cromozomială clasică, aCGH are avantajul că nu necesită celule în diviziune (deci timpul de obținere al rezultatelor este mai scurt) şi pot fi utilizate şi probe biologice ce nu conțin celule vii (celule recoltate prin biopsie, autopsie sau provenite de la produşi de avort). În plus, prin aplicarea aCGH pe sânge periferic se pot detecta mozaicuri cromozomiale care nu pot fi identificate prin cariotip, deoarece metoda analizează toate celulele nucleate din sânge, în timp ce cariotipul standard e realizat doar pe limfocitele T cultivate *in vitro*, după stimulare cu fitohemaglutinină.
- cantitatea de produs biologic necesară metodei este foarte mică, majoritatea platformelor de aCGH permițând efectuarea analizei şi în condițiile unor probe ce conțin doar câteva micrograme de ADN genomic.
- oferă rezultate definitive într-un timp scurt şi se pretează la automatizare (11).

Dezavantajele aCGH includ:

- imposibilitatea detectării anomaliilor cromozomiale echilibrate (inversii, translocații) şi a poliploidiilor (12, 13).
- costurile per analiză sunt mult mai mari decât cele pentru un cariotip clasic, în special pentru platformele care acoperă întregul genom la o rezoluție foarte mare.
- în cazul identificării unor CNV, este necesară validarea prezenței rearanjărilor genomice prin tehnica FISH sau qPCR, tehnici care permit detecția țintită a secvențelor specifice de ADN (9). Alte tehnici moleculare bazate pe PCR (precum MLPA, MAPH) create pentru evaluarea concomitentă a mai multor loci de interes pot fi de asemenea utilizate, dar au o sensibilitate şi specificitate reduse (10).

APLICAȚIILE METODEI aCGH

Retardul mental şi/sau anomaliile congenitale multiple. O aplicație importantă a tehnologiei aCGH este identificarea anomaliilor cromozomiale la pacienții cu retard mental (RM) idiopatic, asociat sau nu cu dismorfie facială sau anomalii congenitale multiple (ACM). Anomaliile cromozomiale reprezintă cea mai frecventă cauză de RM, iar rearanjările subtelomerice sunt responsabile pentru 5-7% din cazuri (14). Evidențierea anomaliilor cromozomiale neechilibrate subtelomerice prin marcaj cromozomial în benzi G este dificilă, deoa-

rece acest tip de marcaj nu vizualizează capetele cromozomiale. Pe de altă parte, folosirea tehnicii FISH impune obținerea unor preparate metafazice de calitate superioară, iar evaluarea tuturor regiunilor subtelomerice de interes este foarte scumpă şi nu se poate face printr-o singură analiză. În schimb, folosind aCGH se pot identifica un număr mare de rearanjări (deoarece sondele de aCGH acoperă un segment subtelomeric mai mare decât cel corespunzător sondelor FISH), este posibilă evaluarea dimensiunii segmentului cromozomial şi pot fi diagnosticate şi rearanjări complexe, precum asocierea dintre deleția unui segment şi duplicația unui segment învecinat (15).

Folosirea metodei aCGH în cazurile de RM (cu sau fără ACM), a permis identificarea unor duplicații şi deleții noi, precum şi stabilirea prevalenței mozaicurilor cromozomiale. Aplicarea aCGH de înaltă rezoluție ca metodă de testare genetică inițială la pacienți cu RM neselectați a permis detecția anomaliei patogenice în 19% din cazuri, valoare mai mare decât cea obținută prin analiză cromozomială (~3,7%) sau tehnica FISH (~9,5%) (12,16,17). În plus, aCGH a detectat mozaicuri la 8-10% din indivizii cu RM şi dismorfie facială din loturi neselectate (11). Deşi tehnica aCGH stabileşte prezența dezechilibrului genomic (surplus sau absență de material genetic) ea nu indică mecanismul prin care a apărut dezechilibrul, aspect important însă pentru acordarea sfatului genetic, de aceea este recomandată efectuarea cariotipului concomitent cu aCGH pentru toate cazurile cu RM (12).

Analiza celulelor fetale. Aplicarea metodei aCGH în diagnosticul prenatal elimină o serie din inconvenientele analizei cromozomiale fetale (timpul lung – 2-3 săptămâni – de obținere a rezultatului definitiv, posibilele eşecuri de cultură, calitatea slabă a preparatelor cromozomiale şi, corelat cu aceasta, obținerea unui număr redus de benzi cromozomiale). Metoda foloseşte ADN genomic din celule fetale (obținute prin amniocenteză sau biopsie de vilozități corionice) şi poate fi aplicată atât direct (imediat după recoltare) cât şi pe celule cultivate. Sensibilitatea metodei este mai mare decât cea a cariotipului, permițând evidențierea anomaliilor cromozomiale neechilibrate (cu excepția poliploidiilor), inclusiv a rearanjărilor subtelomerice (18). Din rațiuni practice (interpretarea unui CNV, descoperit prin aplicarea unei rețele pan-genomice, este deosebit de dificilă) pentru diagnosticul prenatal se folosesc doar rețele „țintite" care conțin clone genomice pentru regiunile subtelomerice şi cele implicate frecvent în sindroamele cu microdeleții/microduplicații (19).

Având în vedere că majoritatea duplicațiilor şi delețiilor asociate cu boli genomice apar *de novo* şi se pare că au aceeaşi frecvență indiferent de vârsta maternă (spre deosebire de aneuploidii, care se asociază cu vârsta maternă avansată), aCGH ar putea înlocui explorările citogenetice în majoritatea cazurilor de diagnostic prenatal (20). Deoarece această tehnică nu poate detecta translocațiile echilibrate şi poliploidiile, s-a sugerat că în cazul ecografiilor fetale anormale cu aCGH normal ar trebui efectuate ulterior tehnici citogenetice (21).

Metoda aCGH are aplicabilitate şi pentru studiul produşilor de avort (care deseori prezintă anomalii genetice), atunci când nu se poate face cariotip din celulele avortonului sau acesta are o anomalie cromozomială criptică (22).

C**ancerul.** Anomaliile de dozaj genic şi rearanjările cromozomiale apar frecvent în celulele canceroase. Identificarea prin aCGH a unor segmente cromozomiale am-

plificate, deleții ale genelor supresoare a creşterii tumorale şi deleții sau rearanjări cromozomiale ce implică protooncogene oferă noi informații privind genele implicate în cancer. Datorită rezoluției şi sensibilității mari a aCGH, aplicarea acesteia în evaluarea dezechilibrelor genomice din cancer asigură îmbunătățirea diagnosticului, schemelor de clasificare a tumorilor, descifrării şi monitorizării mecanismului de apariție şi de progresie a neoplaziei (23, 24). Totuşi, în condițiile în care în numeroase forme de cancer apar translocații cromozomiale complexe, aplicarea acestei tehnologii în scop prognostic este limitată, datorită incapacității de a detecta translocațiile echilibrate (13).

Descoperirea unor noi sindroame şi identificarea genelor candidat. În ultimii ani, implementarea aCGH a permis descoperirea de sindroame plurimalformative determinate de microdeleții sau microduplicații cromozomiale. Majoritatea acestor anomalii sunt nedetectabile prin cariotip clasic sau se găsesc în regiuni genomice pentru care nu au fost produse sonde FISH specifice. Utilizarea aCGH pe loturi mari de pacienți a facilitat descrierea unor noi sindroame printr-o „dismorfologie inversă", în care descoperirea anomaliei citogenetice precede definirea fenotipului corespunzător (10). Practic, după analizarea rezultatelor şi definirea unei regiuni cromozomiale absente/ duplicate la un grup de pacienți, fenotipurile acestora sunt comparate pentru a determina trăsăturile clinice esențiale, pe baza cărora pot fi identificați ulterior alți pacienți cu aceeaşi anomalie.

Capacitatea aCGH de a detecta dezechilibre cromozomiale mici facilitează şi identificarea genică. De exemplu, gena CHD7 (implicată în sindromul CHARGE) a fost localizată prin identificarea prin aCGH a două microdeleții suprapuse parțial, la doi pacienți cu sindrom CHARGE (25).

CONCLUZII

Datorită identificării precise a anomaliilor genomice cantitative, cu o rezoluție înaltă şi un „debit" mare, aCGH a revoluționat diagnosticul genetic şi a oferit noi informații pentru elucidarea structurii şi funcției genomului uman în stare normală şi patologică. Tendința actuală în cercetarea genetică este aplicarea pe scară cât mai largă a metodei aCGH, dar utilizarea ei în scop diagnostic rămâne încă limitată de prețul prohibitiv al aparaturii şi consumabilelor. Totuşi, fiind mai rapidă şi având o rezoluție mai mare decât tehnicile de citogenetică moleculară, aCGH va înlocui în mod cert unele dintre aceste tehnici atunci când laboratoarele vor fi dotate cu aparatura necesară.

NOTĂ

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PRESENT AND FUTURE OF RECOMBINANT VACCINE TECHNOLOGIES

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PRESENT AND FUTURE OF RECOMBINANT VACCINE TECHNOLOGIES (Abstract): Vaccination still has the potential to make a bigger contribution to global health. The routine uses in industrialized countries of hepatitis B and human papillomavirus vaccines successfully marked the prevention of liver and cervical cancer, is a very good example. All technological and scientific acquisitions in recombinant DNA technology over the last few years have resulted in new vaccines, thus making vaccination available to an increasing number of patients. Some recombinant vaccines have been already approved for medical use and others are in different stages of preclinical or clinical evaluation. Nowadays, novel approaches are being explored, such as: reverse vaccinology, bioconjugation technology, the use of generalized modules for membrane antigens and the use of RNA vaccines. By modifying the genome of the agents of infectious diseases new vaccines, safer, more effective, lower cost and convenient delivery, will emerge. **Keywords:** RECOMBINANT VACCINES, DNA TECHNOLOGY, RECOMBINANT VECTORS, SUBUNIT VACCINES.

One of the milestones of modern medicine is undoubtedly the vaccines that saved the lives of millions of people. The experience of the last 200 years has shown that the discovery and use of vaccination on a world scale has surpassed in efficiency the discovery of antibiotics or other inventions and achievements in the medical field. Thus, in some countries, because of systematic immunization, there was a significant reduction in morbidity and mortality from diphtheria, tetanus, pertussis, measles, rubella, or yellow fever (1).

Effective vaccines must induce immunity without pathogenesis. In other words, this is done either by attenuating or inactivating viral or bacterial pathogens, or by using the toxin or subunit components of the pathogen. The success of a vaccine depends both on the stability of the antigen and on its ability to stimulate or cause immunological memory (2).

Generally, all these strategies highlight their advantages and disadvantages and

their use depends on knowing the mechanisms of infection of the target pathogen and the immune response required for protection.

This paper will present new approaches in recombinant DNA technology for vaccine development.

A VERY SHORT HISTORY

At the beginning of the 20th century five human vaccines were used: smallpox, rabies, typhoid, cholera and plague vaccines. The golden age in vaccine development began after the World War II, when at least 23 vaccines were discovered and used (1, 2, 3).

In 1986, the first recombinant hepatitis B vaccine was licensed in the United States. Another important moment was the authorization of the recombinant human papillomavirus vaccine (HPV) in 2006. Since then other recombinant vaccines are available. The recombinant human vaccines development is presented in Table I (1).

TABLE I
Development of recombinant human vaccines

Type of genetically engineered vaccines	Approval year
Hepatitis B surface antigen recombinant	1986
Cholera (recombinant toxin B)	1993
Lyme OspA *(withdrawn)*	1998
Human papillomavirus recombinant (quadrivalent)	2006
Human papillomavirus recombinant (bivalent)	2009
Meningococcal group B proteins	2013
Pertussis toxine subunit 1 recombinant (monovalent)	2016
Zoster, adjuvanted (recombinant subunit)	2017

Recombinant vaccine development

Advances in molecular genetics and genetic engineering have made possible the identification of genes encoding the synthesis of certain antigenic proteins. The emergence of recombinant DNA technology has assured extensively the production of polypeptides normally present on the surface of certain pathogens (then used as subunit vaccines).

Some recombinant vaccines have been already approved for medical use (vaccines against hepatitis B monovalent or in combination with other antigens, cholera, human papillomavirus, group B meningococcal disease, *Bordetella pertussis*, herpes zoster) and others are in different stages of preclinical/clinical evaluation, as it can be seen in table II (3).

Vaccine development using recombinant DNA technology requires an in-depth knowledge of the antigens that will induce protection. It is also important to understand the host immune response to ensure that the vaccine induces an adequate immunological response.

Recombinant vaccines can be classified as follows (1, 3):

- live genetically modified vaccines;
- inactivated (subunit) recombinant vaccines;
- genetic (DNA) vaccines.

TABLE II

Recombinant vaccines development stage

Type of vaccines		Examples	Development stage	
			Preclinical or clinical evaluation	Approved
Live vaccines	Recombinant virus	Herpes simplex virus	X	
		Poliovirus	X	
	Recombinant viral vector	HIV	X	
		Ebola virus	X	
		Cytomegalovirus	X	
	Recombinant bacteria	*Vibrio cholerae*	X	
		Shigella	X	
	Recombinant bacterial vector	*Salmonella typhi*	X	
		Vibrio cholerae	X	
		Shigella flexneri	X	
		Streptococcus gordonii	X	
		Listeria monocytogenes	X	
		BCG	X	
Subunit vaccines	Recombinant polypeptides	Hepatitic B virus		X
		Borrelia burgdorferi		X*
		Human papilomavirus		X
		Vibrio cholerae		X
		Bordetella pertussis		X
		Varicella zoster virus		X
		Neisseria meningitidis serogroup B		X
		Herpes simplex virus	X	
		Streptococcus pyogenes group A	X	
		Streptococcus pneumoniae	X	
		HIV	X	
		Allergy	X	
		Diabetes	X	
Genetic vaccines	DNA “naked”	Influenza viruses	X	
		Hepatitis C virus	X	
	DNA facilitated	Hepatitis B virus	X	
		HIV	X	
		Cancer (renal)	X	
		Influenza	X	
	DNA viral delivery	HIV	X	
		Plasmodium spp.	X	
		Cancer (skin and prostate)	X	
	DNA bacterial delivery	*Shigella flexneri*	X	

* withdrawn

Live genetically modified vaccines

This category includes recombinant viruses, recombinant bacteria and recombinant viral and bacterial vectors (3).

Recombinant viruses. In the viral genome specific changes or gene deletions can be made so that the virus will be more attenuated and stable. Thus, by deleting the gene encoding a glycoprotein responsible for pathogenicity of the herpes simplex virus (HSV) a non-replicating HSV strain was obtained, which can initiate infection *in vivo* without being able to replicate. Polioviruses with unoptimized codons in the capsid region produce more attenuated viruses which stimulate the production of antibodies.

Recombinant bacteria. The attenuation strategy consists in deletion of genes responsible for bacterial virulence or modulation of their expression *in vivo*. By attenuation of *Vibrio cholerae* strains live vaccines against cholera were developed. The attenuation was performed by deletion of genes encoding virulence factors (cholera toxin). There have been attempts to develop *Shigella* strains in live vaccines by certain chromosomal or plasmid gene mutations to reduce pathogenicity.

Recombinant viral vectors. The purpose of creating such vectors is to stimulate the immune system to respond to the antigen in the context of a live virus infection. The viral vector vaccinia virus could express human immunodeficiency virus type 1 (HIV-1) by glycoprotein 160. The fowlpox and canarypox viruses had been developed recently as live viral vectors for use in humans. Adenovirus, one of the most potent vector was designed to express foreign polypeptides particularly for HIV and hepatitis C virus (HCV). Another viral vector is alphavirus which can express cytomegalovirus glycoprotein (CMV), being developed as a candidate for CMV vaccine.

Recombinant bacterial vectors. Important for these vectors is to retain sufficient virulence for replication and to express foreign polypeptides to elicit an immune response against pathogens. *Salmonella typhi* vectors are the most used strains, and the strains of *Vibrio cholerae*, *Shigella flexneri*, *Listeria monocytogenes* and BCG vaccine strain were engineered as a live vector for clinical assessment.

Subunit recombinant vaccines

The use of recombinant polypeptides as subunit vaccines is the most widely applied and accepted reliance of recombinant DNA technology for the development of modern vaccines. Subunit vaccines are effective for some pathogens but have poor immunogenicity, thus more doses with higher amounts of antigen are required.

Virus-like particles. The assembly of proteins as virus-like particles (VLPs) was a major progress. The proteins are expressed in yeast, insect, plant or mammalian cells. VLPs, which lack the viral genome, are a highly effective type of subunit vaccines that elicit strong humoral and cellular response and are a much safer alternative.

The first application was hepatitis B surface antigen (HBsAg) obtained by expressing the gene in *Saccharomyces cerevisiae*. The recent trend is to express the gene in transgenic plants (potato tubers, tobacco plants etc.) (4, 5).

Another example of efficient use of VLPs is the L1 protein of HPV obtained by expressing the gene in insect cell lines (*Spodoptera frugiperda*, *Trichoplusia ni*) or in *Saccharomyces cerevisiae* cells after infection with recombinant baculoviruses

(*Autographa californica)* (6).

Natural sequences. The outer surface protein A (OspA) of *Borrelia burgdorferi* expressed in *E. coli* as recombinant protein was approved for Lyme disease prophylaxis (the vaccine was later withdrawn from the market). Recombinant HSV glycoproteins derived from Chinese hamster ovary (CHO) cells and recombinant HIV-1 glycoprotein 120 expressed in CHO cells were clinically evaluated.

Fusion proteins. Recombinant fusion proteins were expressed as multi-epitope peptide vaccines targeting multiple antigens. Fusion proteins from *Neisseria meningitidis* serogroup B, *Streptococcus pneumonia, Streptococcus pyogenes* group A have been evaluated in clinical studies (7, 8).

Noninfectious disease. Nowadays, clinical trials evaluate the utility of vaccines in such noninfectious diseases as diabetes, cancer, allergy etc.

Genetic vaccines

DNA vaccination means that the antigenic molecule is produced in the host by injecting DNA. The antigen encoded gene is linked to a plasmid together with a strong mammalian promoter and then transfected cells express the antigen resulting in an immune response.

"Naked" DNA. A naked DNA encoding a vaccine antigen is injected so cells synthesize an antigen and an immunologic response is induced. Naked DNA vaccine for influenza is in clinical trials and the obtained data are promising. Recently, plasmids encoding HCV NS3/4a (ChronVac-c) (hepatitis C virus) were identified as potential therapeutic vaccines for HCV in animal models (9).

"Facilitated" DNA. To increase cellular uptake of DNA this molecule can be incorporated into microprojectiles and shot into cells via a gene gun ballistic device or coated into gold microparticles and delivered into the epidermis with a needle-free device or coated with cationic lipids which facilitate transport across membranes. These DNA vaccines may be useful in hepatitis B, cancer, flu, SIDA etc.

DNA viral delivery. The poxvirus family (fowlpox virus, canarypox virus) can deliver DNA in avian cells, mammalian cells to express the recombinant protein (10, 11).

DNA bacterial delivery. Some attenuated bacterial species can deliver DNA into mammalian cells for expressing vaccine antigens. A strain of *Shigella flexneri* is in preclinical studies to deliver recombinant plasmids in mammalian cells (12).

NOVEL APPROACHES

A novel approach to produce new vaccines is the use of reverse vaccinology which involves sequencing the genome of a target pathogen and scanning for genes to select the best vaccine antigens by in silico approaches may be useful to obtain new vaccines (13, 14).

Another way to develop vaccines is the bioconjugation technology. Recently, a phase I study revealed that a novel bioconjugate vaccine against *Shigella flexneri* 2a (containing polysaccharide of the *Shigella flexneri* 2a O-antigen conjugated to the exotoxin protein A of *Pseudomonas aeruginosa)* elicit a robust humoral response and induce functional antibodies (15).

The use of Generalized Modules for Membrane Antigens (GMMA) for the development of vaccines against bacterial pathogens is another affordable technology. The production of outer membrane vesicles can be enhanced by genetic muta-

tion which leads to the production of GMMA. A phase II trial evaluated a *Shigella sonnei* vaccine based on GMMA as a delivery system for *Shigella sonnei* O antigen which proved to be highly immunogenic and well tolerated (16).

Finally, is the use of RNA vaccines, both synthetic mRNA and self-amplifying RNA to induce an immune response. Synthetic mRNA encoding only the protein of interest has an immediate translation of the antigen eliciting a good antiviral protection (17, 18). Self-amplifying RNA is virally derived, encodes the antigen of interest and proteins enabling RNA vaccine replication and induces an immune response (19, 20).

CONCLUSIONS

Our study revealed that recombinant vaccines are designed to be safer and more effective than traditional vaccines. By modifying the genome of infectious agents' new vaccines with higher costs will emerge, which will be a major challenge to developing countries.

All the technological and scientific acquisitions in recombinant DNA technology over the last few years have resulted in new vaccines, thus making vaccination available to an increasing number of people. The benefits to society are population immunity, prevention of epidemics and reduction of costs associated with medical care.

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NEWS

IMPACT OF ACUPUNCTURE IN POLYCYSTIC OVARY SYNDROME

This randomized clinical trial compares the effect of true acupuncture with metformin and sham acupuncture on the insulin sensitivity of women with polycystic ovary syndrome. The study lasted 4 months, and included 3 days per week of true acupuncture (profound needls stimulated manually and electrically) or sham acupuncture (superficial needles without manual or electrical stimulation) and 3 times per day metformin or placebo. The primary objective of the study is to demonstrate that the treatment with true acupuncture and metformin placebo improves insulin sensitivity. Some protocols have been used in the treatment application: regular physical exercise, a balanced diet, fixed acupuncture protocols, and measurements have been applied that have been statistically analyzed to determine the efficacy of the treatments. Finally, it was confirmed that true acupuncture which combines Electrical and Manual Acupuncture improves insulin sensitivity, hormone levels and anxiety in patients with PCOS. (Li J, Hung Yu Ng E, Stener-Victorin E *et al*., Acupuncture treatment for insulin sensitivity of women with polycystic ovary syndrome and insulin resistance: a study protocol for a randomized controlled trial. *Trials*. 2017; 18: 115).

Valerica Volosan (Ilies)

HIGH-RESOLUTION RESPIROMETRY WITH MULTIPLE SUBSTRATES TITRATION IN PERMEABILIZED MYOCARDIAL FIBERS

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HIGH-RESOLUTION RESPIROMETRY WITH MULTIPLE SUBSTRATES TITRATION IN PERMEABILIZED MYOCARDIAL FIBERS (Abstract): The present study was purported to standardize the high-resolution respirometry technique for the analysis of oxidative phosphorylation (OXPHOS) in saponin-skinned cardiac fibers. Preparation of permeabilized myocardial fibers allows the assessment of respiratory function of the entire population of mitochondria in their normal intracellular position. Adult male rat ventricular bundles were permeabilized with saponin (50 µg/ml) and samples (1-3 mg wet weight) were transferred into the Oxygraph-2k (OROBOROS Instr., Austria) chambers containing air saturated incubation buffer in order to measure Complex I (CI) and II (CII) dependent respiration. The following values (expressed in pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$) were obtained: CI_LEAK, 67.18±5.12 (CI dependent basal respiration, after glutamate and malate addition); CI_P, 247.37±49.90 (CI_OXPHOS state after ADP addition); CI_Pc, 252.036±53.13 (the intactness of the outer mitochondrial membrane assessed after cytochrome *c* addition); CI+II_P, 342.90±62.48 (maximum OXPHOS state obtained after succinate addition by activating convergent electron flow from CI+II into the Q junction of the electron transport system (ETS); CII_P, 302.26±50.16 (CII dependent OXPHOS state obtained after the addition of a CI inhibitor - rotenone, with the subsequent entry of electrons from CII only into the Q junction); ETS capacity, 331.11±62.39 (uncoupled respiration). The standardized technique will allow the systematic characterization of mitochondrial respiratory dysfunction associated with several cardiac pathologies in both animals and humans. **Key words:** HIGH-RESOLUTION RESPIROMETRY, PERMEABILIZED MYOCARDIAL FIBERS.

Cardiovascular diseases and coronary heart disease, in particularly, remain the leading cause of mortality due to myocardial infarction and morbidity due to heart failure worldwide. Mitochondria are recognized to be directly involved in pathophysiology of ischemia-reperfusion injury (1-3) and also in cardioprotection (4). In the past decades, analysis of mitochondrial respiratory (dys)function has become a common research tool in basic cardiology, and strategies able to reversibly modulate respiration in order to protect mitochondria during the postischemic reperfusion are widely investigated (5).

The present study was aimed to standardize the technique for preparation of permeabilized fibers from rat hearts view subsequent studies of high-resolution respirometry (HRR). Standard procedures of

mitochondria isolation based on differential centrifugation allow precise characterization of several mitochondria functions (6). However, one of the most important drawbacks of this technique is related to the fact that some of these functions (including the respiratory one) are impaired by the isolation steps; these effects can be even more pronounced in mitochondria isolated from tissue damaged by a pathological process, such as ischemic injury as well as other cardiac pathologies (6).

At variance from the classical method of differential centrifugation, in saponin-skinned cardiac fibers, intracellular structures (cytoskeleton, contractile apparatus) are well preserved and the morphological appearance of mitochondria is similar to the one *in vivo* (7). Last but not least, the latter method uses only small amounts of tissue (1-3 mg wet weight) making it particularly useful for human studies (8).

MATERIAL AND METHODS

1. *Principle of selective plasma membrane permeabilization*

Cholesterol-complex forming agents such as saponin interact with cholesterol molecules which are abundantly present in plasma membrane. This interaction induces a loss of membrane integrity (permeabilization) and the barrier between the intracellular space and surrounding medium disappears (9). Since the cytosol is washed out, the composition of the intracellular space is equilibrated with the experimental incubation medium (10). Mitochondria cholesterol content is considerably low (11, 12) and the cholesterol-complex-forming agent in the concentration used for cell membrane permeabilization does not disrupt these organelles, allowing thus investigation of their function (Fig.1).

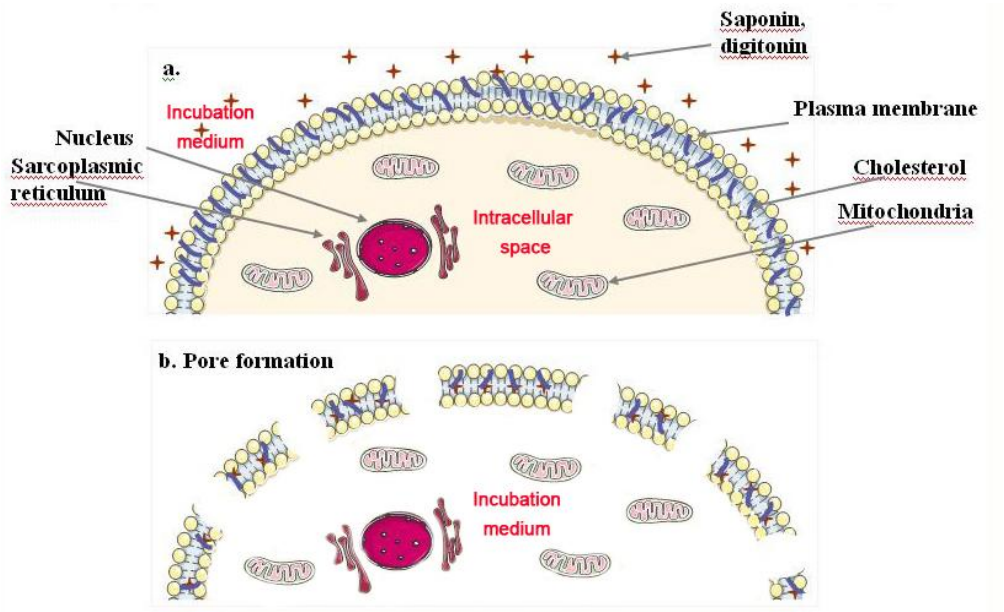

Fig. 1. Principle of selective plasma membrane permeabilization: (a) cells before and (b) after treatment with saponin. (Figures were produced using *Servier Medical Art*)

2. Preparation of permeabilized rat myocardial fibers

All investigations conformed to the Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Ethics Committee of our university.

Adult male rats (n=12, 250-350 g) were anesthetized with xylazine (5 mg/kg) and ketamine (30 mg/kg) I.P. Hearts were rapidly excised and immersed in the isolation buffer containing 10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phosphocreatine (pH 7.1 adjusted with 5 N KOH at 0°C). Atria were discarded and ventricular muscle strips were further dissected into the ice-cold buffer to form thin bundles (a period of maximum 4 minutes is required for the preparation of fiber bundles, thus avoiding damage to the external mitochondrial membrane). The ventricular tissue should remain connected in a mesh-like framework (Fig. 2 A and B).

The fiber bundles were further transferred into ice-cold isolation buffer, containing 50 μg/ml of saponin stock solution (5 mg/ml) and shaken by gentle agitation on ice for 30 min (13). After this period, all samples were transferred into the incubation buffer (0.5 mM EGTA, 3 mM MgCl2.6H2O, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH2P04, 20 mM HEPES, 110 mM sucrose, 1 g/l BSA, essentially fatty acid free, pH = 7.1, at 30 °C + 280 U/ml catalase lyophilized powder, 2,000-5,000 units/mg protein H2O2) and further gently shaken for 10 min on ice (in order to wash out the saponin excess). Fiber bundles were then weighted (optimal amount in the oxygraph chamber for one experiment is between 1-3 mg wet weight) (14).

a. b.

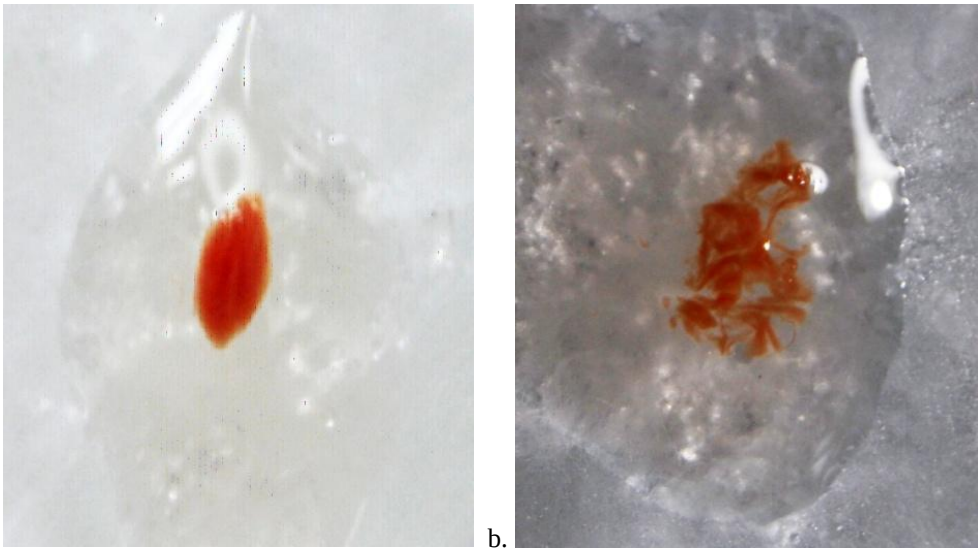

Fig. 2. Steps of isolation of ventricular cardiac fibers:
(a) Ventricle strip in a Petri dish placed on ice.
(b) Mesh-like framework of thin rat ventricle bundles before saponin permeabilization.

3. Measurements of respiratory rates by high resolution respirometry

At the beginning of the experiment oxygen sensors were calibrated with MiR06 at experimental temperature in the "open" chamber (gas phase above the stirred medium, with a partially inserted stopper) of the Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria). Samples were quickly transferred into the Oxygraph-2k chambers containing air saturated incubation buffer. Respiratory flux of muscle fiber bundles is limited by oxygen diffusion even above 50% air saturation (O_2 concentration of 100 μM) (15). Other authors also recommended the prevention of the oxygen limitation in permeabilized fibers experiments by maintaining oxygen levels in the chamber in the range of 200–500 μM (14, 16). In our experiments we used a high-oxygen regimen: before closing the chamber, the oxygen concentration was increased to 300–400 μM, by injecting pure O_2 through the stopper. Five to ten minutes were required for stabilization of the signal. During the recording period, we prevented oxygen drop below 200 μM by intermittent H_2O_2 titrations (100 μM).

Substrate combinations that match physiological intracellular conditions were applied for the evaluation of coupling control, OXPHOS and ETS capacity at 37°C, respectively. The Substrate-Uncoupler-Inhibitor Titration (SUIT) protocol used was as follows: Addition of glutamate and malate as complex I (CI) substrates – a step referred as basal respiration (the classical state 2) or CI_LEAK state (CI_L); Addition of ADP to activate oxidative phosphorylation (OXPHOS) – a step known as active respiration (the classical state 3) and is further referred as CI_OXPHOS state (CI_P); Cytochrome c addition to assess the intactness of the outer mitochondrial membrane (CI_Pc). Mitochondrial integrity may become compromised during mechanical separation of fibers, resulting in damage to the outer membrane and release of cytochrome c which then becomes a limiting factor in mitochondrial respiration. Since cytochrome c cannot penetrate an intact outer mitochondrial membrane, addition of exogenous cytochrome c represents a quality control measure of mitochondrial integrity (17). The absence of an increase of respiration rate after adding 5-32 μM cytochrome c indicates a high quality preparation; Succinate, a complex II (CII) substrate addition to further stimulate the respiration by activating convergent electron flow from CI+II into the Q junction, a step referred as maximum CI+II OXPHOS capacity (CI+II_P); Rotenone (CI inhibitor) addition to measure OXPHOS state with entry of electrons from CII only into the Q junction (CII_P); ATP synthase inhibition with oligomycin (Omy); Uncoupled respiration obtained by FCCP titration, a step known as Electron Transport System capacity (ETS); Inhibition of respiration with antimycin A (Ama), a state referred to as Residual OXygen capacity (ROX state).

Most of the chemicals were purchased from Sigma-Aldrich and were used without further purification. Results are expressed as means ± S.D.

RESULTS AND DISCUSSION

Twelve animals were used and a number of 4 tracings were recorded for each animal according to the above mentioned protocol. Each day 2 animals were sacrificed in order to perform respirometry measurements. No significant differences were found among the daily values for respiration states and RCR, respectively (*p*

> 0.05, ANOVA).

A representative tracing of the SUIT protocol for high-resolution respirometry (HRR) measurements in permeabilized fibers is depicted in Figure 3.

Our results showed the following values for basal, CI_L, 67.18 ± 5.12 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight and, active respiration, CI_P, 247.37 ± 49.90 $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight, respectively. Respiratory control ratio (RCR), a good parameter indicating the coupling between oxygen consumption and oxidative phosphorylation was further calculated as the ratio between OXPHOS and LEAK respiration rates. Accordingly, RCR for NAD-linked substrates was 3.65±0.51, values similar to the ones reported in literature (9, 18, 19).

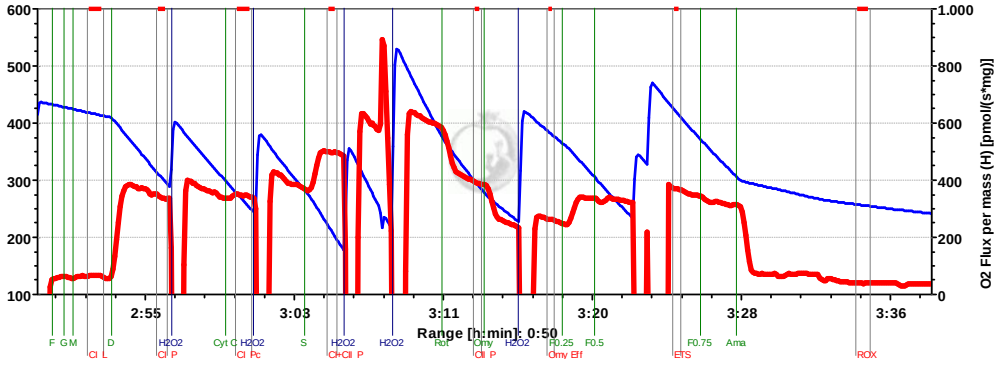

Fig. 3. Original trace of a SUIT protocol in permeabilized cardiac fibers

Oxygen concentration (upper line, left scale) and O_2 flux per mass (lower line, right scale) are represented as a function of time. The additions are as follows:

G – glutamate (10 mM), **M** – malate (0.2 mM), **D** – ADP (5 mM), **Cyt C –** cytochrome c (10 µM), **S** – succinate (10 mM), **Rot** – rotenone (0.5 µM), **Omy** - oligomicin (2 µg/µl), **F** – FCCP titration in steps of 0.5 µM optimal concentration, **Ama** – antimycin A (2.5 µM).

The addition of cytochrome *c*, used as validation of proper tissue preparation, showed an increase with 1.88 ± 6.47 % (CI_Pc, 252.036 ± 53.13 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight), values that are also in agreement with data reported by other researchers; slight elevations in respiratory rates of 5–15% are considered acceptable (8). However, in ischemia/reperfusion experiments an increase in respiration rates after cytochrome *c* addition due to the damage of mitochondria especially during postischemic reperfusion is generally admitted (20, 21). Indeed, the damage of the outer mitochondrial membrane represents an early event in ischemia/reperfusion injury of the heart (20, 22).

In classic respirometry experiments, conventional measurements of state 3 with either NAD+ (CI) or FAD (CII) linked substrates, were reported to underestimate OXPHOS capacity (23). Therefore, in our experiments of HRR, in order to obtain the maximal ADP-stimulated mitochondrial capacity, we measured respiration depending on both CI and CII, by adding succinate immediately after cytochrome *c* (CI+II_P, 342.90 ± 62.48 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight). Establishing the maximum cou-

pled respiration state provides the proper basis for the evaluation of specific enzymatic defects in mitochondrial respiratory physiology and pathology (23).

Inhibition by rotenone determined a decrease in OXPHOS capacity since the electron input is through CII exclusively (CII_P, 302.26 ± 50.16 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight). Addition of oligomycin (which blocks the proton channel of ATP synthase, thus inhibiting ADP phosphorylation) was used not only to check alterations in LEAK respiration, but also the integrity of the inner mitochondrial membrane that could eventually be damaged during the mechanical preparation of fibers (8). Sensitivity of oxygen to oligomycin suggests the intactness of the inner mitochondrial membrane (Fig. 3).

Uncoupled respiration was obtained by stepwise titration of carbonyl cyanide-p-trifluorocarbonyl-cyanide methoxyphenyl hydrazone (FCCP) with an ETS value of 331.11 ± 62.39 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$ wet weight. Increments in O_2 flux above CII_P indicates a limitation in respiratory capacity by ATP synthase, adenine nucleotide translocases or phosphate transporters since under the effect of FCCP, mitochondrial oxidative capacity through complex II can be determined in the absence of the potential control exerted by all of these (24).

The limitations of the present technique are briefly mentioned. During the permeabilization process the cytosolic components of the cardiac fibers which regulate mitochondrial function are lost, resulting in the inability to assess mitochondria in an exact *in vivo* environment; however, this limitation is also encountered in experiments using isolated mitochondria. *In situ* mitochondrial respirometry assesses the total mitochondrial population without distinguishing between the two subpopulations (subsarcolemal and interfibrillar) that can be separated by successive centrifugations and are reported to be differentially affected by ischemia/reperfusion injury (8). Another important issue which must be taken into consideration in order to compare different literature data is that respiratory measurements may be expressed per wet weight or dry weight. We have chosen to express our data in wet weight, since fiber wet weight is easily measured before their addition into the Oxygraph chamber, while measurement of dry weight depends on the complete recovery of the samples after the experimental measurements (23).

CONCLUSIONS

We have standardized for the first time in our country the high-resolution respirometry technique in saponin-permeabilized cardiac fibers. The utilization of HRR according to the SUIT protocol is a highly reproducible method that allows the systematic assessment of mitochondrial respiratory function while cellular architecture is preserved. This method will be further used to characterize abnormalities in mitochondrial function in both animal and human cardiac pathologies and, also to investigate the effects of cardioprotective interventions.

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STUDIU COMPARATIV ÎNTRE TIMPII DE REACŢIE PSIHOMETRICI ŞI POTENŢIALELE EVOCATE VIZUALE COGNITIVE ÎN LEXIE

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REACTION TIMES (RTs) AND COGNITIVE VISUAL EVOKED POTENTIALS DURING READING – A COMPARED STUDY (Abstract): **Objective**: The main aim of the experiment is to prove the Paivio's theory about the concrete-abstract effect. **Methods**: A psycho-verbal stimulation interface, created by us, was experimented as an IT extension of an *EEG / EMG device* dedicated for the human brain Evoked Potentials acquisitions *(EPs)* and reaction times techniques in reading mechanisms assessment. **Results**: The shortest reaction time was achieved in tests at which the reading has no access to the meaning of words, for concrete word, in both hemisphere. But, in left hemisphere the reaction times for abstract words was shorter than for the abstract word in right hemisphere. EPs acquisition exhibits more negativity of N400 for concrete word and more reverberation of P650 – N750 for abstract words. **Conclusions**: The difference in mean reaction times sustain the Paivio's theory and the difference in amplitude of N400, P650-N750 for concrete and abstract nouns show that the electric activities of brain are correlated in time and in amplitude with the same effort of processing the words. The psycho-verbal stimulation interface can be used as a medical research tool for studying and assessment the cognitive processes of reading, memory or learning using the endogenous visual event related potentials and the psychometric reaction times.
Key words: PSYCHO-VERBAL VISUAL EVOKED POTENTIALS (EPs), PSYCHOMETRIC REACTION TIMES (RTS), READING PROCESS.

În domeniul psiho-lingvistic s-au făcut o serie de determinări în timpii de reacţie psihometrici, privind procesarea informaţiei vizuale şi auditive în funcţie de parametrii psihologici individuali şi de dezvoltarea neuropsihică. Metoda determinării vitezei de reacţie psihometrice a fost folosită de asemenea în evaluarea performanţei sportive şi ca metodă de antrenament încă din anii '50. Metoda nu oferă totuşi o evaluare obiectivă a performanţei de procesare senzorială, datorită implicării lanţului motor în răspuns. Timpul de reacţie nu a putut oferi date directe privind mecanismul neuropsihic al procesării informaţiei lexicale, a mecanismului recunoaşterii, memoriei şi nici a mecanismelor patolingvistice implicate în afectarea limbajului, în special în afazii (1).

Apariţia tehnicii de potenţiale evocate a reprezentat un moment de cotitură în evaluarea neinvazivă a integrităţii analizatorilor. Totuşi, metoda în varianta clasică, utilizând stimuli nediferenţiaţi, nu dă date privind procesarea sensorial-semantică a in-

formației (2).

În anii ‘80 - 90, utilizarea stimulilor cu încărcătură semantică (imagini, cuvinte) au permis corelarea unor componente tardive ale potențialelor evocate - P300, N400 cu procesarea psihică a informației recepționate vizual. Tehnica, deşi nu are standarde de implementare clinică, este în prezent folosită în cercetarea proceselor neurocognitive, pentru buna rezoluție temporală a procesării stimulului cognitiv şi, alături de tehnicile neuroimagistice PET, SPECT, constituie instrumentele de bază pentru desluşirea mecanismelor limbajului, memorie şi învățării (3).

Teoria codificării duale a informației a lui Paivio (1971, 1986, 1991), postulează existența unor sisteme distincte de procesare a stimulilor verbali şi neverbali, dar interconectate funcțional şi probabil anatomic (4). Conform teorii codificării duale, toți stimulii verbali concreți sau abstracți sunt inițial procesați de un sistem unic de reprezentare verbală. Cuvintele concrete au acces la ambele sisteme: în sistemul de asociație verbală, cât şi în sistemul de asociație non-verbal. De aceea, cuvintele concrete generează cu uşurință imagini mentale. Cuvintele abstracte au în special conexiuni asociative doar în sistemul verbal şi de aceea nu generează cu uşurință imagini mentale. Implicarea celor două sisteme în decodificare, şi prin aceasta, şi în reprezentarea semantică, determină o performanță superioară de procesare a cuvintelor concrete în teste ce impun recunoaşterea sau evocarea - redarea semantică a informației (5). Teoria nu face precizări privind distribuția celor două sisteme în creierul uman (6). Obiectivul experimental a fost demonstrarea existenței unor structuri şi mecanisme cerebrale distincte în procesarea diferitelor categorii lexicale şi gramaticale în zone cerebrale diferite, exprimată în determinările timpilor de reacție şi componentele tardive N400, P650, N720 a potențialelor evocate vizual printr-o metodă în premieră în România. Originalitatea metodei a fost utilizarea stimulilor psiho-verbali în culegerea potențialelor evocate, corelată cu determinarea vitezei de reacție printr-o interfață hardware-software de concepție personală.

MATERIAL ŞI METODĂ

Studiul a fost efectuat pe un lot de 20 de persoane dreptace, cu studii superioare, cu vârste între 20 şi 40 de ani, cu abilități lexicale şi vizuale integre. Persoanele au fost selecționate prin discernământ voluntar şi au fost plătite pe perioada experimentului. La înscrierea în experiment, fiecare participant a prezentat o adeverință de la specialistul oftalmolog privind integritatea funcției vizuale şi a completat un chestionar care a probat calitățile de dreptaci. Comisia de etică universitară a aprobat experimentul prin faptul că este neinvaziv şi nu implică modificări ale discernământului, ale stării de sănătate sau de conştiență a indivizilor testați.

Metodele folosite au fost de stimulo-detecție a timpii de reacție psihometrici şi a potențialelor evocate vizuale cognitive, utilizând o interfață de stimulare psiho-verbală concepută şi brevetată, conectată la un aparat EMG - MYTO I – EBNeuro. Stimularea s-a realizat cu 500 de cuvinte cu frecvență lexicală crescută, fără domeniu specific, prezentate 1000 ms, pauză 1000 ms, font 120 galben pe fond negru, expunere stabil reperată (colț stânga-jos ecran) la 60 cm la nivelul central al câmpului vizual (fig.1). S-au folosit 8 liste-cuvinte integrate aleator (4 liste pentru timpii de reacție şi 4 liste pentru achiziția de potențiale evocate)

pentru fiecare din cei 20 de subiecți (10 bărbați şi 10 femei): în listele 1 - 4 s-au folosit substantive concrete imaginabile, iar în listele 5- 8 au fost introduse substantive abstracte neimaginabile. Timpii de reacție au fost determinați prin stimulare în hemicâmp vizual, iar potențialele evocate cognitive s-au înregistrat prin stimulare binoculară.

Montajul şi setările achiziției au fost următoarele: electrozii referință (+) Cz, activul (-) Oz, *ground nasion*, înregistrare pe 4 canale, sensibilitate canal 2 μV, filtre cut-down 10 Hz, cut-up 3kHz, *sweep* 100 ms, impedanța electrozi < 10 kΩ, achiziție prin mediere coerentă

Fig. 1. Procedura achiziției de potențiale evocate cognitive vizuale

REZULTATE

Determinările s-au efectuat în paralel pentru timpii de reacție şi pentru potențialele evocate. Timpii de reacție s-au efectuat în hemicâmp vizual şi au fost comparați ca timpi de reacție medii. S-a determinat media lor la cuvinte concrete, abstracte, şi mediile timpilor de reacție la cuvintele cu 1- 4 silabe (7).

Determinările în potențiale evocate au vizat identificarea componentelor tardive P300, N400, P650, N720 şi studiul comparativ în latență, durată şi amplitudine în stimulare cu cuvinte concrete şi abstracte.

Sarcina subiectului în efectuarea testului a vizat accesul semantic lexical prin identificarea cuvintelor cu apartenență categorială (ex. cuvinte exprimând *ustensile*) şi lipsa accesului semantic lexical prin testul de identificare literală.

S-au obținut următoarele diferențe în timpii de reacție medii: timpii medii de reacție sunt mai buni în lipsa accesului lexical; sunt direct proporționali cu numărul silabelor; viteza medie de reacție la cuvintele concrete a fost mai bună decât la cuvintele abstracte. Un procent de 20% din timpii de reacție la cuvintele concrete sunt mai mari decât cei la cuvintele abstracte şi acest fapt s-a observat în cazul cuvintelor cu frecvență lexicală redusă. În privința determinărilor în hemicâmp vizual, timpul de reacție mediu la cuvintele concrete din hemicâmpul vizual drept este sensibil egal cu cel de la cuvintele abstracte în hemicâmpul vizual drept. Viteza de reacție medie la cuvintele abstracte din hemicâmpul vizual drept este mai bună decât cea de la cuvintele abstracte în hemicâmpul stâng (*idem Day 77*). Timpul de reacție mediu la cuvintele concrete în hemicâmpul vizual stâng este sensibil egal cu timpul de reacție mediu la cuvintele concrete în hemicâmpul vizual drept. Valorile la cuvintele concrete sunt mai mici decât cele de la cuvintele abstracte în hemicâmpul vizual stâng (tab. I) (*idem Chiarello, Senehi & Nuding 1987*) (2).

TABELUL I

Timpii de reacție medii

Timp reacție	***Cuvinte concrete***	***Cuvinte abstracte***
Emisfer drept	370 ms ± 20 ms	490 ms ± 30 ms
Emisfer stâng	380 ms ± 20 ms	390 ms ± 35 ms

Din punct de vedere statistic există puternice diferenţe semnificative între timpul de reacţie la cuvinte abstracte în emisferul drept faţă de cuvintele concrete din acelaşi emisfer ($p < 0,001$). Comparând aspectul negativităţii N400 se observă că este mai pronunţat în cazul cuvintelor concrete, în schimb pentru cuvintele abstracte sunt mai pronunţate P650 şi N720 la 75% din subiecţii testaţi (fig. 2).

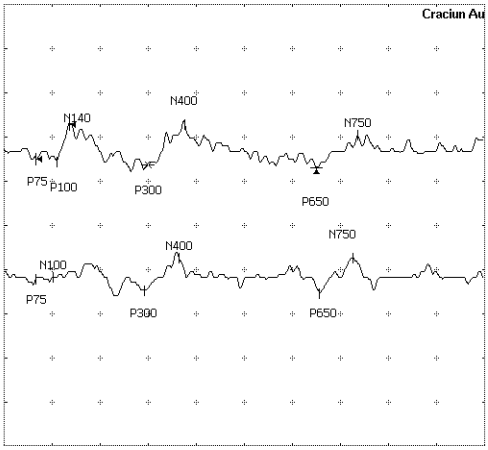

Fig. 2. Potenţiale evocate cognitive la stimularea prin cuvinte concrete (sus) şi prin cuvinte abstracte (jos)

DISCUŢII

Experimentul în timpii de reacţie verifică validitatea teoriei lui Paivio cu privire la procesarea superioară a cuvintelor concrete. Teoria lui Paivio nu oferă o explicaţie de ce cuvintele concrete sunt procesate la fel de bine biemisferic. O ipoteză ar fi că legăturile dintre sistemul non-verbal şi cel verbal ar fi la fel de bine realizate biemisferic încât sistemul verbal permite cuvintelor concrete un acces direct şi o procesare identică biemisferică. Paivio arată că nu concretețea ci imaginabilitatea cuvintelor conferă o performanţă superioară în recunoaştere. Un număr neglijabil de cuvinte concrete au fost cotate puţin imaginabile şi un număr redus de cuvinte abstracte au fost cotate imaginabile. Procesele imaginative sunt generate de ambele emisfere cerebrale, de aceea cuvintele concrete sunt amintite identic când sunt prezentate pe fiecare hemicâmp (1).

Teoria nu explică de ce cuvintele abstracte sunt procesate mai lent de emisferul drept (hemicâmpul stâng) faţă de emisferul stâng. O explicaţie ar fi: cuvintele abstracte au acces iniţial direct doar la sistemul verbal cu localizare predominantă în emisferul stâng, unde conexiunile cu sistemul nonverbal sunt mai puternice, ceea ce permite o procesare mai rapidă pe emisferul stâng şi o specializare pentru procesarea abstractă la dreptaci. Dovezile clinice şi anatomice arată că totuşi în procesele de memorizare şi evocare, rolul primordial îi revine regiunii temporale drepte (hipocamp).

CONCLUZII

Interfaţa de stimulare psiho-verbală este o extensie indispensabilă achiziţiei de potenţiale cognitive. Standardizarea tehnicii impune criterii flexibile adaptate mediului lexical al individului, ceea ce impune colaborarea cu neurolingvişti. Interfaţa de stimulare psiho-verbală asigură posibilitatea studiului proceselor cognitive asociate lexiei, recunoaşterii, memorie şi validitatea unor teorii susţinute de alte tehnici (10).

Studiul în PE a aspectului abstract-concret susţine procesarea diferită a cuvintelor concrete şi abstracte. Cuvintele concrete generează o negativare mai amplă şi de mai lungă durată a lui N400 faţă de cuvintele abstracte (8). Cuvintele abstracte generează reverberaţii tardive la P650 şi N720 (9). Efectul este mai amplu în testele cu acces lexical semantic faţă de cele fără acces (recunoaştere literală). La 25% din subiecţi nu s-a identificat modificările

amintite ceea ce sugerează existența unor *pattern*-uri diferite de procesare, dar nu se exclude posibilitatea culegerilor similare în alte derivații, sau statusul psihologic şi implicarea diferită a subiecților.

Experimentul a arătat că există o corelație între studiile de potențiale evocate şi timpii de reacție în procesarea aspectului concret - abstract. Experimentul nu a vizat studiul lateralității în utilizarea PE, dat fiind lipsa evidențierii lateralității cerebrale în lexie. Se poate confirma existența unor generatori mai extinşi implicați în limbaj (modelul Hebbian), orice *pattern*-ului electric poate avea ca origine diverse configurații ale surselor sau poate fi expresia atenuării prin craniu şi creier a volum - conducerii electrice.

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EXPRESIA RECEPTORILOR HORMONALI (A-ESTROGEN, B-ESTROGEN ŞI PROGESTERON), Ki-67 ŞI p 53 ÎN ENDOMETRUL PACIENTELOR CU CARCINOM MAMAR TRATAT CU TAMOXIFEN

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EXPRESSION OF HORMONAL RECEPTORS (α-ESTROGEN, β-ESTROGEN, PROGESTERON), Ki-67 AND P 53 IN ENDOMETRIUM OF TAMOXIFEN TREATED BREAST CANCER PATIENTS (Abstract): Tamoxifen is the most commonly prescribed adjuvant therapy for women with breast carcinoma ER+. It has agonist activity on the endometrium and is associated with an increased risk of endometrial carcinoma. The aim of this study was to evaluate the immunohistochemical changes on the histological endometrial modification from the patients treated with tamoxifen. **Material and method**: A group of 2o women selected from 125 patients with breast carcinoma, with adjuvant therapy. The criteria of selection were the histopathological proliferative changes of endometrium from these symptomatic patients. The 20 specimens of endometrium were evaluate immunohistochemycal by estrogen receptor alpha, beta, progesterone receptor, Ki-67 and P53. **Results and conclusions**: The 20 endometrium specimen showed histological proliferative endometrial lessions, including polyps, hyperplasia and carcinoma. Immunohistochemical, benign lessions showned expression of estrogen α and β receptor, low expression Ki-67 and absent expression of P53. These tests were negative in clear cell carcinoma, but were intens positive for Ki-67 and P53. In general, tamoxifen associated endometrial adenocarcinoma were characterized by a lower expression of estrogen receptor alpha, higher expression of progesterone receptor, and more frecquent expression of estrogen receptor beta than endometrial spontaneous tumors. **Key words**: ENDOMETRIUM, TAMOXIFEN, EXPRESSION OF ESTROGEN RECEPTORS, PROGESTERON, Ki-67, P53.

Prin efectele sale antiestrogenice, tamoxifenul este utilizat ca tratament adjuvant pentru carcinomul mamar cu receptori pozitivi la estrogeni (ER+). Într-un studiu anterior (1) am prezentat modificările endometriale histopatologice curente la 125 paciente simptomatice cu carcinom mamar (ER+).

În acest studiu ne-am propus să evidenţiem expresia receptorilor hormonali: α -estrogen, β – estrogen şi progesteron. De asemenea, prin utilizarea reactivilor Ki-67 şi P 53 am încercat să stabilim tendinţa la proliferare a endometrului de la aceste paciente.

MATERIAL ŞI METODĂ

Din 125 paciente tratate cu tamoxifen, în 20 cazuri, pe secţiunile histologice ale produselor de chiuretaj au foat aplicaţi

reactivi imunohistochimici: receptori α- şi β- estrogeni, progesteron, Ki-67 şi p53.

Toate pacientele au prezentat hemoragii uterine disfuncţionale atât în postmenopauză, cât şi în premenopauză în cursul tratamentului cu tamoxifen de 40 mg zilnic într-o perioadă cuprinsă între 8 şi 72 de luni. Diagnosticul histopatologic a fost precizat pesecţiunile colorate cu hematoxilin-eozină.

Pentru evidenţierea imunohistochimică (IHC) a antigenelor tisulare, metoda utilizată este bistadială, bazată pe un sistem de vizualizare cu reţea polimerică (DAKO EnVision) (tab. I).

TABELUL I

Tehnica imunohistochimica de evidenţiere a antigenelor tisulare

Antigen	Clona	Specificitate	Producător	Diluţie	Pretratament
ER alfa	CloneSP1	nucleii celulelor epiteliale în receptorii de estrogeni α	DAKO, Carpinetria CA, USA	1:200	Fierbere în target Retrieval high pH, DAKO
ER beta	Clone PP65/10	nucleii celulelor epiteliale în receptorii de estrogeni β	DAKO, Carpinetria CA, USA	1:10	Fierbere în target Retrieval S 1700
PGR	Clone 16	nucleii celulelor epiteliale glandulare şi stromale	Novocastra Leica-microsystems. com	1:100	Fierbere în tampon cittrat pH 6
Ki 67	Ac monoclonal 410-5M	Trusă de lucru NovoLink Min Polymer Delection System (50 tests)			
P 53	Ac monoclonal 195-5M				

REZULTATE

Diagnosticul histopatologic curent al celor 20 de paciente evidenţiază tendinţa la proliferare a endometrului ceea ce a determinat selectarea lor pentru IHC (tab. II)

Rezultatele imunohistochimice au relevat reacţie pozitivă la receptorii de estrogeni α şi β - dar mai intensă la β – în nucleii celulelor epiteliale glandulare atât în polipii adenomatoşi endometriali şi hiperplazie (fig. 1), cât şi în adenocarcinoamele endometrioide diferenţiate (fig. 2).

TABELUL II

Diagnosticul histopatologic al celor 20 de paciente

Nr crt.	Diagnostic histopatologic	Nr paciente
1	Polipi adenomatoşi endometriali	4
2	Hiperplazie endometrială simplă	6
3	Hiperplazie endometrială complexă	3
4	Hiperplazie endometrială complexă atipică	1
5	Adenocarcinom endometrioid diferenţiat	5
6	Adenocarcinom mixt endometrioid diferenţiat şi carcinom cu celule clare solid	1

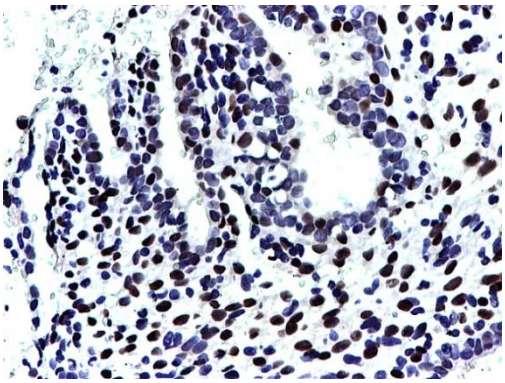

Fig. 1. Hiperplazie endometrială simplă. Reacţie intens pozitivă în nucleii celulelor epiteliale şi al unor celule stromale. (IHC - receptor de progesteron, ob. x20)

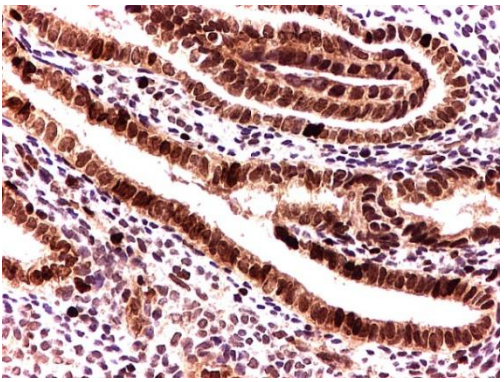

Fig. 2. Hiperplazie endometrială complexă. Reacţie intens pozitivă în nucleii celulelor epiteliale (IHC-receptor beta-estrogeni, ob. x20)

În ariile de carcinom endometrial cu celule clare marcajul pentru receptori α şi β estrogeni a fost negativ. Receptorii pentru progesteron au fost intens pozitivi în nucleii celulelor epiteliale şi în celulele stromale (fig. 3) din polipi, hiperplazii şi adenocarcinoamele endometrioide şi negativ în ariile de carcinom cu celule clare (fig. 4).

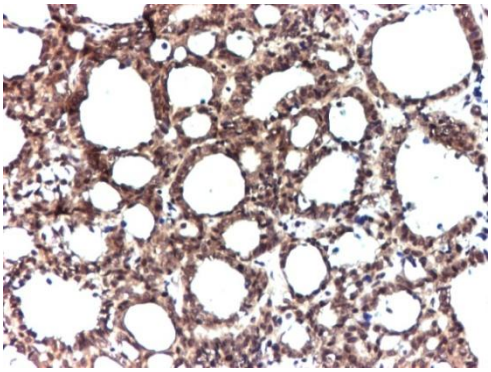

Fig. 3. Carcinom endometrioid diferenţiat. Marcaj intens pentru receptorii beta estrogeni din nucleii celulelor epiteliale. (ob. x10)

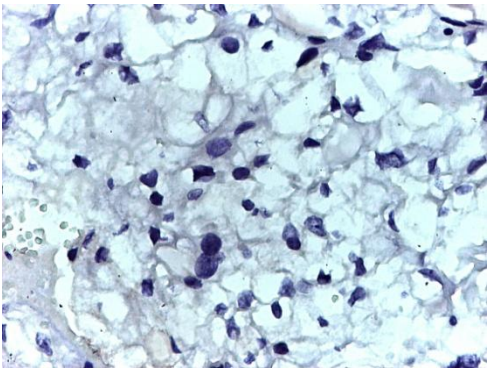

Fig. 4. Carcinom endometrial cu celule clare Receptor β estrogeni negativi. (ob. x20)

Marcajul pentru Ki-67 a fost slab în focar în polipi şi hiperplaziile simple, foarte intens şi continuu în toate tipurile de adenocarcinom (fig. 5).

În ceea ce priveşte proteinele genei mutante p 53, acestea au fost absente în polipii endometriali şi hiperplaziile complexe, moderat în nucleii adenocarcinoamelor endometrioide şi intens în carcinomul endometrial cu celule clare (fig. 6) (tab. III).

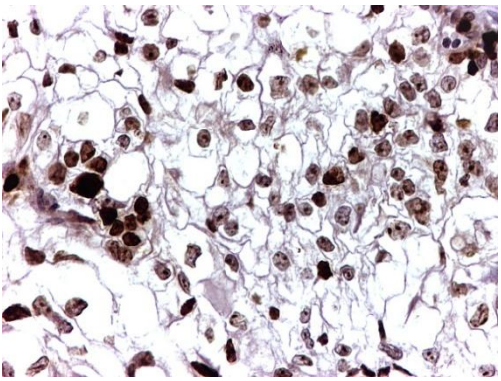

Fig. 5. Carcinom endometrial cu celule clare. Marcaj intens pozitiv pentru Ki 67 în nucleii celulelor tumorale. (ob. x20)

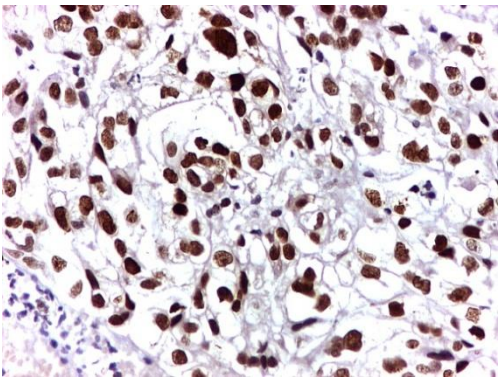

Fig. 6. Carcinom endometrial cu celule clare. Marcaj intens pentru p53. (ob. x20)

În ceea ce priveşte proteinele genei mutante p 53, acestea au fost absente în polipii endometriali şi hiperplaziile complexe, moderat în nucleii adenocarcinoamelor endometrioide şi intens în carcinomul endometrial cu celule clare (fig.6) (tab. III).

TABELUL III

Diagnostic imunohistochimic*

Nr. crt.	Tipul de leziune	Receptori α-estrogen	Receptori β-estrogen	Receptori progesteron	Ki-67	p53
1	Polipi adenomatoşi endometriali	+ +	+ + +	+ + +	+ în focar	N
2	Hiperplazie endometrială	+ +	+ + +	+ + +	+ în focar	N
3	Adenocarcinom endometrioid grad 1,2	+ +	+ + +	+ + +	+ +	N
4	Carcinom cu celule clare endometrial	N	N	N	+ + +	+ +

(*) N = negativ; intensitatea marcajului: +, ++, +++.

DISCUŢII ŞI CONCLUZII

Numeroase studii au arătat că utilizarea tamoxifenului ca adjuvant în terapia post-mastectomie pentru carcinom de glandă mamară creşte riscul apariţiei unor modificări histologice endometriale cu caracter proliferativ (în general benigne) până la leziuni premaligne şi maligne (2, 3). Tamoxifenul are un efect asupra endometrului când este în competiţie cu secreţia ovariană de estrogeni, dar concomitent are un efect paradoxal estrogenic în absenţa unei secreţii estrogenice ovariene (4).

Expresia receptorilor de estrogeni (ER) şi progesteron e moderată până la intensă în carcinoamele endometrioide, dar este absentă sau slab exprimată în carcinoamul cu celule clare (5, 6).

De asemenea, expresia ER şi PR e redusă în carcinoamele endometrioide slab diferenţiate (grad 3), ca şi în carcinoamele seroase şi cu celule clare (7).

În leziunile proliferative endometriale (polipi şi hiperplazie) s-a observat o tendinţă relativ redusă la marcajul cu Ki-67 şi negativă la p53. În adenocarcinomul endometrioid diferenţiat grad 1,2 se constată un marcaj moderat pentru Ki-67 şi p 53.

Un test IHC pentru p 53 permite recunoaşterea proteinei p 53 anormală, ce rezultă dintr-o mutaţie a genei, cu acumularea în nucleii celulelor tumorale, probabil printr-o degradare scăzută sau stabilizare.

Există expresia intensă a imunoreactivităţii p 53 în adenocarcinoamele endometrioide în paralel cu creşterea gradelor. Astfel, adenocarcinoamele de grad 3 se vor colora intens (8).

Carcinomul cu celule clare prezintă supraexpresia Ki-67 şi marcaj p 53 intens.

În general, studiul IHC a arătat că adenocarcinomul endometrioid, care apare în cursul terapiei cu tamoxifen se caracterizează printr-o expresie mai scăzută a receptorilor α estrogeni şi mai intensă a receptorilor de progesteron şi mai frecventă a receptorilor β estrogeni decât în tumorile endometriale spontane.

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PPARS : STRUCTURĂ, MECANISME DE ACȚIUNE ŞI DE REGLARE

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PPARs: STRUCTURE, MECHANISMS OF ACTION AND CONTROL.Note I (Abstract). PPARs (peroxisome proliferator activated receptors) are proteine receptors that act as transcription factors activated by ligands. There are three known isoforms of PPARs (α, β/δ, γ) with similar modulated structure, consisting of distinct regions with specific functions. PPARs activate transcription of their target genes by forming cytoplasmatic heterodimers (PPARs:RXR) with his partner RXR (retinoid X receptor), and once translocated into the nucleus bind to specific DNA sequence called PPRE (peroxisome proliferator response elements) and modulate the expression of genes. Each PPAR is differently expressed in various tissues. Modulatory function of PPARs is induced by natural or synthetic ligand binding. Additional activator proteins are recruited to form a complex that coordinates and regulates the expression of many genes.. Moreover, nuclear receptors' activity is also regulated by posttranslational changes. **Key words**: PPARs, STRUCTURE, LIGANDS, COREGLATORS, POSTTRANSLATIONAL CHANGES

PPARs: (peroxisome proliferator-activated receptors) sunt reprezentați de o familie de receptori nucleari (NR) proteici care acționează ca factori de transcripție activați de ligand (1, 2).

Nomenclatura, tipuri şi distribuția PPARs.

Subfamiliei de receptori nucleari PPARs aparțin 3 membri (izotipuri, izoforme) principali : PPARα (NR1C1= NR subfamilia 1, grupa C, numărul receptorului), PPAR β/δ (NR1C2) cunoscut şi sub alte denumiri – NUC-1 (nucleare abnormal-1) sau FAAR (fatty acid activated receptor) şi PPARγ (NR1C3) (3).

Aceste izoforme, codificate de gene specifice separate, etalează distribuție tisulară, funcții fiziologice şi profile farmacologice distincte care depind de genele țintă.

- PPAR α se exprimă preponderent în țesuturile cu catabolism activ al acizilor graşi : ficat, inimă, muşchi, țesut adipos, rinichi, precum şi în celulele endoteliale vasculare, VSMC (vascular smooth muscle cells), monocite/macrofage din peretele arterial (1, 4).
- PPAR β/δ este distribuit ubicuitar (5) în principal în creier, țesut adipos, piele.
- PPAR γ se exprimă în mai multe forme (γ1, γ2, γ3, γ4, γ5, γ7,)(6, 7) distribuite astfel :

- γ 1 se exprimă în toate țesuturile, incluzând inimă, muşchi, colon, rinichi, pan-

creas, splină ;

- γ 2 se exprimă în pricipal în țesutul adipos ;

- γ 3 se exprimă în macrofage, intestin gros, țesut adipos alb ;

- în macrofage au fost identificate tipurile de PPAR γ4, γ5, γ7 (γ6 nu a fost detectat) sugerând contribuția la funcția macrofagului (8).

În multe tipuri de celule se exprimă mai mult decât o izoformă.

Trăsăturile structurale ale PPARs şi mecanism de acțiune.

În ciuda diferențelor de localizare şi funcționale, PPARs prezintă aceeaşi structură generală (9). Ca şi alti receptori nucleari, au structură modulară cu mai multe domenii funcționale distincte (A/B, C, D, E)(domeniul F este absent din PPARs) (10), tipică membrilor superfamiliei de receptori nucleari (9,11) (Fig. 1):

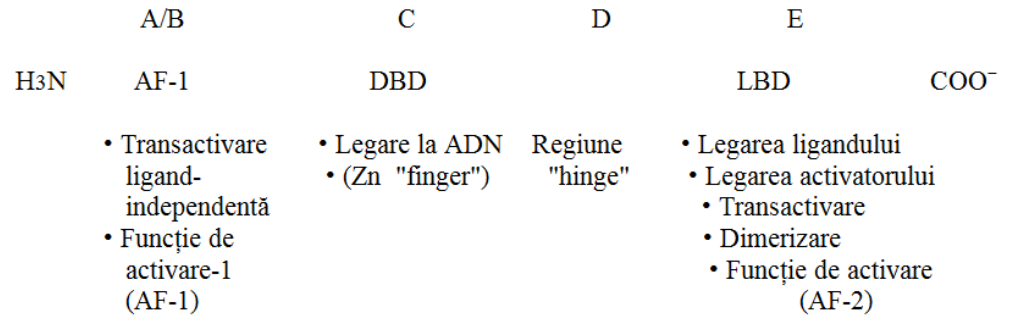

Fig. 1. Structura clasică a PPARs

Pentru fiecare din aceste domenii au fost stabilite funcțiile cheie (12).

● Domeniul A/B : regiune N-terminală, cu secvență foarte variabilă între diferite tipuri de receptori, este dinamică, lipsindu-i structura pliată în ciuda proprietății de activare a genei (13). Are funcție activatoare transcripțională independentă de ligand denumită AF-1 cu specificitate tisulară care leagă cofactorul. Activarea tisulară este foarte slabă, dar sinergică cu a AF-2 din domeniul E (LBD) (14).

● Domeniul C: DBD (DNA binding domain) este un domeniu înalt conservat, cu o structură caracteristică de meandru (helix-loop-helix) stabilizată de 2 atomi de Zn (Zn "finger") care asigură interacțiunea cu o secvență specifică **genomică** (tipar) numită PPRE (peroxisome proliferator response elements). În citozol PPARs formează cu pertenerul lui RXR (NR2B) (retinoid X receptor) heterodimerul PPARs:RXR care translocat în nucleu este recunoscut şi fixat pe secvența PPRE din ADN (15). Asocierea PPARs cu RXR apare independentă de legarea ligandului iar nu necesită întotdeauna legarea la ADN (16).

PPRE amplasat în protomerul genei țintă este constituit din repetarea unei secvențe scurte alcătuită din două unități hexamere – AGGTCA**N**AGGTCA (DR-1 şi DR-2 – direct repeat-1 şi direct repeat-2), spațiate de 1-2 nucleotide oarecare (**N**) (17).

● Domeniul D ("hinge", balama), nespecific, conectat la DBD asigură flexibilitatea între partea N-terminală şi C-terminală a moleculei, este implicat în legarea corepresorului (10).

● Domeniul E: LBD (ligand binding

domain) cu o structură moderat conservată în secvenţă, dar înalt conservată în structura tridimensională (18). LBD este o regiune complexă care conţine câteva activităţi bine integrate şi interdependente: un buzunar (cavitate) hidrofob (ă) de legare a liganzilor naturali sau sintetici, o interfaţă de dimerizare şi o interfaţă de transactivare ligand-dependentă (AF-2)(14).

Funcţiile PPARs sunt determinate de configuraţia precisă a domeniului care leagă ligandul (activatorul) indusă după legarea acestuia şi de un număr de proteine auxiliare denumite cofactori (corepresori şi coactivatori) a căror prezenţă potenţează transcripţia sau inhibă funcţia receptorului respectiv (19) şi mediază contactul cu heterodimerul PPAR:RXR, cromatină şi "maşinăria" transcripţională (20, 21, 22).

Acţiuni non-genomice

PPARs prezintă unele mecanisme de acţiune, mai puţin aşteptate, la nivel non-genomic (independente de ADN). Aceşti receptori pot media efecte represive indirecte denumite transrepresie. Transrepresia poate fi determinată de inhibarea legării factorilor de transcripţie la ADN prin interacţiune proteină-proteină (16) sau prin sechestrarea cofactorilor necesari activităţii lor (23, 16).

Mecanisme de reglare transcripţională

Controlul transcripţional este un proces care se desfăşoară în mai multe etape, reflectat, de specificitatea şi disponibilitatea ligandului, recrutarea cofactorilor, modificarea posttranslaţională care afectează stabilitatea şi potenţa receptorului.

Liganzii sunt compuşi naturali sau sintetici care induc o conformaţie distinctă a receptorului. Există o oarecare specificitate între liganzi şi subtipurile de PPARs determinată de structura şi aminoacizii din buzunarul de legare a izotipurilor de receptori (24).

În categoria liganzilor naturali endogeni pentru cele trei izoforme de PPARs sunt incluşi: PPARs α: acizi graşi şi compuşi derivaşi de la acizi graşi, eicosanoide naturale derivate de la acid arahidonic pe calea lipoxigenazei ca leucotriena B4 şi fosfolipide xidate din lipoproteine oxidate (25); PPARγ: acizi graşi şi compuşi derivaţi de la acizi graşi. De asemenea este receptor pentru metaboliţi eicosanoidici formaţi pe calea ciclooxigenazei precum prostaglandine (PGJ2, PGH1, PGH2) (26) şi pe calea lipoxogenazei; PPARα, -γ, -β/δ sunt receptori pentru acizi graşi nesaturaţi. În plus de exprimarea preferenţială în anumite ţesuturi, PPARs sunt senzori capabili de adaptare a exprimării genei la diferiţi stimuli: stres şi ritmul circadian (27), post (28), frig (29). De asemenea, exprimarea PPAR este afectată de insulină şi corticosteroizi (30), diferite citochine (31).

Liganzii sintetici (agoniştii sintetici) sunt reprezentaţi de hipolipidemiante, antiinflamatoare, compuşi insulino-sensibilizatori, antidiabetice. Agoniştii sintetici ai PPARs sunt folosiţi în tratamentul unor afecţiuni metabolice precum diabetul de tip 2 şi dislipidemie. Glitazonele antidiabetice (rosiglitazone, pioglitazone), sensibilizatori de insulină sunt liganzi cu mare afinitate pentru PPARγ (32). Fibraţii, droguri hipolipidemice, sunt liganzi PPARα (25). Beneficiile clinice incontestabile ale agoniştilor PPARγ în tratamentul diabetului de tip 2 sunt dublate de efecte colaterale precum creşterea în greutate şi edem, fapt ce a stimulat interesul pentru obţinerea de modulatori care să reţină eficacitatea de sensibilizatori la insulină dar să minimalizeze efectele adverse (33).

Recent s-au sintetizat o serie de agonişti PPAR cu mare afinitate subtip-specifici

(34). Diversitatea liganzilor găzduiţi în buzunarul de legare a ligandului precum şi varietatea modificărilor induse de ligand, reflectă şi diversitatea funcţiilor în care PPARs sunt implicaţi.

Reglarea PPARs de cofactori

În afară de configuraţia precisă a domeniului de legare a ligandului (activatorului) indusă după legarea acestuia, funcţiile PPARs sunt influenţate şi de un număr de cofactori (coactivatori şi corepresori) a căror prezenţă poate stimula sau inhiba funcţia receptorului respectiv (8, 20) prin legarea factorilor de transcripţie la "maşinăria" transcripţională şi modificarea specifică a structurii cromatinei (21). Cofactorii (coreglatorii) sunt proteine funcţionale care asigură controlul transcripţional PPAR-mediat al genelor ţintă (12, 20). In cursul ultimilor 15 ani au fost identificaţi mai mult de 300 de cofactori (corepresori şi coactivatori) care interacţionează cu receptorii nucleari şi le mediază activitatea transcripţională (35), dar funcţia reglatorie fiziologică a multora dintre aceste molecule rămâne de explorat.

Corepresori**.** În absenţa ligandului, heterodimerul receptorului nuclear este asociat cu corepresorul (i), proteină (e) care inhibă proprietatea transactivatoare. În această stare inactivată, în unele dar nu în toate tipurile de celule, PPARS pot fi localizaţi mai curând în citozol decât în nucleu . Heterodimerul formează complexe cu corepresori precum NCoR (nuclear receptor corepressor), RIP 140 (receptor interacting protein 140) sau SMRT (silencing mediator of retinoid and thyroid receptors) ce recrutează histon deacetilaza, determinând represia transcripţiei (12, 36, 37). Este posibil să prezinte un rol şi interacţiunile cu "maşinăria" bazală transcripţională (38). Altă clasă de corepresori ligand-dependenţi sunt recrutaţi prin intermediul unor secvenţe scurte L/VXX-I/V-I (L=leucina, V=valina, I=izoleucina, X=un aminoacid oarecare) sau LXXXI/LXXXI/L (19) care represează activitatea receptorului prin competiţie cu coactivatori. Deşi corepresorii prezintă o mare diversitate structurală, mecanisme de represie şi funcţii fiziologice, ei recrutează efectori din aval precum histon deacetilaza pentru a-şi exercita efectele represive (19).

Coactivatori**.** Transactivarea este indusă prin mecanisme ligand-dependente şi ligand-independente, acestea din urmă mai puţin înţelese. Recent s-au identificat proteine NCoEx (nuclear corepressor exchange factors) (39), care asociate cu receptorul nuclear favorizează schimbul corepresor cu coactivator, schimb ce promovează tranziţia de la starea represivă la cea activă. Legarea ligandului induce o modificare conformaţională a heterodimerului care exclude corepresorii din complex şi expune locuri de legare ale coactivatorului nedisponibile în absenţa ligandului. Coactivatorii conţin 3 secvenţe de LXXLL care mediază recunoaşterea şi legarea la AF2 (40), dar nu toţi coactivatorii utilizează această secvenţă pentru interacţiunea cu diferiţi receptori nucleari.

PPARs interacţionează cu un mare număr de complexe proteice coactivator cu multiple subunităţi cu funcţii diferite: unele prezintă activitate histon acetiltransferazică sau metiltransferazică intrinseci, altele facilitează remodelarea cromatinei ATP-dependentă sau folosesc ca punţi de legare cu "maşinăria" transcripţională. Perturbarea exprimării coactivatorilor poate afecta funcţia PPARs (20).

Proteinele implicate în modificarea cromatinei sunt complexele SWI/SNF (switch/ sucrose non-fermeting) (16) sau factori

care modifică covalent histonele prin acetilare, metilare,fosforilare, ubiquitinare, modificări ce pot apare într-un mare număr de combinaţii, adesea în mod interdependent (41). În consecinţă, recrutarea coactivatorilor duce la răspunsuri biologice distincte (42).

Reglarea PPARs prin modificare posttranslaţională

În plus de liganzi şi cofactori, activitatea PPARs este reglată prin modificări posttranslaţionale care pot contribui la afectarea activităţii acestor receptori nucleari.

Fosforilarea. La nivel molecular, efectele fosforilării PPARs-mediate prin diferite mecanisme, includ modificarea afinităţii pentru liganzi (43), RXR (44,) şi cofactori (45) care adesea se datorează modificării conformaţionale a întregului receptor (46). PPARα şi PPARγ sunt fosfoproteine, a căror grad de fosforilare afectează activitatea transcripţională (47).

Ubiquitinarea. Ubiquitina este o proteină mică care se leagă covalent la proteine expunându-le la degradare mediată de proteasomi (48). Ubiquitinarea şi degradarea PPARs proteasomi-mediată sunt legate de activitatea transcripţională a receptorului nuclear prin reglarea nivelului celular al receptorului. Spre exemplu, tiazolidindionele induc ubiquitinarea receptorului PPARγ şi ulterior degradarea de proteasomi (49), fibraţii inhibă ubiquitinarea şi cresc timpul de înjumătăţire a proteinei PPARα (49).

Sumoilarea. SUMO (**s**mall **u**biquitin-like **mo**difiers) alcătuit dintr-o familie de proteine omologe cu ubiquitina reglează diferite mecanisme celulare, inclusiv represia transcripţiei (50). Represia transcripţională de către SUMO este în general mediată de recrutarea corepresorilor şi a histon deacetilazei (51).

Nitrarea resturilor tirozil din PPARγ inhibă translocarea acestuia din citozol în nucleu (52) reducându-i capacitatea de a influenţa transcripţia genei.

Izoformele PPARs codificate de gene separate care se deosebesc prin distribuţie, selectivitate şi sensibilitate la liganzi şi recrutarea de proteine distincte (corepresori şi coactivatori) contribuie la reglarea diferitelor seturi de gene specifice (53) implicate în variate funcţii celulare metabolice şi non-metabolice: metabolism energetic (lipidic, glucidic, proteic), termogeneză, diferenţiere celulară, precum şi în exprimarea genelor cheie în diferite stări de boală (16, 54, 55, 56).

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BROMINATION-A VERSATILE TOOL FOR DRUGS OPTIMIZATION

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BROMINATION-A VERSATILE TOOL FOR DRUGS OPTIMIZATION (Abstract): Scientific literature presents many naturally occurring brominated substances exhibiting different biological activities. Most natural brominated derivatives have been isolated from the marine biosphere, being metabolites of algae, sponges and corals. These molecules became structural models for obtaining synthetic derivatives with superior bioactive potential. The present paper is an analysis of previously published studies on the influence of insertion of bromine atoms in different molecules on their bioactivity. In general, the results confirmed the positive effect of bromination on the bioactive potential of substances. The mechanisms explaining these results are still insufficiently elucidated, several possibilities being mentioned: increased lipophilicity and permeability through biological membranes, increased half-life or the possibility of intermolecular bond formation (attractive interactions) between the electrophilic region of the molecule containing bromine atoms and various nucleophilic active sites of biomolecules. In conclusion, bromination may be a way to increase the potency of therapeutic agents and research in the field of molecular design of brominated compounds will certainly continue. **Keywords**: BROMINE DERIVATIVES, BIOLOGICAL ACTIONS, DRUG DESIGN.

There is constant preoccupation for discovering new therapeutic agents, both naturally-occurring and synthetic compounds. The substances isolated from the natural environment can become structural models for the synthesis of new derivatives, this research field being an area of great interest. The present review focuses on the importance of bromine-containing compounds and is divided into two main sections: naturally occurring bromo-derivatives and synthetic bromo-derivatives with important biological activities. Many brominated derivatives are mentioned and characterized in the scientific literature. They were isolated from natural sources or chemically obtained and exhibited many biological activities. Nowadays, computational tools have been intensively employed in the field medicinal chemistry, QSAR (Quantitative Structure-Activity Relationship) modelling being the most popular. There is evidence linking the presence of halogen atoms in molecules with a positive influence on various biological activities, halogenation becoming an important tool for drug optimization (1).

Naturally occurring bromo-derivatives

In the late nineteenth century, Drechsel, cited by Murphy (1), was the first to report

the isolation of a halogen-containing natural product - diiodotyrosine - from the coral *Gorgonia cavolii*. In 1968, around 50 examples of halogenated natural products were reported. The discoveries in this area have increased exponentially over the last decades, changing the perception that organohalogens found in the environment were mostly of anthropogenic origin. Most of the halogen-containing natural substances were isolated from the marine biosphere (2, 3). The marine biosphere is a medium rich in halogenated compounds, about 5000 being identified so far. Marine organisms (algae, sponges, corals) produce a wide range of bioactive metabolites in response to ecological pressure (4), 45 percent of them being bromo-metabolites due to the high concentration of bromine in sea water (1). Excellent reviews on marine natural compounds were recently published (2, 4, 5, 6).

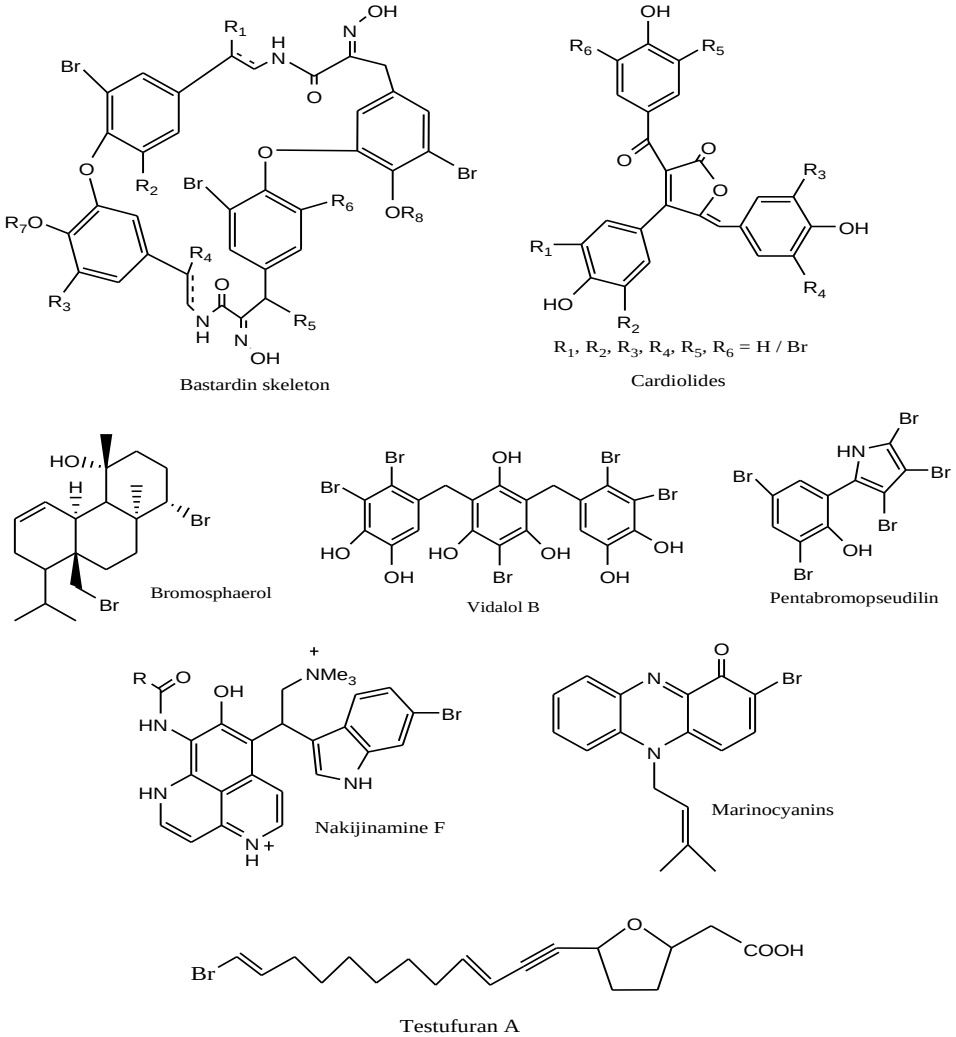

Fig. 1. Representative natural brominated compounds

Chemically, the bromo-metabolites of marine flora include phenols (vidalol A and B), tyrosine derivatives (bastadins), furanones (cadiolides), pyrrole derivatives (pentabromopseudilin), diterpenes (bromosphaerols), alkaloids (keramadine), phenazinone derivatives (marinocyanins), imidazole derivatives (massadine), indole derivatives (nakijinamines), polyunsaturated lipids (xestonarienes), carboxylic acids (testufuran A), glycopeptides (theonellamide G), macrolides (callyspongiolide), brominated monocarbocyclic oxylipins (bromovulone). In figure 1 are presented some of the most important bromine-containing natural molecules. The purpose of the current review is not an exhaustive presentation of the brominated compounds isolated from natural sources, but to stress upon the diversity and biological importance of these compounds.

Bromo-compounds found in the natural environment exhibited a wide range of biological activities: antibacterial, antifungal, antiparasitic, antiviral, antitumor, antioxidant, anti-inflammatory and enzymatic activity (protein kinase inhibition, acetylcholinesterase inhibition) (2, 7, 8, 9, 10, 11). Several natural brominated substances were also active inhibitors of bacterial biofilm formation (bromoageliferin, brominated furanones, bromopyrrole alkaloids) (12).

Synthetic bromo-derivatives with important biological activities

The large number of natural brominated metabolites with many applications in the pharmaceutical field encouraged the research in synthesis and biological evaluation of bromine-containing compounds. There are many studies proving that adding halogen atoms to a pharmacophore can be beneficial for its bioactivity, halogen insertion being considered a traditional tool in drug optimization. In recent years, there is an ever-growing interest in halogenated drugs.

The halogenation degree also influences the lipophilicity and cell membrane solubility of the compounds. Surface activity and permeability coefficient are other parameters used to show that halogenation of drugs improves membrane binding, permeation and diffusion in general. *In vivo* half-life of the substance is also influenced (13, 14). The usefulness of a drug depends on its ability to reach appropriate receptors in the target tissue and remain metabolically stable to produce the desired effect. In this regard, halogenated enkephalin analogues involved in antinociceptive processes were synthesized to improve the stability and bioavailability of the compounds. The study was focused on the investigation of the effect of halogenation on stability, lipophilicity and *in vitro* blood-brain barrier permeability of the novel enkephalin analogues. Bromine and chlorine additions significantly enhanced *in vitro* blood-brain barrier permeability, providing evidence for improved delivery to the central nervous system (14).

Besides the steric and lipophilic contributions of halogens, organobromines also have the capacity to form halogen bonds (non-covalent interactions) with different biomolecules, improving drug-target binding affinity. Studies confirmed that halogen bonding interactions are responsible for different conformations of molecules in the active site. Halogen bonds also affect binding between drugs and transport proteins, playing an import role in distribution. In

addition, halogen bonds were found to influence metabolic and detoxification processes (15, 16).

Several biological actions were distinguished for bromine-containing compounds: antimicrobial, antioxidant, antitumor, antiplasmodial, gastroprotective.

Antimicrobial activity. Infectious diseases currently represent a great concern for the medical world. The increase in the occurrence of different infections overlaps with the overprescribing phenomenon that has made antibiotics less effective and microbes resistant to one or more antimicrobial agents. In this context, there is a constant search for new antimicrobial agents. There are many examples of compounds with bromine atoms that exhibited a good antimicrobial potential. QSAR studies analyze the comparative biological effects of different substances depending on the nature of the substituents present in their molecules and there is substantial data supporting the hypothesis that the presence of bromine substituents has a positive influence on the biological activity.

Many natural derivatives present antimicrobial activity. The fimbrolides isolated from *Delisea pulchra* inhibit the quorum sensing (QS), a regulatory communication system between bacteria of several bacterial species and are a good starting point for chemical manipulation. Steenackers *et al*. demonstrated the importance of bromo-substituents on fimbrolides for the bacterial QS inhibitory activity, the introduction of a bromine atom drastically enhancing the activity of the furanones (17). Other brominated heterocycles (furanones, pyrrolones and pyridazinones) were synthesized as antibacterial agents, with anti-quorum sensing activity (18). The dibromomethylene group was found to be important for the anti-QS activity of some pyridazinones, disrupting bacterial cell-to-cell communication and affecting biofilm formation (19).

Based on the natural model provided by bromophenolic dimers isolated from red algae of *Rhodomelaceae* family, several brominated resorcinol dimers with antibacterial activity were synthesized. The presence of bromine atoms on the phenol ring was important for the antimicrobial activity (20).

Several halogenated derivatives of 3-methyleneflavanone were synthesized and the substitution of one ring with chlorine and bromine atoms was found to enhance antibacterial activity, the chloro- and bromo-analogues being approximately twice as effective as their parent compounds against *S. aureus*, and four times more active against *Enterococcus faecalis* (21).

The bromination of 2-(2′-hydroxybenzoyl) pyrrole led to a series of derivatives, related to monodeoxypyoluteorin, whose antimicrobial activity against *Staphylococcus aureus* increased with the degree of halogenation. The most active member of the series was the pentabromo-derivative, exhibiting a MIC (minimum inhibitory concentration) against *S. aureus* ATCC 25923 of 0.005 μg/mL and promising activity against preformed *S. epidermidis* and *S. aureus* biofilms (22).

New compounds with Bromo pyrrole and 1,3,4-oxadiazole moieties were synthesized and their antimicrobial potential was determined. The hybrids showed promising antibacterial activity against *S. aureus*, *E. coli* and *Mycobacterium tuberculosis* and antifungal potential (23). Of the nitrovinylfurans, 2-bromo-5-(2-bromo-2-nitrovinyl)

furan was remarked for its broad-spectrum antibacterial and antifungal properties. The bromo(nitro)methyl unit was found to be essential for the antibacterial activity and MurA enzyme inhibition (24). It was observed that 5- and 6-bromo substituted compounds possessing a central benzimidazole ring showed a good antimycobacterial activity, probably due to the higher hydrophobicity of bromine, making the molecule more lipophilic and more cell-permeable. 5- and 6-bromo-1-((phenyl) sulfonyl)-2-((4-nitrophenoxy) methyl)-1H-benzimidazoles are potential antitubercular agents (25).

The antimicrobial activity of three new ferrocene derivatives containing 2,3-dihydroquinolin-4(1H)-ones was tested. They showed a very strong and unselective activity against all tested microorganisms (Gram-positive and Gram-negative bacteria and *Candida albicans*). The bromine-containing compound, the most active of all three, had MIC values lower than tetracycline for all bacterial strains (26).

Four N-cinnamoyl amino acid amides were brominated and the antimicrobial activity of the resulting α, β-dibromohydrocinnamoyl amino acid amides against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* was tested. Two of the compounds were active against *S. aureus*, being good candidates for further studies, one of them emerged as a highly active agent, displaying a strong inhibition potential against *E. coli* as well (27). There are also reports that the bromination of the double bond of cinnamic acid and its derivatives caused remarkable enhancement of the antimicrobial activity (28, 29).

Staneva *et al*. described the peripheral modification of a second-generation polypropylene amine dendrimer with eight 4-bromo-1,8-naphthalimide units and its copper and zinc complexes, revealing promising antimicrobial activities against some pathogenic Gram-positive and Gram-negative bacteria and *Candida lipolytica* yeasts (30).

Salicylanilides presented significant *in vitro* antimicrobial and antifungal activities. The influence of different substituents on the salicylanilide molecule on the antimycobacterial and antimicrobial activities was highlighted. The presence of chlorine and bromine atoms on the salicylic ring had a positive impact on bacterial growth inhibition. Bromine derivatives were more active than their chlorine analogues, regardless of the position of the halogen, most likely because of the higher calculated ClogP values. 5-bromo-2-hydroxy-N-(4-(trifluoromethyl) phenyl)-benzamide and 5-chloro-2-hydroxy-N-(4-(trifluoromethyl) phenyl)-benzamide were the most active salicylanilides (31).

Eight brominated 2'(4')-nitro-3-hydroxydiphenyl ethers were analyzed in terms of their antimicrobial potential and the compounds with more halogen atoms had a higher antibacterial activity than those less halogenated (32).

Antioxidant potential. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases, counteracting the negative effects of excess free radicals. Several research groups have proved that halogens are important for cellular antioxidant activity.

Barettin (isolated from the marine sponge *Geodia barretti*) is a molecule with a polar arginine and a non-polar brominated tryptophan end, presenting a strong

antioxidant activity, while its debrominated synthetic analogue did not display the same activity, indicating that bromine is important for the antioxidant potential (33). Similarly, the bromination of flavonoids gave derivatives with a higher free radical scavenging activity and higher lipophilicity than the parent compounds. Brominated flavonoids can diffuse easily through membranes, increasing the intracellular concentration of the compounds (34).

Another study analyzed the antioxidant properties of some phenolic Schiff bases and the impact of different substituents on the antioxidant activity. The conclusion of the study was that bromine substitution may slightly enhance the antioxidant activity (35). The results of a research concerning the antioxidant activity of a series of quinazolin-4-(3H)-ones showed that brominated compounds exhibited a better activity than unsubstituted ones. 6,8-dibromo-2-phenyl-3-(4-phenylthiazol-2-yl) -quinazolin-4(3H)-one exhibited the highest scavenger activity among the tested compounds (36). The evaluation of the antioxidant activity of some 1,2,4-triazole derivatives revealed that the insertion of a halogen atom (chlorine or bromine) had a positive impact on the antioxidant potential of the compounds (37, 38).

Although most studies indicate a positive influence of bromine atoms in the molecule, there are also opposite results in literature. For example, a series of chalcone fatty acid esters were synthesized and their antioxidant potential was evaluated. The results indicated that the addition of bromine to the double bond in the molecule caused the antioxidant activity to decrease (39). Another research team evaluated the antioxidant activity of some 3,5-disubstituted-2-pyrazolines. They concluded that the presence of halogens as substituents caused a reduced antioxidant activity (40). In another paper, the antioxidant and radical scavenging effects of some bromophenols were investigated, the activity exhibited by phenols with bromine and without bromine atoms being similar (41).

Anti-tumor activity. Cancer is responsible for a large percentage of all deaths worldwide and the percentage is still increasing. Therefore, there is a constant need for new compounds that can serve as leads for new anticancer drugs. New antitumor therapies point towards new compounds that demonstrate selective anticancer actions, exhibiting a cytotoxic effect on malignant cells, without damaging normal cells. The synthesis of brominated derivatives is considered a common way to gain cellular potency of anti-cancer agents, several bromine-containing compounds with cytotoxic potential being reported in literature. Pipobroman is an anti-cancer drug with bromine atoms in the molecule that probably acts as an alkylating agent.

Paclitaxel is an antitumor agent approved by the Food and Drug Administration for the treatment of several cancer forms. Nano-formulations of paclitaxel have been investigated for safer and more effective chemotherapy. Lipophilic paclitaxel conjugates bearing carbon chains at the 2′-position have the advantage of an increased stability. The incorporation of a bromine atom in the paclitaxel conjugates was also evaluated, the bromoacyl derivatives being 50 to 250-fold more cytotoxic than the molecules without Br in the human MCF-7 breast carcinoma cell line, indicat-

ing Br-induced hydrolysis. Peng *et al*. studied the 2′-(2-bromohexadecanoyl)-paclitaxel conjugate (Br-C16-PX) incorporated into lipid-based nanoparticles for the treatment of non-small cell lung cancer. The Br-C16-PX nanoparticles had a superior therapeutic efficacy and an improved pharmacokinetic profile compared to paclitaxel in the orthotopic xenograft model, the bromine group on the fatty acid chain enabling faster hydrolysis to release the active substance (42).

Noscapine, a microtubule-interfering agent, can arrest mitosis, to induce apoptosis and has a potent antitumor activity. Two brominated derivatives of noscapine, 5-bromonoscapine ((S)-3-((R)-9-bromo-4-methoxy-6-methyl-5,6,7,8-tetrahydro (1),(3) dioxolo(4,5-g)isoquinolin-5-yl)-6,7-dimethoxyiso-benzofuran-1(3H)-one) and reduced 5-bromonoscapine ((R)-9-bromo-5-((S)-4,5-dimethoxy-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetra-hydro-(1),(3)di-oxolo-(4,5-g) isoquinoline), exhibited higher tubulin binding activity than noscapine and were able to arrest cell cycle progression at mitosis at concentrations much lower than noscapine. 5-Br-noscapine and reduced 5-Br-noscapine were active in inhibiting the proliferation of various human cancer cells, including those that were resistant to paclitaxel and epothilone (43). 9'-bromonoscapine is known as EM011 and it was introduced in clinical trials. The efficacy of EM011 was also assessed in CNS malignancies - low grade gliomas (a heterogeneous group of tumors, representing the most common form of neoplasms in the central nervous system among children). The results of the study indicated that EM011 decreased both tumor-cell proliferation and invasion indices, making it an appealing molecule for the treatment of pediatric low-grade gliomas (44). A third generation of di-substituted bromo analogues of noscapine was also synthesized, the novel di-substituted noscapinoids showing significant antiproliferative activity (45). N-(3-bromobenzyl)-noscapine, a third-generation noscapine analog, was slightly more potent than the first-generation clinical candidate EM011. The efficacy of noscapine and its analogs has been enhanced through nanoscale-based drug delivery systems (46).

Psammaplin A is also a natural bromine-containing cytotoxic compound. Hybrid agents are compounds designed to inhibit simultaneously multiple cellular targets relevant to tumor growth and survival. Cincinelli R. and her team designed a camptothecin-psammaplin hybrid with enhanced anticancer potential, targeting both topoisomerase I and HDAC enzymes (47).

Aroylhydrazones obtained by condensation between salicylaldehyde and different hydrazides possess diverse pharmacological activities, including anticancer. The cytotoxic effects of 5-bromosalicylaldehyde-4-hydroxybenzoylhydrazone and 5-bromosalicylaldehyde isonicotinoyl hydrazone were comparable to that of Cisplatin, the inclusion of a bromine atom greatly increasing the activity of the compounds (48).

Many synthetic heterocyclic compounds presented antitumor activity. Zhang *et al*. tested the ability of a series of synthetic brominated chalcone derivatives to inhibit the growth of cancer cell lines. Some compounds exhibited antiproliferative activity at lower concentrations than 5- fluorouracil, used as

the positive control. One of the compounds, H72, exhibited selective cytotoxicity against gastric cancer cells versus normal human gastric epithelial cells (49). The cytotoxicity of 5-bromo-6-(4-chlorophenyl)-2-propyl-imidazo (2,1-b) (1,3,4) thiadiazol was evaluated and compared to the standard antitumor drug Bleomycin. The results indicated relatively close cytotoxicity index values with the standard and lower cytotoxicity towards normal cells compared to Bleomycin, which represents an important advantage (50).

4,5,6,7-tetrabromo-1H-1,2,3-benzotriazole, 4,5,6,7-tetrabromo-1H-benzimidazole and 4,5,6,7-tetrabromo-1H-benzimidazole-2-N,N-dimethylamine derivatives are examples of brominated compounds with protein kinase inhibitory activity; they are specific inhibitors of casein kinase 2. It is an established fact that abnormal phosphorylation of proteins mediated by kinases may lead to cancer and, in consequence, the inhibition of protein kinases is a potential target in cancer treatment (51). The structure activity relationship analysis performed for new 3,6-disubstituted-1,2,4,5-tetrazine derivatives with antitumor activity revealed that the insertion of a bromine atom in the molecule significantly increased the cytotoxicity (52).

A few 6-bromine derivatives of 7-anilino-1-arylisoquinoline-quinone presented an antiproliferative effect against some cancer cell lines. The conclusion of the study was that the insertion of bromine atoms in the 6-position of the quinone ring of the isoquinoline-quinone scaffolds is an important factor for the antiproliferative activity (53). The influence of bromine substitution on the cytotoxic activity of some isoquinolinequinone-amino acid derivatives also depended greatly on the insertion position. One brominated analogue showed potent and selective cytotoxic activity on lung and bladder cancer cells, appearing as a new lead compound in the aminoisoquinoline-quinone series (54).

Antiplasmodial activity. Malaria is the most prevalent insect-borne parasitic disease, caused by protozoa belonging to the genus *Plasmodium*, a large percentage of the world population being at risk, mainly in the poorest countries. Malaria therapy is affected by the appearance of chloroquine-resistant *Plasmodium* strains, making the discovery of new drugs a priority. A series of halogenated 4-quinolones was synthesized and their activity against *Plasmodium falciparum* was investigated. The introduction of a bromine atom on the quinolone skeleton caused an increase in the antiplasmodial activity (55). The antimalarial potential of a series of 1,2-substituted-4(1*H*)-quinolones was also investigated. The presence of an aliphatic group containing a bromine atom at C-2 enhanced the *Plasmodium falciparum in vitro* inhibition. On the other hand, introduction of a phenyl group in the aliphatic side chain at C-2 dramatically increased the antimalarial potency; the compound with a phenyl group and a bromine atom at the end of the aliphatic side chain appeared to be the most potent antimalarial agent (56).

Ellipticine is an alkaloid first extracted from *Ochrosia elliptica* and *Rauvolfia sandwicensis*. Ellipticine and its analogues exhibit antimalarial potential. Ring brominated and nitrated ellipticine derivatives also presented good *in vitro* inhibition of *Plasmodium falciparum* (57). Other alkaloids with antiplasmodial activity are cryptolepine and neocryptolepine, isolated from *Cryptolepis sanguinolenta*. Cryptolepine derivatives with a halo-

gen attached to the quinoline ring and a halogen or a nitro group on the indole ring presented enhanced antiplasmodial activity. 2,7-dibromocryptolepine was very potent and possessed encouraging *in vivo* activity in mice infected with *P.berghei* (58). Neocryptolepine presented lower cytotoxic potential than cryptolepine, so it can become a starting point for the development of new antimalarial agents. 2-bromo- and 3-bromo-neocryptolepine proved to be more active than neocryptolepine itself, but not as active as cryptolepine. 2-bromoneocryptolepine showed selectivity towards the *Plasmodium* parasite, justifying its position as the most promising lead now in the series of the neocryptolepine derivatives. Furthermore, 2-bromoneocryptolepine showed a low affinity for DNA and no inhibition of human topoisomerase II, revealing no obvious cytotoxicity (59).

Anti-inflammatory. Based on the structural resemblance of noscapine to colchicine, a tubulin-binding anti-inflammatory drug, noscapine and its semi-synthetic brominated analogues were examined for *in vitro* anti-inflammatory activity. Brominated noscapine analogues were found to inhibit cytokine and chemokine release from macrophage cell lines but did not affect cell viability. Brominated noscapine analogues displayed a significantly higher anti-inflammatory activity compared to the parent drug - noscapine (60).

Chrysin is a naturally occurring flavone with an anti-inflammatory effect. Some 6,8-dihalogenated chrysin derivatives were synthesized and their anti-inflammatory activities were evaluated in comparison to the initial compound. Although the dihalogenation of chrysin increased the *in vitro* anti-inflammatory activity, no significant difference between chrysin and dibromochrysin or diiodochrysin was recorded in *in vivo* carrageenan-induced inflammatory activity test. One possible explanation of the phenomenon could be the low permeability through the skin to the target inflammation sites of the dihalogenated chrysin derivatives, due to their large molecular structures (61).

The anti-inflammatory potential of several halogenated isatin derivatives was tested, the activity varying according to type of halogen and the position of the substituent. The investigated parameters were the inhibition of TNFα, NO and PGE_2. The results suggested that bromine C5- and C6-substituted isatin analogues displayed an increased anti-inflammatory activity (62).

Gastroprotection. Bromine substituted Schiff base - copper and zinc complexes showed amazing gastroprotective activity. The presence of bromine atoms coordinated to the zinc metal ion seemed a possible active site for the complex due to the electron donating properties of the halogens by resonance, making the lone pair of electrons more available to a plausible electron transfer (63, 64).

CONCLUSIONS

The field of bromine-containing substances constitutes a research area of great interest due to the importance of these derivatives as bioactive agents. Many natural or synthetic compounds have been studied so far and very interesting biological properties have been revealed. The insertion of bromine atoms in the molecule is usually associated with the enhancement of the bioactive potential, bromination becoming an important tool for the design of new drug candidates.

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NEWS

3D BIOPRINTED CARTILAGE: A NEW ERA IN BIOENGINEERING

The advent of 3D bioprinting has revolutionized the area of tissue bioengineering and it is becoming a linchpin in reconstructive surgery as surgeons are using bioengineering techniques to push standard flaps over their limits. Despite the many advantages of 3D bioprinting, the lack of a patent vascular network makes it difficult to grow 3d bio printed materials *in vivo* based only on diffusion of nutrients. However, cartilage tissue survives only on diffusion and thus should be a perfect candidate for 3D bioprinting, especially when co-cultured with bone marrow-derived mesenchymal stem cells (hBMSC) whose trophic role in cell differentiation and proliferation is widely appraised. Moller T. *et al* proved *in vivo* chondrogenesis by subcutaneously implanting 3D bio printed cellulose/alginate scaffolds co-cultured with hBMSC in rats. The scaffolds were followed-up histologically, immunohistochemically and showed enhanced deposits of glycosaminoglycans and human collagen II, leaving us with many ideas of how 3D bio printed cartilage could become a standard in reconstructive procedures (Moller T, Amoroso M, Hagg D, Brantsing C, Rotter N, Apelgren P, Lindahl A, Kolby L, Gatenholm P. *In Vivo* Chondrogenesis in 3D Bio printed Human Cell-laden Hydrogel Constructs. *Plast Reconstr Surg Glob Open* 2017; 5(2): e1227).

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CORELAŢIA ALIMENTAŢIE – VENIT PE MEMBRU DE FAMILIE LA UN LOT DE ADOLESCENŢI DE LA LICEUL „DIMITRIE CANTEMIR" DIN IAŞI

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FOOD INTAKE - FAMILY INCOME IN A GROUP OF TEENAGERS FROM IAŞI "DIMITRIE CANTEMIR" HIGH SCHOOL (Abstract): Food intake is influenced by a series of factors, including family, income, school and mass-media. **Material and method**: A group of 78 teenagers were investigated using a food intake questionary, correlating the results with family income. **Results**: Our results show that the food intake is influenced more by family dietary habits rather than family income, with no significant differences between families with different socio-economic status. **Conclusion**: Educational programs for changing the nutritional habits must be implemented. **Key words**: FOOD INTAKE, INCOME, NUTRITIONAL HABITS

În abordarea generală a problematicii alimentaţiei adolescenţilor trebuie să pornim de la factorii ce o influenţează, factori reprezentaţi de familie, venit, grupul de prieteni, şcoală, mass-media. Familia reprezintă un model, mai ales pentru primii ani de viaţă. În acest mediu se formează unele obiceiuri alimentare ce persistă toată viaţa (1,2). Cu vârsta rolul familiei scade din ce în ce mai mult, apărând orientarea către grupul de prieteni. Venitul disponibil reprezintă o problemă mai ales pentru familiile sărace unde se consumă dominant alimente ce asigură doar necesarurile cantitative. Influenţa grupului de prieteni devine tot mai puternică în perioada adolescenţei. Prietenii asigură frecvent sprijinul necesar realizării unor schimbări majore în alimentaţie (modificări ce pot fi pozitive sau negative). Şcoala poate avea o influenţă pozitivă prin programele realizate (laptele şi cornul) sau în situaţia în care la cantină se oferă o masă caldă (3,4). Mass--media şi reclamele au un impact puternic asupra adolescenţilor care petrec mult timp în faţa televizorului. Frecvent, se oferă adolescenţilor anumite modele ce contribuie la formarea unei imagini nerealiste asupra aspectului corporal (se insită asupra tinerelor slabe şi a tinerilor cu mase musculare dezvoltate). Aspectele prezentate sunt complexe şi trebuie atent abordate. Doar o insistenţă din partea familiei, a medicului sau a şcolii nu rezolvă problemele, ci le încurcă şi mai mult.

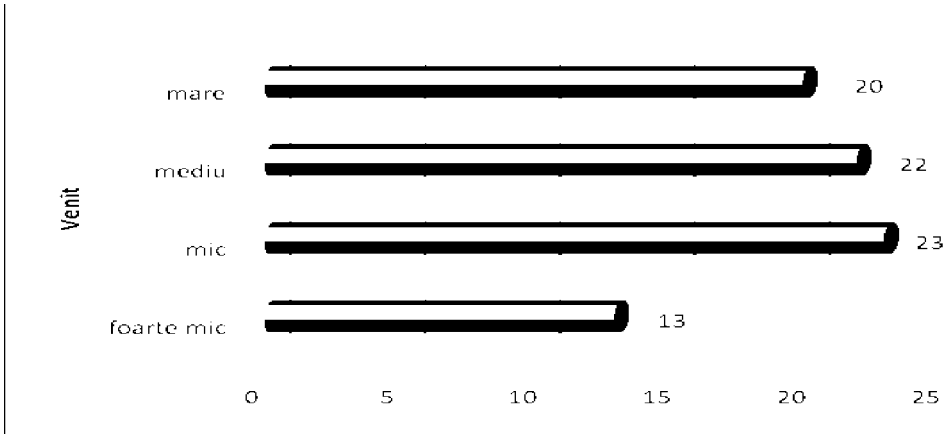

Fig. 1. Repartiţia cazurilor în funcţie de venitul pe membru de familie

TABELUL I
Frecvenţa consumului săptămânal de lapte

Consum	Zero	1 dată	2-3 ori	4-7 ori	Total
Venit foarte mic	1	2	8	2	13
Venit mic	1	6	11	5	23
Venit mediu	4	5	5	8	22
Venit mare	2	1	8	9	20
Total Nr.	8	14	32	24	78
%	10,25	17,94	41,02	30,76	

MATERIAL ŞI METODĂ

Studiul s-a realizat pe 78 de adolescenţi cu vârste cuprinse între 14 şi 16 ani ce învaţă la liceul D.Cantemir din Iaşi. Aceştia au completat un chestionar ce cuprinde întrebări referitoare la venitul pe membru de familie şi la alimentaţie. Venitul pe membru de familie a fost considerat foarte mic (sub 150 roni), mic (160 – 500 roni), mediu (500 – 1000 roni) sau mare (peste 1000 roni). Aprecierea alimentaţie s-a făcut cu ajutorul unui chestionar de frecvenţă săptămânală a consumurilor, prin care se apreciază aportul alimentar obişnuit şi nu cel real. Tehnica impune chestionarea subiectului în legătură cu frecvenţa consumului fiecărui aliment de pe o listă prestabilită. (5) Prelucrarea statistică a rezultatelor s-a realizat cu ajutorul testului Pearson.

REZULTATE ŞI DISCUŢII

In mod obişnuit venitul familiei influenţează alimentaţia, locuinţa şi calitatea îngrijirilor medicale acordate copiilor şi tinerilor. În familiile sărace alimentaţia asigură doar necesarul caloric, locuinţa este frecvent insalubră, iar tratamentele sunt urmate doar atunci când sunt gratuite. (6)

Pornind de la aceste considerente este important să cunoaştem, în primul rând nive-

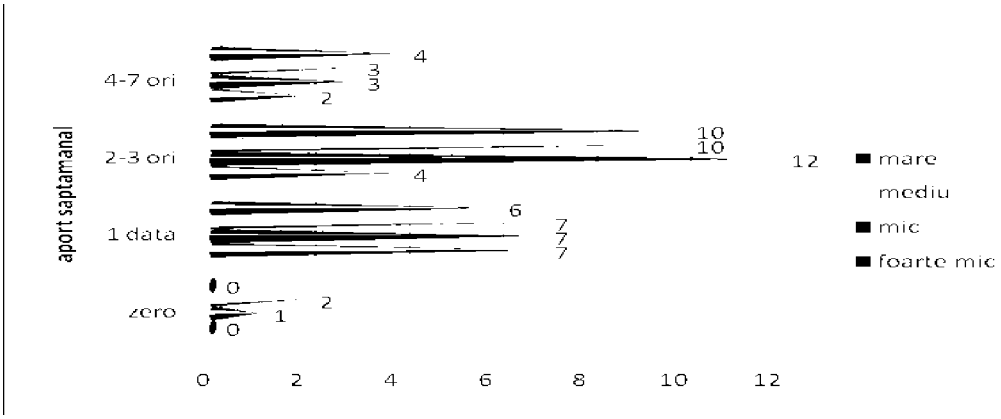

Fig. 2. Distribuţia cazurilor în funcţie de aportul săptămânal de brânzeturi

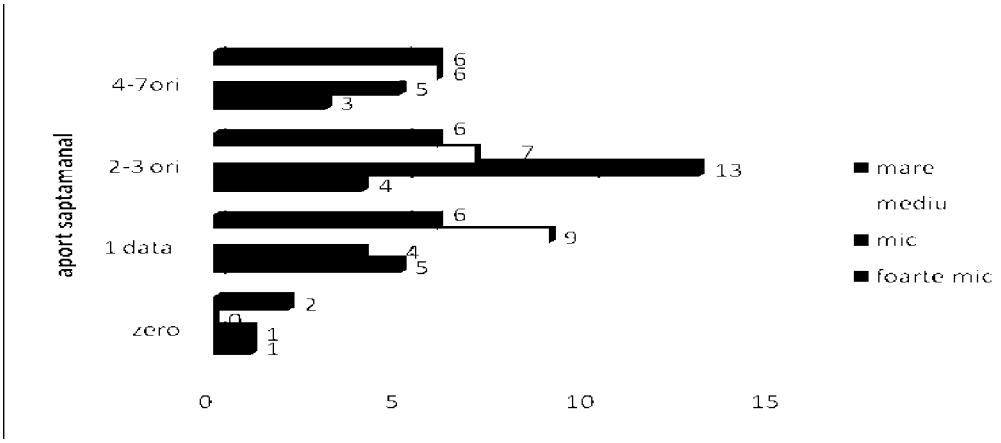

Fig. 3. Frecvenţa consumului de ouă la lotul de studiu

lul socio-economic al familiilor adolescenţilor din lotul de studiu. Distribuţia cazurilor în funcţie de venit este relativ uniformă, fiind dominante familiile cu un venit mic (29,48%) sau mediu (28,2%) (fig. 1).

Am insistat asupra consumului de alimente reprezentat de lapte, brânzeturi, ouă, cartofi şi fructe. Laptele este asigurat în majoritatea cazurilor de 2-3 ori pe săptămână (41,02%). Există situaţii în care acest aliment nu este consumat de adolescenţi (10,25%), astfel că se marcheaza varianta zero. La polul opus se plasează 30,76% tineri ce afirmă un consum de lapte de 4 – 7 ori pe săptămână. In funcţie de venit diferenţele constatate sunt nesemnificative statistic ($p > o,o5$, χ^2 - 11,968) ceea ce ne orientează către obiceiuri alimentare asemănătoare. Practic, în familiile cu venituri mari nu apare o creştere semnificativă a consumului săptămânal de lapte (tab. I).

Dominant, consumul săptămânal de brânzeturi este de 2-3 ori (46,15%) sau de 1 dată (34,61%). Atrag atenţia 3,84% răspunsuri de zero ce orientează către absenţa brânzeturilor din alinentaţia unor adolescenţi. La polul opus se plasează 15,38% răspunsuri de consum frecvent al acestor

TABELUL II
Consumul săptămânal de cartofi

Consum	Zero	1 dată	2-3 ori	4-7 ori	total
Venit foarte mic	0	2	5	6	13
Venit mic	0	8	10	5	23
Venit mediu	2	6	8	6	22
Venit mare	0	3	8	9	20
Total nr.	2	19	31	26	78
	2,56	24,35	39,74	33,33	

TABELUL III
Consumul săptămânal de fructe la elevii chestionaţi

Consum	Zero	1 dată	2-3 ori	4-7 ori	total
Venit foarte mic	0	1	2	10	13
Venit mic	0	1	4	18	23
Venit mediu	0	2	3	17	22
Venit mare	1	1	5	13	20
Total nr.	1	5	14	58	78
	1,28	6,41	17,94	74,35	

produse ce ajunge la de 4 – 7 ori pe săptămână (fig. 2). Testul Pearson indică diferenţe nesemnificative statistic ($p > o,o5$, χ^2 - 4,504) ceea ce ne orientează către ideea existenţei unor obiceiuri alimentare asemănătoare în familiile elevilor chestionaţi. Laptele şi brânzeturile sunt produse bogate în proteine, elemente minerale (în special calciu), lipide, glucide şi vitamine. Consumul lor este benefic pentru elevi, dar el depinde de obiceiurile alimentare ale familiei. La lotul de studiu nu apare o creştere a aportului acestor alimente în familiile cu un nivel economic ridicat.

Ouăle sunt alimente cu o mare valoare biologică (ele sunt bogate în proteine, lipide, vitamine, elemente minerale). La adolescenţi se recomandă un consum de un ou pe zi. La elevii chestionaţi aportul majoritar de ouă este de 2-3 ori pe săptămînă (38,46%). Situaţia cea mai gravă apare în 5,12% cazuri unde se bifează varianta zero ce indică absenţa acestui aliment din alimentaţia elevilor. Remarcăm doar 25,64% tineri ce au un aport adecvat de ouă (de 4-7 ori pe săptămână) (fig. 3).

Valorile sunt distribuite uniform în funcţie de venit, diferenţele constatate fiind nesemnificative statistic ($p > o,o5$, χ^2 - 7,392). Rezultatele obţinute relevă o puternică fixare în tradiţii, ceea ce duce la respectarea unor obiceiuri învăţate în copilărie şi perpetuarea lor în propria familie.

În alimentaţie produsele de origine animală sunt asociate cu cele de origine vegetală. Cartoful este o legumă aparte pentru ţara noastră, deoarece se cultivă pe arii extinse. Consumul majoritar de cartofi este de 2-3 ori pe săptămână (39,74%) sau de 4-7 ori (33,33%). Răspunsul zero este prezent în 2,56% din cazuri ceea ce ne atrage atenţia în mod deosebit, mai ales pentru zona Moldovei unde cultivarea cartofului este bine dezvoltată. Diferenţele nesemnificative statistic ($p > o,o5$, χ^2 - 11,035) ne orientează tot către obiceiuri alimentare ase-

mănătoare. La lotul de studiu observăm un consum asemănător de cartofi, chiar dacă familiile au posibilităţi financiare diferite. Familiile elevilor chestionaţi sunt profund ancorate în tradiţiile învăţate de la părinţi, astfel că nu reacţionează la schimbarea nivelului economic prin modificarea alimentaţiei (tab. II).

Fructele proaspete asigură un aport important de vitamină C şi oferă un aport caloric mic ceea ce este un element favorabil pentru menţinerea constantă a greutăţii corporale. La lotul de studiu consumul dominant de fructe este de 4-7 ori pe săptămână. Este alarmantă situaţia a 1,28% elevi ce afirmă absenţa consumului de fructe. Diferenţele nesemnificative constatate ($p > o,o5$, χ^2 - 5,597) ne orientează tot către obiceiuri alimentare asemănătoare. Cumpărarea fructelor nu este o problemă în anumite familii, dar consumul lor este dependent de tradiţia familiei (tab. III).

CONCLUZII

La lotul de studiu consumul alimentar este asemănător chiar dacă venitul pe membru de familie este diferit. Familiile cu un venit foarte mic fac eforturi pentru a asigura o alimentaţie adecvată copiilor lor. Din păcate, în familiile cu un venit mediu sau mare nu se observă un salt calitativ. Elaborarea unor programe naţionale de modificare a obiceiurilor alimentare trebuie să pornească de la particularităţile propriei populaţii (chiar a celor de la nivel local – regiuni istorice). Programele care nu iau în calcul aceste particularităţi au prea puţine şanse de reuşită.

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EXPERIMENTAL STUDIES ON FORMULATION OF MODIFIED-RELEASE AMIODARONE TABLETS

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EXPERIMENTAL STUDIES ON FORMULATION OF MODIFIED-RELEASE AMIODARONE TABLETS (Abstract): Drug formulations and their technological processes must ensure quality and reproducibility in the conditions of the information transfer between research institutions and industry for the preparation of large amounts of pharmaceutical product. **Aim:** Analysis of the influence of formulation factors on flow parameters of the powder mixture in order to obtain amiodarone tablets. **Material and methods:** Data fitting and calculation of statistical indicators for the validation of the experimental plan. The study was carried out using the MODDE 10.1 optimization software. The quantitative variables were amiodarone, amiodarone complex with hydroxypropyl-β-cyclodextrin, Kollidon® SR, Chitosan, Aerosil®, magnesium stearate, and Avicel®, while the qualitative variables were the following flow and compressibility parameters of the mixtures subjected to compression: flow time, coefficient of friction, angle of rest, Hausner index and Carr index. The matrix for optimizing the formulation based on the presented parameters was based on a D-optimal experimental design. **Results and Discussion:** The results of the study on the influence of formulation factors on flow time confirmed that Kollidon® SR concentration levels decrease the flow time, also confirmed for Chitosan but on a smaller scale. Of the formulation factors, Avicel® and Kollidon® SR variables had the greatest influence on the friction coefficient, increasing the concentration of these parameters generating a decrease in the friction coefficient. **Conclusions:** The use of experimental designs in the formulation of amiodarone tablets and the formulation analysis based on flow and compressibility parameters allows establishing a hierarchy based on the importance of qualitative variables, influenced by quantitative variables, to identify the optimal formulation and technology for the preparation of tablets. **Keywords:** AMIODARONE, MODIFIED RELEASE, FORMULATION, EXPERIMENTAL PLAN.

The qualitative formulation elements of a pharmaceutical product include documentation of physicochemical and biopharmaceutical properties of both drug substances and excipients, as well as establishing optimum working conditions that would facilitate the transfer of the pharmaceutical formulation from the research and development phases to the production stage during industrial process scale-up.

Besides being a screening technique, optimization is a selection method of the best combination of raw materials and of the most appropriate technological process,

in order to obtain the optimal formulation of the pharmaceutical product (1).

The formulation of drugs and their technological processes must ensure quality and reproducibility under the conditions of large-scale preparation. The number of experiments necessary to investigate the drug formulation should be minimized in order to collect the technical information specific to the formulation. The use of experimental plans and optimization methods, which ensure the progressive acquisition of information, reduces the number of experiments without affecting the quality of results. Experimental plans effectively minimize classical method errors by changing the level of all variables in each experiment in a programmed and justified manner (2, 3). The advantages of using experimental plans are: reducing the number of experimental determinations, the possibility of studying a large number of variables, the possibility of identifying possible interactions between various factors, the possibility of generating optimal values for each variable, obtaining a good accuracy of results and optimizing the drug formula in terms of quality, quantity, technology (4, 5).

The aim of the study was to analyze the influence of the qualitative formulation factors on the flow parameters of the powder mixture in order to obtain modified-release tablets with amiodarone complex with hydroxypropyl-β-cyclodextrin (6-10).

MATERIAL AND METHODS

Data fitting and calculation of statistical indicators for the validation of the experimental plan was carried out using the MODDE 10.1 optimization program. The quantitative variables were amiodarone, amiodarone complex with hydroxypropyl-β-cyclodextrin, Kollidon® SR, Chitosan, Aerosil®, magnesium stearate, Avicel®, while the qualitative variables were the following flow and compressibility parameters of the mixtures subjected to compression: flow time, coefficient of friction, angle of rest, Hausner index and Carr index (11-14).

The study of the influence of formulation factors on flow and compressibility parameters of the powder mixtures subjected to compression was carried out in two stages (15, 16). During the first stage, the reduced experimental plan, which included seven factors on three levels, was implemented. It enabled identifying the factors that influence the process of tablet manufacturing.

The study focused on the influence of the independent quantitative variables (tab. I) on the qualitative variables (17-20) (tab. II).

TABLE I

Quantitative variables

Factors	Code	%		
		1	2	3
Amiodarone complex	X1	0	33.33	-
Amiodarone	X2	0	33.33	-
Kollidon® SR	X3	40	50	60
Chitosan	X4	3	5	7
Aerosil®	X5	constant (1)		
Mg stearate	X6	constant (0.5)		
Avicel®	X7	22.17		

TABLE II
Qualitative variables

Factors	Code
Flow time (seconds)	Y1
Friction coefficient	Y2
Angle of rest α (°)	Y3
Hausner ratio	Y4
Carr index (%)	Y5

Tables III and IV illustrate the formulation of tablets with either amiodarone or hydroxypropyl-β-cyclodextrin amiodarone complex, and the studied flow parameters. Table V describes the matrix of the experimental plan designed to optimize the formulation by using a D-optimal experimental design.

TABLE III
Formulation factors of amiodarone complex tablets

Nº	X1	X2	X3	X4	X5	X6	X7
1	0	33.33%	40%	3%	1%	0.5%	22.17%
2	0	33.33%	40%	5%	1%	0.5%	20.17%
3	0	33.33%	40%	7%	1%	0.5%	18.17%
4	0	33.33%	50%	3%	1%	0.5%	12.17%
5	0	33.33%	50%	5%	1%	0.5%	10.17%
6	0	33.33%	50%	7%	1%	0.5%	8.17%
7	0	33.33%	60%	3%	1%	0.5%	2.17%
8	0	33.33%	60%	5%	1%	0.5%	0.17%
9	0	33.33%	60%	7%	1%	0.5%	0.15%
10	33.33%	0.00%	40%	3%	1%	0.5%	22.17%

TABLE IV
Flow parameters studied during the analysis of experimental plans

Nº	Y1	Y2	Y3	Y4	Y5
1	0.138889	0.461538	25.00	1.178571	15.15152
2	0.117647	0.475	23.70	1.300000	23.07692
3	0.153846	0.451220	24.50	1.346154	25.71429
4	0.333333	0.714286	35.50	1.391304	28.12500
5	0.666667	0.692308	34.80	1.333333	25.00000
6	0.217391	0.725000	36.00	1.315789	24.00000
7	0.125000	0.810811	39.20	1.574074	36.47059
8	0.147059	0.885714	41.50	1.596774	37.37374
9	0.105778	0.888889	41.70	1.666667	40.00000
10	0.120428	0.578947	30.00	1.250000	20.00000

TABLE V
Matrix of the reduced experimental plan

Nº	Run Order	Independent variable						
		X1	X2	X3	X4	X5	X6	X7
N1	7	0	33.33%	60%	3%	1%	0.5%	2.17%
N2	6	0	33.33%	50%	7%	1%	0.5%	8.17%
N3	4	0	33.33%	50%	3%	1%	0.5%	12.17%
N4	9	0	33.33%	60%	7%	1%	0.5%	0.15%
N5	10	33.33%	0.00%	40%	3%	1%	0.5%	22.17%
N6	1	0	33.33%	40%	3%	1%	0.5%	22.17%
N7	3	0	33.33%	40%	7%	1%	0.5%	18.17%
N8	5	0	33.33%	50%	5%	1%	0.5%	10.17%
N9	2	0	33.33%	40%	5%	1%	0.5%	20.17%
N10	8	0	33.33%	60%	5%	1%	0.5%	0.17%

RESULTS AND DISCUSSION

During the study, an experimental plan that included seven factors (independent variables) and more than three levels were used.

The influence of formulation factors on the mechanical strength can be assessed by the coefficients of polynomial equation (mathematical model) which was carried out using the experimental fitting (centered and scaled factors).

According to the data obtained from the analysis of the influence of formulation factors according to the reduced experimental plan it was appreciated that the greatest influence on the characteristics of matrix tablets was carried by the pharmacotechnical variables X3 and X7 (Kollidon® SR and Avicel®).

The results on the influence of quantitative factors on flow time confirmed that the concentration levels of Kollidon® SR decrease the flow time, also noticed for Chitosan but on a smaller scale.

Of the analyzed formulation factors, Avicel® was the variable that had the greatest influence on the friction coefficient. Increasing its concentration resulted in a decrease of the friction coefficient.

The Kollidon® SR variable had a significant positive influence on the friction coefficient, increasing Kollidon® SR concentration in the formulation resulting in higher friction coefficients.

The pharmacotechnical parameters - diameter and thickness - were not significantly influenced by the independent variables of the experimental plan, as they were primarily defined by the working conditions and the technical characteristics of the tableting machine.

Very good results were obtained for the Hausner ratio, which had a validity value model very close to 1 (tab. VI). According to the data presented in table VII, good results were also found for the friction coefficient, angle of rest, flow time and Carr index.

TABLE VI

The result obtained by fitting data for validating the experimental plan

Variable	R2	Adjusted R2	Q2	SD	RSD	Model validity	Reproducibility
Y1	0.73012	0.360817	0.452125	0.249055	0.199117	0.10529	0.624684
Y2	0.950057	0.9200923	0.852641	0.179401	0.050448	0.135784	0.952423
Y3	0.827836	0.727407	0.628324	8.45044	4.41201	0.0348447	0.848697
Y4	0.95487	0.928545	0.921543	0.165729	0.0443011	0.0751738	0.95904
Y1	0.948478	0.918424	0.911777	8.27953	2.36476	0.0969724	0.952417

TABLE VII

Matrix of correlation coefficients

Variable	X1	X2	X3	X4	X7	Y1	Y2	Y3	Y4	Y5
X1	1	-1	-0.361	-0.361	0.420	-0.186	-0.185	-0.160	-0.315	-0.326
X2	-1	1	0.361	0.361	-0.420	0.186	0.1858	0.160	0.315	0.326
X3	-0.361	0.361	1	0.130	-0.983	0.0436	0.9658	0.954	0.9076	0.903
X4	-0.361	0.361	0.130	1	-0.295	-0.020	0.1259	0.103	0.2585	0.274
X7	0.420	-0.420	-0.983	-0.295	1	-0.054	-0.948	-0.934	-0.907	-0.909
Y1	-0.186	0.186	0.043	-0.020	-0.054	1	0.0796	0.1245	-0.180	-0.136
Y2	-0.185	0.185	0.965	0.125	-0.948	0.079	1	0.995	0.851	0.849
Y3	-0.160	0.160	0.954	0.103	-0.934	0.124	0.995	1	0.817	0.816
Y4	-0.315	0.315	0.907	0.258	-0.907	-0.180	0.851	0.817	1	0.995
Y1	-0.326	0.326	0.903	0.274	-0.909	-0.136	0.849	0.816	0.995	1

CONCLUSIONS

We have used experimental plans in the formulation of amiodarone tablets and the analysis of formulation based on flow and compressibility parameters, which allowed us to establish a hierarchy based on the importance of quantitative variables, as they influenced the qualitative variables, in order to achieve optimal formulation and technology for tablet manufacturing.

We found Avicel® to have the greatest influence on tablet formulation, followed by Kollidon® SR and Chitosan. They positively influenced the values of the analyzed qualitative variables that defined the preparation of the tablets by direct compression method.

Taking also into account the results of the analysis of the influence of formulation factors on flow properties, it was considered that the independent variables were selected in corresponding concentration ranges to obtain matrix tablets.

The results of the experimental plan recommended lower optimal values corresponding to Kollidon® SR 40% and Chitosan 3%.

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UNELE EVALUĂRI EPIDEMIOLOGICE ŞI MEDICO-LEGALE ASUPRA CAZURILOR DE VIOLENŢĂ DOMESTICĂ, ÎNREGISTRATE ÎN MUNICIPIUL PIATRA-NEAMŢ, ÎN PERIOADA 1997-2009

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SOME EPIDEMIOLOGICAL AND FORENSIC ASSESSMENTS ON CASES OF DOMESTIC VIOLENCE, RECORDED IN PIATRA NEAMŢ, BETWEEN 1997-2009 (Abstract): Authors highlighted some aspects regarding domestic violence in an urban community, during 13 years. **Material and method**: The study included 31406 individuals from the county, with various aggressions, from which 1433 (53.43%) from Piatra Neamţ Town and 2682 (8.53%) were victims of domestic violence < 20-61 aged. **Results**: Domestic violence prevalence in Piatra Neamţ Town, comparatively with county, had values of 6.21% in 1997 and 7.10% in 2009. Cumulatively for 13 years, female victims were 1081 (75.43%), comparatively with males 352 (24.56%). A percent of 79.51% of cases interested 21-60 aged active social groups. The gravity of domestic violence lesions were distributed in 4 categories, from 10 to 61 days of hospitalization. **Conclusions**: Negative social economical involvements of domestic violence for individual, family and community, presented various aspects, some of them difficult to quantify (hospitalisation, absenteeism, temporary work incapacity a.s.o). **Key words**: DOMESTIC VIOLENCE, PREVALENCE, FORENSIC MEDICINE

Violenţa umană, cu expresia ei domestică, este o componentă a naturii umane care, însă, poate şi trebuie prevenită şi combătută (1,2,3). Perioada de tranziţie a României spre economia de piaţă a determinat mutaţii profunde de ordin politic şi socio-economic care s-au asociat şi cu schimbări în instituţia familiei, exprimată adeseori printr-o frecvenţă tot mai mare a exceselor comportamentale, dominate de violenţă fizică sau psihologică, cu impact negativ asupra evoluţiei vieţii de familie. Deşi este un fenomen care a însoţit dintotdeauna construirea şi dinamica familiei, doar în ultimele decenii, ştiinţele sociale şi juridice se referă la reglementările, de nivel naţional şi internaţional, privind violenţa domestică. Astfel, OMS defineşte violenţa ca fiind ameninţarea sau utilizarea intenţionată a forţei fizice sau a puterii împotriva propriei persoane, a unei alte persoane, împotriva unui grup sau împotriva comunităţii şi care antrenează un risc crescut de a produce un traumatism, un deces, o daună psihologică, o dezvoltare anormală sau o privaţiune (4, 5, 6). Între numeroasele documente elaborate la nivelul Comunităţii Europene, important este cel din anul 2004, privind programul de acţiune Dafne II, pentru perioada 2004-2008, urmat de actualizări, care includ măsuri preventionale şi combatere a violenţei împotriva copiilor, tinerilor şi fe-

TABELUL I

Distribuţia anuală, a cazurilor de violenţă domestică, comparativ, în judeţul şi municipiul Piatra Neamţ, în perioada 1997-2009

Anul	Număr total agresiuni/judeţ	Din care cazuri de violenţă domestică/ Municipiul Piatra Neamţ	
		Nr.	*%*
1997	3116	195	6,75
1998	2574	188	6,82
1999	2566	211	8,22
2000	2492	204	8,18
2001	2437	251	10,25
2002	2409	230	9,54
2003	2242	197	8,87
2004	2283	206	8,02
2005	2232	233	10,43
2006	2018	186	9,21
2007	1971	160	8,11
2008	2041	219	10,73
2009	2845	202	7,10
Total cumulativ	**31.406**	**2682**	**8,53**

meilor, pentru protecţia victimelor şi a grupurilor cu risc.

Şi în România, relaţiile din cadrul familiei au fost reglementate şi protejate dintotdeauna dar în ultimii ani, Codul Penal a inclus prevederi de protejare a familiei împotriva violenţei domestice iar Hotărârea Guvernului din anul 2009 se referă la măsurile de prevenire şi combatere a violenţei în familie, care vor fi implementate prin Autoritatea Naţională pentru protecţia familiei şi a drepturilor copilului (7, 8, 9, 10).

Lucrarea îşi propune să evidenţieze unele aspecte privind violenţa domestică într-o comunitate urbană din Moldova.

MATERIAL ŞI METODE

În cercetările noastre am inclus un lot de 31406 persoane din judeţul Piatra Neamţ, supuse diferitelor agresiuni dintre care 1433 (53,43%) au provenit din municipiul Piatra Neamţ, iar 2682 (8,53%)au fost victime ale violenţei domestice având vârsta de < 20 la = 61 ani, şi 1081 (75,43%) fiind de sex feminin.

Informaţiile au fost obţinute de la Serviciul Judeţean de Medicină legală şi Fundaţia „Columna", Piatra Neamţ, pentru perioada 1997-2009.

Prelucrarea şi interpretarea datelor acumulate a fost realizată prin metode epidemiologice (ancheta, incidenţa şi prevalenţa) şi statistico-matematice (regresia liniară, probabilitatea statistică etc.).

REZULTATE ŞI DISCUŢII

1. Distribuţia anuală a victimelor diverselor agresiuni, cu violenţă domestică, în judeţul şi municipiul Piatra Neamţ. Din totalul cumulativ pentru cei 13 ani, agresiunile au totalizat 2682 (8,53%) pentru municipiul Piatra Neamţ dar dintre acestea, violenţa domestică a înregistrat 1433 (53,43%) cazuri, dovedind existenţa unor factori de risc cu o pondere semnificativ mai ridicată în mediul urban ($p \leq 0,05$).

În municipiul Piatra Neamţ, atât pentru victimele agresiunilor variate cât şi cele ale violenţei domestic, valorile prevalenţei anuale au avut diferenţe nesemnificative ($p = 0,05$).

Din totalul victimelor agresiunilor pentru întregul judeţ, prevalenţa violenţei domestice, în municipiul Piatra Neamţ, a avut valori de 6,25% în anul 1997 şi 7,10% în 2009.

În perioada luată în studiu, din 2682 cazuri de violenţă domestică înregistrate

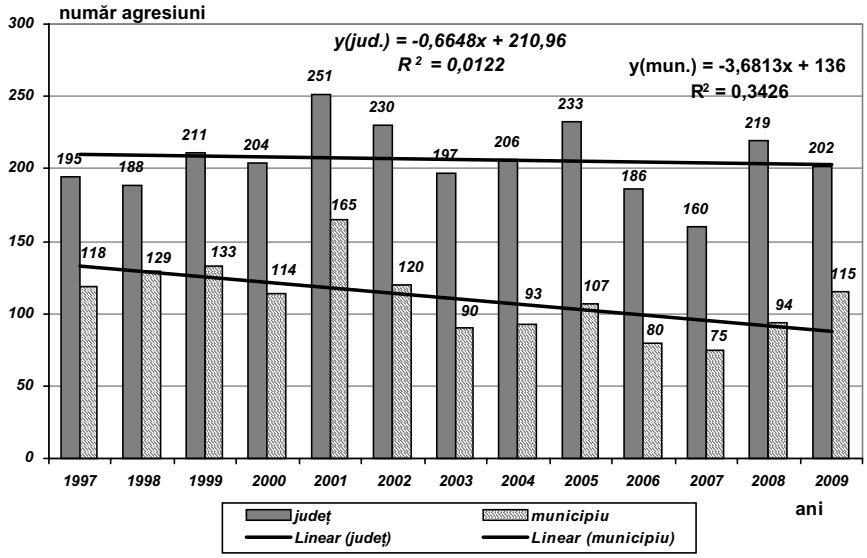

Fig. 1. Distribuţia anuală, comparativă, a cazurilor de violenţă domestică, din judeţul şi municipiul Piatra Neamţ, în perioada 1997-2009

la nivel judeţean 1433 (53,43%) s-au produs în mediul domestic din municipiul Piatra Neamţ (tab. I, fig. 1).

Ancheta epidemiologică şi medico-legală a evidenţiat cauzele principale ale prevalenţei ridicate a cazurilor de violenţă domestică în municipiul Piatra Neamţ, ca fiind în relaţie cu deteriorarea situaţiei socio-economice (scăderea veniturilor, şomaj) care a determinat creşterea nivelului agresivităţii, nemulţumirilor şi neînţelegerilor din mediul familial. De asemenea, urmare a realizării unor programe informative-educaţionale, a crescut adresabilitatea victimelor spre structurile medico-judiciare.

2. Analiza repartiţiei pe sex, a cazurilor de violenţă domestică, a dovedit cu un nivel ridicat de semnificaţie statistică ($p < 0,005$), faptul că femeile au suportat cel mai frecvent agresiunile intrafamiliale iar bărbaţii, îndeosebi, cele extrafamiliale.

Cumulativ, pentru cei 13 ani incluşi în studiu, victimele violenţei în familie au înregistrat 1081 (75,43%) pentru femei faţă de 352 (24,56%) la bărbaţi. Distribuţia anuală arată diferenţe nesemnificative în cazul femeilor ($p = 0,05$) dar acestea sunt evidenţiate când se compară situaţia între cele două sexe.

3. Repartiţia pe grupuri de vârstă. Violenţa domestică a fost semnalată, cu valori diferite în toate grupurile de vârstă. Din total cumulativ, 79,51% din cazuri au interesat grupurile active social (21-60 ani), urmate de = 61 (14,44% şi 0-20 (6%) (fig. 2).

Victimizarea vârstnicilor, precum şi a grupurilor de vârsta de până la 20 ani, prezintă particularităţi fizice şi psihologice. Îndeosebi, consecinţele asupra dezvoltării psiho-comportamentale sunt deosebit de grave în cazul implicării, directe sau indirecte, în actele de violenţă domestică a copilului.

4. Evaluări asupra gravităţii leziunilor produse prin violenţa domestică. În raport cu prevederile legislaţiei, variatele leziuni neletale produse prin acte de violenţă domestică sunt evaluate în ce priveşte gravitatea în raport cu numărul zilelor de îngrijiri medicale necesare vindecării, şi incluse în 4 categorii: *uşoare* (excoriaţii, echimoze, hematoame, plăgi superficiale); *medii* (fracturi costale, plăgi sau hematoame compli-

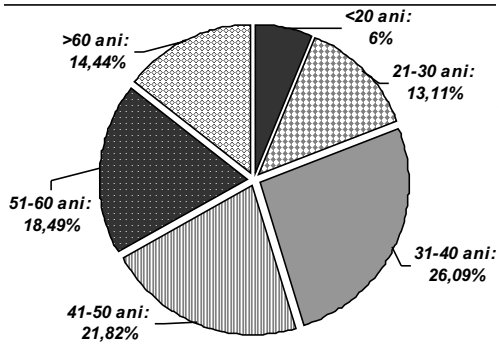

Fig. 2. Distribuţia pe grupuri de vârstă a cazurilor de violenţă domestică înregistrate, cumulative, în municipiul Piatra Neamţ, în perioada 1997-2009

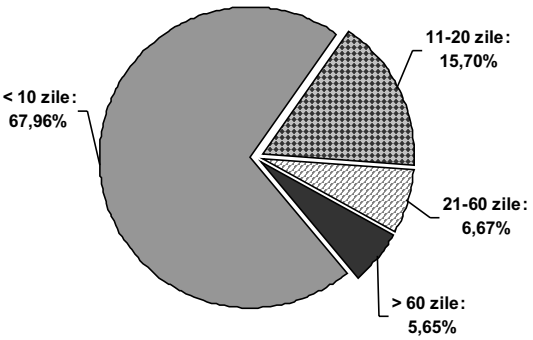

Fig. 3. Eşalonarea, pe categorii de gravitate, a leziunilor produse în 1433 cazuri de violenţă domestică, înregistrate în municipiul Piatra Neamţ, cumulativ în perioada 1997-2009

cate) ; *grave* (contuzie cerebrală medie, fracturi complicate) şi *foarte grave* (traumatism cranio-cerebral cu fracturi multiple, contuzie cerebrală gravă, arsuri, fracturi complicate). În raport de categoria de gravitate numărul de zile îngrijiri medicale vor varia de la 10 la peste 61 zile (Fig. 3).

Implicaţiile socio-economice negative ale violenţei domestice, pentru individ, familie şi comunitate, prezenta numeroase aspecte, unele dificil de cuantificat (costuri pentru : consultaţia medico-legală şi a altor specialităţi, investigaţiile paraclinice, spitalizările, absenteismul ocupaţional, incapacitatea temporară de muncă, perturbările grave ale vieţii de familie etc..

Din lotul de 1433 cazuri de violenţă domestică, 253 (17,65%) au necesitat spitalizări cu durată de 1-58 zile iar 413 (28,82%) au apelat la investigaţii variate în ambulator.

Studiul pe un sublot de 364 cazuri a arătat că 147 (40,38%) cazuri de violenţă în familie s-au finalizat prin divorţ şi 47 (12,91%) cu diferite acţiuni în instanţele de judecată.

Analiza situaţiei socio-economică a victimelor violenţei domestice evaluată pe un sublot de 388 cazuri, a evidenţiat că 75 (22,18%) nu aveau nici un suport financiar ; 125 (36,90), cu salariu minim ; 115 (34,02) cu salariu mediu sau pensie şi numai 23 (6,89%) posedau un venit care depăşea salariul mediu pe economie.

Rezultă că proporţii importante (57,98%) dintre victimele violenţei domestice sunt dependente financiar de agresor ; motiv pentru care un număr semnificativ de cazuri nu sunt raportate, la acestea adăugându-se costurile imposibil de raportat pentru a beneficia de prestările medico-legale şi cele dependente de acestea.

CONCLUZII

Transformările profunde şi alerte, înregistrate la nivel planetar, afectează grav multe din aspectele de echilibru în familie, îndeosebi pe seama unor dificultăţi socio-economice în strânsă corelaţie cu variate modificări comportamentale.

Violenţa domestică semnalată de-a lungul vremurilor, a cunoscut în ultimele decenii, o recrudescenţă semnificativă în toate ariile geografice, cu o prevalenţă mai ridicată în mediul urban, unde relaţiile interumane au particularităţi care, adesea, favorizează violenţa. Astfel, în municipiul Piatra Neamţ, în perioada 1997-2009, violenţa domestică a înregistrat 53,43% din totalul agresiunilor semnalate la nivelul judeţului, cu evidenta afectare a femeilor (75,43%)

comparativ cu bărbaţii (24,56%).

Cumulativ pentru cei 13 ani luaţi în studiu, grupurile de vârstă cele mai active socio-economic (21-60 ani) au fost afectate de violenţa în familie 79,51% din total cazuri.

În cele 6% din cazuri consecinţele asupra dezvoltării psiho- comportamentale sunt deosebit de grave în cazul implicării, directe sau indirecte, în actele de evidenţa domestică a copiilor şi adolescenţilor.

În 1433 cazuri de violenţa domestică, gravitatea leziunilor (uşoară, medie, gravă, foarte gravă) a fost evaluată după numărul de zile de spitalizare (< 10, 11-20, 21-60, = 61) având proporţii de: 15,70; 60,67; 5,65%.

Implicaţiile socio-economice negative ale violenţei domestice, pentru individ, familie şi comunitate, au prezentat numeroase aspecte, unele dificil de cuantificat (costuri pentru: consultaţiile medico-legale şi a altor specialităţi, investigaţiile paraclinice, spitalizările, absenteismul ocupaţional, incapacitatea temporară de muncă, perturbările grave ale vieţii familiale etc.).

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IN MEMORIAM

IN MEMORIAM, DR. EMIL GHEORGHIȚĂ

Dr. Emil Gheorghiță, passed away in November 2018. He was an eminent physician, founder of clinical hospitals and medical schools, a pioneer in the medical world and, above all, a very special man, whose generosity, talent, breadth of knowledge and kindness endeared him to colleagues, patients, relatives and friends alike.

He started his residency training in Obstetrics and Gynecology at the "Cuza-Vodă" Clinical Hospital for Obstetrics and Gynecology in Iași, under the direct guidance of Professor Vasile Dobrovici, a famous specialist who became his mentor and whom he had always held in high esteem. During the same period, he was entrusted with the position of director of the same hospital, which he completely reorganized and modernized.

In 1975, Dr. Emil Gheorghiță founded, together with Professor Gheorghe Teleman, the "Elena Doamna" Clinical Hospital of Obstetrics and Gynecology which he coordinated with professionalism and abnegation until the late 1990s. Within this hospital, Dr. Emil Gheorghiță set up (with Dr. Dan Sorin Tomoșoiu), organized and led the Regional Centre for Reproductive Health and Family Planning, which soon became a national landmark in this field.

In 1976, he defended his doctoral thesis entitled *Visual Disorders in Puerperality*, a topic that had not been treated before, which impressed at that time by its originality and modernity of approach. Besides his medical and organizational activity, Dr. Gheorghiță found the time to sit for further examinations in order to become a "primary physician" in the Romanian medical system (which corresponds, more or less, to a British "consultant") as well as a university reader. Although he successfully passed both exams, he could not pursue an academic career because all the academic positions for which contests had been organized in universities were blocked. Even so, his scientific concerns materialized not only in the books and monographs which he coauthored, or in the numerous articles that he published in specialized journals, but also in his initiative of organizing the "Vasile Dobrovici" National Conference, an outstanding scientific event that has gathered specialists in the field up to this day.

With over 40 years of medical experience as an obstetrician-gynecologist, he helped thousands of women fulfil their dream of having a healthy baby. He brought the smile on the lips of other thousands of women who asked him for help, some of them being saved from certain death.

His love of life and people, his mentoring vocation and talent were manifest both in the way he practiced his profession and in his constant wish to share his professional knowledge with his younger colleagues. Whatever the team he may have worked with, he encouraged an atmosphere of emulation and understanding, of mutual trust, love of life and dynamism. Through his way of stimulating his collaborators and disciples, he gained their confidence and gratitude. An altruistic and life-loving spirit, he enjoyed being surrounded by friends, whom he cherished, loved and helped. All that remains to those who loved and admired him is to follow his example and decipher with the same meticulousness the holy dimension of medical care.

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HERNIA AMYAND – O ENTITATE ANATOMOCLINICĂ RARĂ DIAGNOSTICATĂ INTRAOPERATOR

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AMYAND HERNIA (Abstract) : The Amyand hernia is an uncommon variant of the inguinal hernia, rarely recognised before the surgical treatment because of the confusion with a strangled hernia. In spite of this, the clinical presentation seems to follow a well determined pathway, so it is possible to state that the uncorrect diagnosis is to be attributed to the ignorance of this variant of hernia. We present two consecutive case reports of acute appendicitis founded in an inguinal hernia sac. The clinical presentation depended on the inflammation extension inside the hernia sac and the presence or not of peritoneal contamination. The patients were admitted for a painful pseudotumor in the inguinal region with irreducibility, mimicking strangled inguinal hernia with acute inflammatory syndrome. Intraoperatively we have found a hernia sac with a phlegmonous/gangrenous appendix inside. Appendectomy was performed, followed by hernioplasty (retrofunicular technique) without prosthetic material). The operation followings were favorable. We conclude that amyand hernia must be considered as differential diagnosis of apparently strangled inguinal hernias. Technical precautions and antibioprophylaxy applied during surgery may prevent septic complications after hernioplasty. The hernia repair must be performed without prosthetic material and using exclusively resorbable sutures. **Key words:** INGUINAL HERNIA, ACUTE APENDICITIS, AMYAND HERNIA

Apendicita acută într-un sac de hernie inghinală, denumită hernie Amyand, este o afecţiune rară, iar când aceasta apare, este deseori diagnosticată eronat ca hernie strangulată.

MATERIAL ŞI METODĂ

Au fost selectate şi analizate retrospectiv două cazuri de hernie inghinală, internate şi operate în urgenţă în Clinica a III-a Chirurgicală a Spitalului "Sf. Spiridon". La aceste cazuri, intraoperator s-a descoperit conţinut herniar reprezentat de cec împreună cu apendicele cecal. Acestea prezentau complicaţii mecanoinflamatorii (ireductibilitatea conţinutului prin strangulare şi inflamaţie acută apendiculară cu aderenţe inflamatorii la sacul herniar) aspecte ce încadrează leziunile în categoria herniilor Amyand.

Prezentare caz 1

Pacientul I.C. de sex masculin în vârstă de 67 ani, cu antecedente patologice cardiovasculare, se internează cu semne clinice de subocluzie intestinală. Examenul local evidenţiază o hernie inghinofuniculară ireductibilă. Se intervine chirurgical în condiţiile unei antibioprofilaxii cu Cefoperazonă + Sulbactam 2 g, asociat cu Metronidazol 0,5 g, în două prize (la inducţie şi după 6 ore). Intraoperator se descoperă un sac herniar inghinal drept, cu un conţinut herniar reprezentat de cec şi apendice cecal, conţinut ce este strangulat şi aderent la sacul herniar la nivelul coletului. Peretele

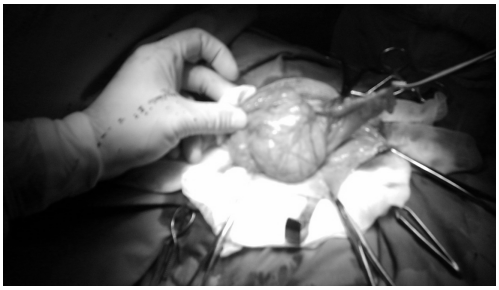

Fig. 1. Cec şi apendice cecal cu leziuni inflamatorii flegmonoase în sacul herniar

cecal şi apendicele prezentau aspect edematos-congestiv, cel mai probabil, datorită stazei venoase cauzate de strangularea la nivelul coletului herniar (fig. 1). Se eliberează şi se reduce conţinutul sacular prin secţionarea inelului herniar, gest urmat de apendicectomie cu bont legat şi înfundat şi reintroducerea cecului în cavitatea peritoneală. Cura herniei se realizează printr-un procedeu retrofunicular cu sutura aponevrozei marelui oblic şi a tendonului conjunct la ligamentul Cooper. Rezultatul anatomopatologic al apendicelui relevă modificări inflamatorii în stadiul cataral spre flegmonos şi hipetrofia foliculilor limfoizi.

Prezentare caz 2

Pacientul P.M. de sex masculin, în vârstă de 58 ani, având comorbidităţi importante (obezitate gradul II, hipertensiune arterială şi cardiopatie ischemică cronică dureroasă), şi antecedente de hernie inghinală dreaptă diagnosticată de aproximativ 1 an, se prezintă la Unitatea de Primire Urgenţe (UPU) a Spitalului „Sf. Spiridon” pentru tumefiere şi durere la nivelul regiunii inghinoscrotale drepte, apărute brusc de aproximativ 24 ore, cu tranzit intestinal prezent. Examenul clinic al abdomenului, dificil de efectuat datorită obezităţii, relevă la nivelul regiunii inghinoscrotale drepte o formaţiune pseudotumorală de dimensiuni mari 15/7 cm, renitentă, dureroasă, ireductibilă spontan sau la manevra de taxis, cu tegumentele supraiacente congestive. Testele de laborator evidenţiază leucocitoză importantă (17.000/mmc). Examinarea ultrasonografică abdominală este dificilă şi neconcludentă. Radiografia abdominală simplă în ortostatism relevă câteva nivele hidroaerice la nivelul intestinului subţire. Aspectul clinic coroborat cu leucocitoza a orientat diagnosticul spre o hernie inghioscrotală dreaptă ireductibilă, cu probabilitate de strangulare de ansă intestinală ileală. S-a intervenit chirurgical în urgenţă, sub anestezie generală, în condiţiile aceleiaşi antibioprofilaxii standard. Abordul inghinal drept a evidenţiat un sac herniar oblic extern prelungit până la nivelul hemiscrotului drept de aproximativ 17/ 4 cm, ce conţine lichid cu aspect tulbure, filant, fetid, în care se regăsesc apendicele cecal şi parţial cecul. Apendicele a prezentat leziuni inflamatorii în stadiul flegmonos, cu aderenţe inflamatorii şi false membrane, cu vârful acolat la capătul sacului herniar ; se mai observă modificări de tiflită, cu o zonă de necroză de 3/2 cm în apropierea inserţiei apendiculare (fig. 2). S-a practicat apendicectomie şi excizie parcelară de perete cecal, urmate de cecorafie cu mezoplastia suturii. Ulterior se disecă şi se rezecă sacul herniar mult îngroşat (aproximativ 1 cm grosime) prin modificări inflamatorii. Refacerea peretelui se realizează printr-un procedeu retrofunicular (Bassini modificat), cu fire lent rezorbabile Vicryl nr. 2. Exami-

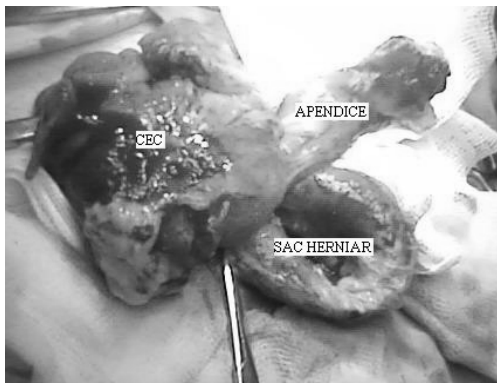

Fig. 2. Cec şi apendice cecal cu inflamaţie gangrenoasă

narea microscopică a confirmat stadiul flegmonos al inflamaţiei apendiculare şi necroza parcelară a peretelui cecal excizat.

Rezultatele tratamentului chirurgical al celor două cazuri au fost favorabile, fără complicaţii septice peritoneale sau la nivelul plăgii operatorii.

DISCUŢII

Prima apendicectomie a fost efectuată de Claudius Amyand (1,2) în 1735 la Spitalul St. George, Londra. Pacientul era un băiat de 11 ani care s-a prezentat cu o hernie inghinală şi fistulă stercorală la acest nivel. Apendicele cecal este estimat a fi găsit în mai puţin de 1% din cazuri cu hernii inghinale la adult. Descoperirea unei apendicite acute într-un sac de hernie inghinală este şi mai rară. D'Alia et al. (3) raportează o rată de 0,08% în 1341 hernii inghinale operate, iar Ryan (4) raportează doar 11 cazuri de apendicită acută asociată la 8.902 pacienţi cu hernii inghinale. Aspectul clinic al acestei afecţiuni variază în funcţie de intensitatea inflamaţiei la nivelul apendicelui şi de prezenţa sau nu a contaminării peritoneale (5,6). Poziţia apendicelui faţă de cec este variabilă : există tipuri de apendice cu situare retrocecală, pelvină, preileală, sau poziţie dreaptă pericolică (poziţia înaltă cecală). Nu s-au identificat poziţii ale apendicelui predispozante pentru angajarea într-un sac herniar inghinal (7,8). Rămâne nedemonstrată corelaţia între aceste variante anatomice de poziţie ale apendicelui şi angajarea acestuia în sacul herniar inghinal cu apariţia apendicitei acute. Fiziopatologia herniei Amyand adaogă la mecanismul obstrucţiei lumenului apendicular de către un coprolit şi strangularea apendicelui la nivelul coletului sacului herniar. Relaţia între încarcerarea şi inflamarea apendicelui nu este deplin dovedită (1,9). Majoritatea autorilor admit însă scenariul conform caruia imediat ce apendicele intră în sacul herniar, acesta devine vulnerabil la traumă iar ulterior este reţinut prin aderenţe inflamatorii (10,11). Angajarea cecului şi apendicelui în sacul herniar favorizează în plus staza conţinutului cecal şi apendicular sau chiar obstrucţia lumenului apendicular. Aportul sanguin poate fi substanţial afectat şi împreună cu edemul interstiţial să conducă la inflamarea apendicelui şi proliferarea bacteriană în lumenul şi în peretele acestuia. Contractarea muşchilor abdominali şi alte creşteri bruşte ale presiunii abdominale pot duce la compresia apendicelui şi inflamarea sa ulterioară. Anamneza şi examenul clinic orientează diagnosticul spre hernia inghinală încarcerată cu peritonită localizată, saculară (4,5). Prezenţa unui panicul adipos abundent face dificilă examinarea clinică şi decelarea semnelor inflamatorii locale sau a celor de abdomen acut. Contaminarea marii cavităţi peritoneale de la lichidul septic intrasacular conduce la apariţia fenomenelor de peritonită. Diagnosticul apendicitei acute este afirmat pe baza exmenului clinic ; prin urmare şi în cazul apendicitei acute în sac herniar examenul clinic local ar trebui sa stabilească diagnosticul, însă datorită rarităţii situaţiei anatomoclinice, raţionamentul care ar trebui sa ne conducă la această entitate este eronat şi, în consecinţă, diagnosticul constituie o surpriză intraoperatorie. Confirmarea diagnosticului la momentul deschiderii sacului herniar impune măsuri sporite de izolare şi protejare a câmpului operator cu evitarea contaminării elementelor anatomice extrasaculare (12). Antibioprofilaxia este de dorit a fi începută la inducţia anestezică în contextul prezenţei semnelor inflamatorii locale (13). Tratarea sacului herniar (disecţia de elementele funiculului spermatic şi rezecţia) poate fi dificilă dacă peritoneul sacular prezintă modificări inflamatorii importante (13,14). Înaintea ligaturii sau suturii acestuia la nivelul coletului, este necesară inspectarea conţinutului marii cavităţi peritoneale. Timpul septic de la apendicectomie contraindică utilizarea materia-

lului protetic sau a firelor de sutură nerezorbabile pentru refacerea peretelui inghinal.

CONCLUZII

Hernia inghinală cu apendicită acută în sacul de hernie este o variantă rară, iar când aceasta survine, este deseori diagnosticată eronat ca hernie strangulată. Tratamentul implică apendicectomia prin herniotomie urmată de cura herniei per primam fără material protetic.

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NOI SISTEME POLIMER-MEDICAMENT PE BAZĂ DE POLIMERI NATURALI ŞI SINTETICI

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NEW POLYMER-DRUG SYSTEMS BASED ON NATURAL AND SYNTHETIC POLYMERS (Abstract) : The great versatility of polymers makes them very useful in the biomedical and pharmaceutical fields. The combination of natural and synthetic polymers leads to new materials with tailored functional properties. The aim of this work consists in the preparation of new drug delivery system based on chitosan (natural polymer) and polybetaines (synthetic polymers), by a simple process, well known in the literature as complex coacervation methods. Also, the adsorption and release studies of two antibiotics as well as the preservation of their bactericidal capacities were performed. **Key words** : CHITOSAN, POLIBETAINES, COMPLEX COACERVATION METHOD, ANTIBIOTICS

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LEVEL OF KNOWLEDGE AND ATTITUDES AMONG DENTISTS REGARDING THE USE OF CBCT IN DENTAL PRACTICE

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LEVEL OF KNOWLEDGE AND ATTITUDES AMONG DENTISTS REGARDING THE USE OF CBCT IN DENTAL PRACTICE (Abstract): Cone Beam Computed Tomography (CBCT) is a revolutionary imaging technology in modern dentistry, providing detailed three-dimensional visualization of dental and maxillofacial structures. Despite its advantages, CBCT adoption is influenced by several factors, including costs, training, and professional experience. This study **aimed** to assess the level of knowledge and attitudes of dentists regarding CBCT usage in dental practice and identify the factors influencing its adoption. **Materials and methods**: A cross-sectional study was conducted using a structured online questionnaire completed by 113 dentists. The questionnaire collected data on demographic characteristics, professional experience, frequency of 2D and CBCT use, perceived benefits and limitations, and training levels. Statistical analyses were performed using *SPSS 26.0*, with a significance level set at $p < 0.05$. **Results:** The results showed that CBCT use was more frequent among specialists (46% weekly use) compared to non-specialists (33.3% daily use of 2D radiographs). Key advantages of CBCT, such as enhanced diagnostic precision and reduced need for exploratory surgical interventions, were recognized by 90.9% of dentists with over 10 years of experience. Barriers included excessive costs (56.6%) and challenges in interpreting 3D images (37.2%). Continuous training was highlighted, with 100% of dentists with 0–5 years of experience participating in specialized courses. **Conclusions**: CBCT is valued for its superior diagnostic capabilities, but its adoption is hindered by costs and training requirements. Standardized protocols and accessible education programs are essential for wider implementation in dental practices. **Keywords:** CBCT, DENTAL RADIOLOGY, IMAGING TECHNOLOGY.

INTRODUCTION

Cone Beam Computed Tomography (CBCT) has become an essential technology in modern dental medicine, providing detailed and three-dimensional imaging of dental and maxillofacial structures. This study aims to assess the level of knowledge and attitudes of dentists regarding the use of CBCT in their daily practice.

Three-dimensional imaging through CBCT is crucial in complex clinical scenarios that require detailed visualization of

anatomical structures. It is frequently indicated for dental implant planning, assessment of bone pathology, and the diagnosis of complex endodontic lesions. Additionally, CBCT is valuable for analyzing the temporomandibular joint (TMJ), advanced orthodontic treatments, and the evaluation of dento-maxillary trauma (1, 2). Furthermore, this method is preferred for the precise localization of mandibular canals, maxillary sinuses, and other surgically critical structures (3).

CBCT provides detailed three- dimensional visualization, enabling more precise clinical planning and execution of treatments. This technology allows for superior analysis of bone structures and complex dental anatomy, improving the diagnosis of complex pathologies and reducing the risks associated with surgical interventions (4, 5). Compared to conventional computed tomography (CT), CBCT involves lower radiation doses and provides images in a shorter time frame (6). Additionally, the images can be digitally stored and analyzed, facilitating their integration into patient documentation and communication with other specialists (7).

Despite its significant advantages, the use of CBCT comes with certain limitations. The high costs associated with equipment acquisition and investigation procedures may pose a barrier for both dental practices and patients (8). Furthermore, interpreting 3D images requires specialized training, and the lack thereof can lead to diagnostic errors (5). Another disadvantage is the large file sizes generated, which may necessitate advanced computer systems for management. Moreover, although the radiation dose is lower compared to conventional CT, it remains higher than that of 2D radiographs, necessitating cautious and justified use (6, 9).

Studies conducted in recent years highlight significant variability in the level of knowledge among dentists regarding the use of CBCT. Research shows that dentists who have participated in continuous education and specialized training programs generally demonstrate a better understanding of the indications, limitations, and advantages of CBCT. For example, a study by Alamri *et al.* (2012)

(10) revealed that while most general dentists are familiar with the basic applications of CBCT, they have limited knowledge when it comes to advanced interpretation of 3D images. This underscores the importance of integrating CBCT-related education into dental school curricula and continuous professional development programs.

Another aspect explored in several studies is the confidence level of dentists in using CBCT. According to research by Pauwels *et al.* (2013) (11), specialists in oral and maxillofacial surgery or implantology exhibit the highest confidence in utilizing this technology due to their experience in interpreting three-dimensional images. However, challenges such as limited access to equipment, excessive costs, and the need for additional training remain significant barriers. Studies also emphasize that only a small percentage of dentists possess detailed knowledge of radiation protection principles associated with CBCT usage, highlighting an urgent need to enhance education in this area (11, 12). In Romania, the number of studies on this topic is extremely limited, which is why the purpose of this study was to investigate the level of knowledge and attitudes of dentists regarding the use of CBCT in dental practice, as well as to identify the factors influencing the use of CBCT

in dental clinics.

MATERIALS AND METHODS

Study design. This cross-sectional study was conducted using a structured questionnaire designed for dentists, aiming to evaluate practices and perceptions regarding the use of 2D and 3D imaging (CBCT) in dental diagnosis and treatment. The questionnaire was developed to collect data on the frequency of use of these imaging methods, their clinical applicability, perceptions of their advantages and disadvantages, as well as the dentists' level of comfort in interpreting radiological images.

The questionnaire included demographic questions (age, gender, specialty, and professional experience) and specific sections on the use of 2D radiography and CBCT, the advantages and limitations of each method, the clinical scenarios in which they are applied, and the challenges encountered. Additionally, it investigated aspects related to professional training, knowledge of radiation protection, and the factors influencing clinical decisions regarding the use of these technologies.

Participation in the study was voluntary and anonymous, open to both general dentists and specialists. Data were collected through self-administered questionnaires, completed online via the Survio platform. The questionnaire included a combination of closed-ended, multiple-choice questions and open-ended questions to allow for detailed expression of opinions. The estimated time for completing the questionnaire was 15–20 minutes.

Participant selection. This analytical cross-sectional study included a representative sample of dentists, varying in age, gender, professional experience, and geographic location. The participants were informed about the purpose of the study, and completing the online questionnaire served as informed and mandated consent, eliminating the need for a signed consent form.

Data collection. The questionnaire was designed to collect information on the frequency of use, perceptions, and experiences of dentists with radiological imaging techniques. It included questions about the demographic and professional characteristics of respondents, the frequency of 2D and 3D radiology use, and the clinical contexts where these methods are essential. Additionally, the questionnaire explored the advantages and challenges associated with these techniques, such as costs, image interpretation, and equipment access.

Another critical aspect evaluated was the respondents' competence in radiological imaging and their level of professional training, with questions about participation in training courses and the need for additional education. The questionnaire also investigated the impact of imaging on therapeutic decision-making, the reduction of exploratory surgical interventions, and the improvement of patient communication. Furthermore, topics related to workplace safety and radiation protection, including known protection methods and the risks associated with radiation exposure, were addressed.

Data collection was conducted via the completion of the questionnaire in physical or electronic format, with participation being voluntary and confidential. The varied structure of the questions, including grids, evaluation scales, and open-ended responses, allowed for detailed insights into the use of radiological imaging in dentistry. The collected results will contribute to understanding current practices

and identifying training needs in this field.

The data analysis was conducted using *SPSS version 26.0 software* (IBM, USA).

Statistical tests such as the chi-square test were employed to compare the frequencies of categorical variables, and the t-test was used to analyze differences in means. The level of statistical significance was set at $p < 0.05$, confirming the relevance of observed differences in the use of 2D radiographs and CBCT based on specialization and professional experience.

RESULTS

The data presented in first table highlight the demographic characteristics of the study sample, which consisted of 113 participants. The average age of the participants was 42.88 years, with a standard deviation (SD) of 7.22 years, indicating a moderate variation in age within the sample; the youngest participant was 30 years old, while the oldest was 57 years old. The majority of participants were women (53.1%, n = 60), while men accounted for 46.9% (n = 53). Of the participants, 55.8% (n = 63) did not indicate having a specific specialization ("no"), while 44.2% (n = 50) reported holding a specialization ("yes"). Most participants (58.4%, n = 66) had more than 10 years of experience, followed by those with 6–10 years of experience (35.4%, n = 40), while only a small percentage (6.2%, n = 7) had between 0 and 5 years of experience.

Table II highlights the use of 2D radiography and 3D imaging (CBCT) in dental practice, correlated with specialization and professional experience. Regarding the frequency of 2D radiography use, most non-specialist dentists reported daily usage (33.3%), whereas specialized dentists predominantly used this technique occasionally (88.0%). Similarly, those with limited experience (0–5 years) used 2D radiography only occasionally (100%), while dentists with 6–10 years of experience tended to use it daily (40%). In terms of CBCT usage, non-specialist dentists utilized the technology both daily (33.3%) and weekly (34.9%), whereas specialized dentists predominantly used it weekly (46.0%).

TABLE I.
Demographic characteristics of the study sample

	No.	%
Age	42.8761± 7.21745 years old (min. age 30 years, max. age 57 years)	
Gender		
Female	60	53.1
Male	53	46.9
Specialty		
No	63	55.8
Yes	50	44.2
Work experience		
0-5 years	7	6.2
6-10 years	40	35.4
> 10 years	66	58.4

The clinical situations in which 3D imaging is considered essential are diverse, with 88.0% of specialized dentists and 65.2% of those with over 10 years of experience frequently selecting the response "All of the above." Additionally, the majority of participants believe that 3D imaging reduces the need for exploratory surgical interventions, with this opinion being more prevalent among non-specialist dentists (76.2%) and those with extensive experience (78.8%). Regarding the advantages of 3D imaging, "All of the above" was the dominant response, chosen by 82.0% of

specialized dentists and 90.9% of those with over 10 years of experience. Decisions regarding the use of 2D or 3D radiology based on costs varied: specialized dentists frequently opted for more financially accessible methods (62.0%), while non-specialists (46.0%) prioritized method efficiency, regardless of cost. These trends were supported by statistically significant values ($p < 0.05$) in most comparisons.

TABLE II.

Distribution of Questionnaire Responses by Specialty and work Experience (I)

	Specialty		Work Experience		
	No	**Yes**	**0-5 years**	**6-10 years**	**> 10 years**
How often do you use 2D radiographs in your dental practice?					
Daily	33.3%	4.0%		40.0%	10.6%
Weekly	25.4%	8.0%		10.0%	24.2%
Occasionally	41.3%	88.0%	100.0%	50.0%	65.2%
p	0.000		0.001		
How often do you use 3D imaging (CBCT) in your dental practice?					
Daily	33.3%			37.5%	9.1%
Weekly	34.9%	46.0%		32.5%	48.5%
Occasionally	31.7%	54.0%	100.0%	30.0%	42.4%
p	0.000		0.000		
In which clinical situations do you consider the use of imaging essential 3D (CBCT)?					
Dental implant planning		2.0%			1.5%
Assessment of bone pathology	33.3%	10.0%		25.0%	24.2%
Treatment of complex endodontic lesions	33.3%			37.5%	9.1%
All of the above	33.3%	88.0%	100.0%	37.5%	65.2%
p	0.000		0.002		
What, in your opinion, is the main advantage of using 3D imaging (CBCT)?					
Detailed visualization of bone structures	9.5%	4.0%		20.0%	
All of the above	73.0%	82.0%	100.0%	50.0%	90.9%
Detailed visualization of bone structures + Precision in treatment planning	17.5%	14.0%		30.0%	9.1%
p	0.427		0.008		
Do you believe that the use of 3D radiology has reduced the need for exploratory surgical interventions?					
Yes	76.2%	52.0%	100.0%	37.5%	78.8%
No		40.0%		15.0%	21.2%
Not sure	23.8%	8.0%		47.5%	
p	0.000		0.000		

	Specialty		Work Experience		
	No	Yes	0-5 years	6-10 years	> 10 years
Have you attended specialized training courses on the use and interpretation of 3D imaging?					
Yes, I have attended multiple courses	11.1%	2.0%	100.0%	2.5%	
Yes, I have attended one course	46.0%	58.0%		30.0%	69.7%
No, but I plan to attend	42.9%	40.0%		67.5%	30.3%
p	0.129		0.000		
To what extent do you consider 3D imaging necessary for accurate diagnosis in dentistry?					
Essential	41.3%	18.0%		85.0%	1.5%
Useful, but not essential	58.7%	82.0%	100.0%	15.0%	98.5%
p	0.008		0.000		
How do you decide to use 2D or 3D radiology based on the associated costs?					
I always choose the most efficient method, regardless of cost	46.0%	22.0%		50.0%	30.3%
I choose the more financially accessible method for the patient	9.5%	62.0%		20.0%	43.9%
I discuss the options with the patient and decide together	44.4%	16.0%	100.0%	30.0%	25.8%
p	0.000		0.000		

Table III presents data on dentists' opinions and practices regarding the use of 2D and 3D imaging, correlated with specialization and professional experience. Most specialized dentists (57.1%) believe that the use of 2D and 3D radiology should be standardized through clear protocols, while non-specialists are more divided, with a significant proportion (34.4%) considering that the decision should remain at the discretion of the dentist. Dentists with limited experience (0–5 years) unanimously support standardization (100%), whereas those with over 10 years of experience tend to favor decision-making freedom (47.6%). The determining factors for using CBCT include the ability to visualize structures in detail, with this option being predominant among specialized dentists (89.8%) and those with 6–10 years of experience (87.5%).

Regarding communication with radiologists, most dentists report contacting them rarely, with a higher percentage among non-specialists (90.2%) and those with limited experience (100%). In cases of disagreement with a radiologist's evaluation, specialized dentists tend to rely on their own judgment (63.3%), while non-specialists prefer to request a second opinion (44.3%). Additionally, discussing CBCT with the patient is considered necessary by a majority of specialized dentists (69.4%) and those with extensive experience (88.9%). In contrast, non- specialists and less experienced dentists (0– 5 years) do not consider patient discussions mandatory, possibly indicating a lack of awareness or differing priorities. Statistically significant p-values ($p < 0.05$) support the relevance of the observed differences.

TABLE III.

Distribution of Questionnaire Responses by Specialty and Work Experience (II)

	Specialty		Work Experience		
	No	**Yes**	**0-5 years**	**6-10 years**	**> 10 years**
Do you believe that the use of 2D and 3D radiography should be standardized in dental practices?					
Yes, there should be clear protocols for the use of each imaging method	31.1%	57.1%	100.0%	32.5%	42.9%
No, dentists should have the freedom to decide based on individual cases	34.4%	42.9%		30.0%	47.6%
Only for certain specialties, such as implantology or maxillofacial surgery	34.4%			37.5%	9.5%
p	0.000		0.000		
What factors influence your decision to use 3D imaging (CBCT) instead of 2D radiographs the most?					
Case complexity	34.4%	10.2%		12.5%	33.3%
The ability to visualize structures in detail	65.6%	89.8%	100.0%	87.5%	66.7%
p	0.003		0.017		
How often, if ever, do you contact the radiologist/ dental and maxillofacial radiology specialist regarding a result?					
Rarely	90.2%	63.3%	100.0%	77.5%	76.2%
Sometimes	9.8%	36.7%		22.5%	23.8%
p	0.001		0.348		
If you disagree with the evaluation of the radiologist/ dental and maxillofacial radiology specialist, what do you do?					
I rely on my own judgment	36.1%	63.3%		65.0%	42.9%
I request another consultation from the same radiologist	44.3%	2.0%	100.0%	15.0%	23.8%
I seek a second opinion from a colleague	19.7%	34.7%		20.0%	33.3%
p	0.000		0.000		
Do you believe a prior discussion with the patient regarding CBCT is necessary?					
Yes	36.1%	69.4%			88.9%
No	63.9%	30.6%	100.0%	100.0 %	11.1%
p	0.001		0.000		

The advantages of using CBCT (Cone Beam Computed Tomography) compared to other radiological evaluation methods, are illustrated in first figure. The most significant advantages highlighted include shorter time for acquisition and interpretation (55.8%), lower radiation exposure compared to other methods (61.1%), a more useful three-dimensional structure (61.1%), and better clarity of anatomical relationships (61.1%).

Additionally, 42.5% of respondents

found the ability to analyze images on a computer beneficial. The option "All of the above" was selected by 80.5% of participants, suggesting that the majority perceive CBCT as offering a comprehensive set of benefits. Notably, no respondent considered CBCT cheaper than other methods or reported being unaware of its advantages, indicating a clear perception of this technology's superiority among the respondents.

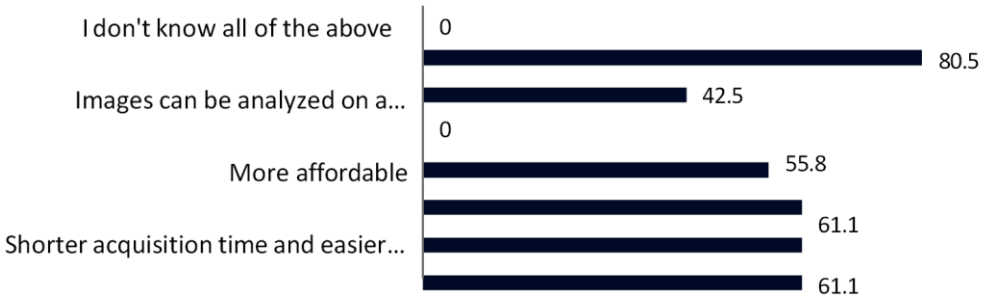

Fig. 1. The advantages of CBCT compared to other radiological evaluation methods

The main reasons why CBCT is not frequently used are presented in second figure. The most significant obstacle identified is the high procedural cost (56.6%), followed by the increased radiation risk (48.7%) and the difficulty in interpreting results (37.2%). Additionally, 27.4% of respondents consider CBCT unnecessary for the type of diagnosis they perform. Factors such as the need for specialized training (7.1%) and lack of access to this technology (7.1%) were mentioned to a lesser extent. Notably, file size and image quality were not considered obstacles, as they were not selected by any respondent.

Furthermore, 35.4% of participants chose the option "All of the above," indicating that for many users, the barriers to CBCT adoption are multiple and interconnected.

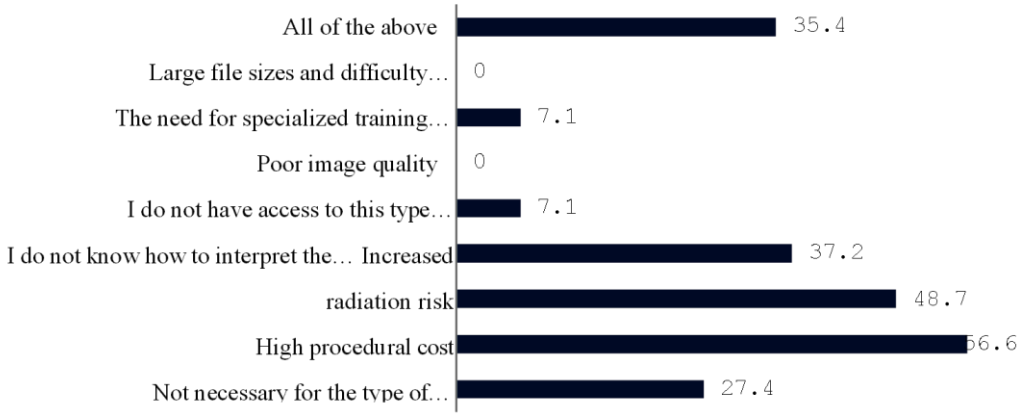

Fig. 2. Reasons for the Infrequent Use of CBCT

Figure 3 reflects users' expectations regarding the information included in a CBCT report. The most requested elements are measurements (54%) and images (54%), indicating the importance of visual and quantifiable details in data interpretation. The description of results is also considered essential (49.6%), while patient information is expected by 24.8% of respondents. The options "All of the above" and "Summary" were selected by 25.7%, suggesting that a significant number of users prefer a comprehensive and well- structured report. In contrast, differential diagnosis was not considered relevant, as it was not selected by any respondent. Other details, such as scan indication and type, were mentioned to a lesser extent (13.3%), indicating that users focus more on the imaging and analytical content of the report.

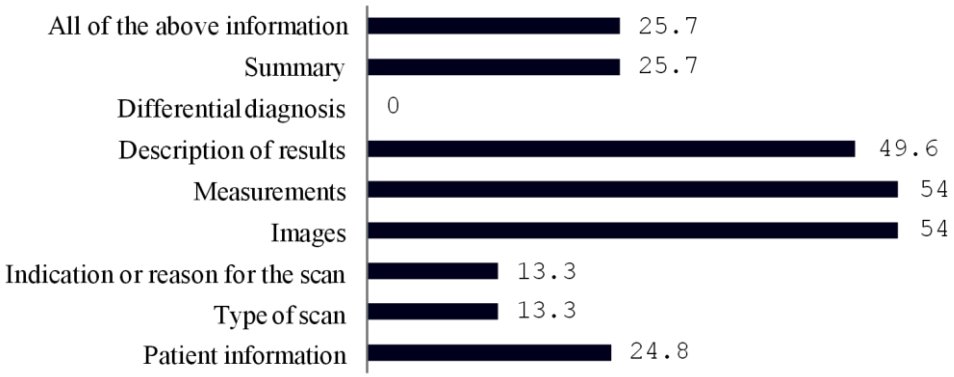

Fig. 3. Users' expectations regarding the information included in a CBCT report

DISCUSSION

Cone Beam Computed Tomography (CBCT) is recognized as a superior imaging technology compared to 2D radiographs due to its ability to provide detailed three- dimensional images of bone and dental structures.

The study results indicate that CBCT usage is more frequent among specialized dentists (46% use CBCT weekly) compared to non-specialists, where daily usage reaches 33.3%. These findings are supported by the scientific literature, which highlights the increasing adoption of CBCT in implantology, orthodontics, and oral surgery due to its enhanced diagnostic accuracy (14,15). Recent studies also emphasize that CBCT is frequently used in dental practice for the evaluation of bone pathologies and orthodontic treatment planning, aligning with the trends observed in this study.

Regarding the frequency of 2D radiograph usage, the data indicate that they are used daily by 33.3% of non-specialist dentists but by only 4% of specialists, who predominantly prefer occasional use (88%). This trend is confirmed by recent studies suggesting that 2D radiographs remain useful for routine diagnostics, whereas CBCT is preferred for complex cases requiring detailed three-dimensional information (16). On the other hand, dentists with 6–10 years of experience use 2D radiographs daily in 40% of cases, indicating a progressive adaptation to new technologies

as professional experience accumulates.

An important aspect identified in the study is the perception of CBCT advantages, with 90.9% of dentists with over 10 years of experience considering that 3D imaging offers superior benefits compared to all other available methods. These findings are supported by the scientific literature, which highlights that CBCT provides enhanced accuracy in treatment planning and reduces risks associated with surgical interventions (17). Additionally, CBCT usage has been correlated with a decrease in the need for exploratory surgical interventions, a trend also observed in this study, where 78.8% of experienced dentists reported that CBCT has significantly reduced the necessity for surgical exploration.

On the other hand, a major obstacle to CBCT adoption remains its high cost, which is perceived as a limiting factor by 56.6% of respondents. The scientific literature confirms this issue, indicating that the high price of equipment and associated scanning costs are barriers to the widespread adoption of CBCT technology (18). Nevertheless, specialized dentists tend to prioritize financial accessibility for patients (62%), whereas non-specialists (46%) prefer efficiency regardless of cost, highlighting differences in professional approaches.

The study also indicates that CBCT image interpretation is perceived as a challenge, with 37.2% of respondents considering it an obstacle. This trend is confirmed by recent literature, which suggests that the lack of specialized training can lead to difficulties in accurately interpreting three-dimensional images (19). Furthermore, studies emphasize the importance of training programs for the optimal use of CBCT, which is supported by the study results, where 100% of dentists with 0–5 years of experience reported participating in training courses.

The study findings are largely consistent with recent scientific literature, confirming the advantages of CBCT in dental diagnostics while also highlighting cost and training-related barriers. The study underscores that, although CBCT is an advanced technology, its optimal use depends on factors such as professional experience, dentist specialization, and financial accessibility.

CONCLUSIONS

The study highlights significant differences in the use of 2D radiographs and CBCT based on specialization and professional experience. Non-specialist dentists frequently use 2D radiographs, while specialists prefer 3D imaging, utilizing it more frequently on a weekly basis. CBCT is considered essential for the diagnosis and planning of complex treatments, particularly among experienced dentists, who recognize its role in reducing the need for exploratory surgical interventions.

Although cost and image interpretation challenges are perceived as barriers, most respondents acknowledge the advantages of CBCT, indicating the necessity of standardizing its use. The study emphasizes the importance of continuous professional training, revealing that dentists with limited experience actively participate in specialization courses. This suggests a growing trend in CBCT adoption in the future of dental practice.

CONFLICTS OF INTEREST AND FUNDING

All the authors declare no funding received and no conflict of interest.

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EPIDEMIOLOGICAL ASPECTS OF SOME HEALTH CONDITIONS THAT CAN BE PREVENTED IN PEDIATRIC PATIENTS FROM REPUBLIC OF MOLDOVA

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EPIDEMIOLOGICAL ASPECTS OF SOME HEALTH CONDITIONS THAT CAN BE PREVENTED IN PEDIATRIC PATIENTS FROM REPUBLIC OF MOLDOVA (Abstract): For the assessment of the effectiveness of different health programs in recent years, population studies as well as data processing by statistical methods are used. The results of such actions in assessing the level of protection of the population against preventable contagious diseases by means of vaccination programs serve as a basis for improvement and immunization services. Evaluating the National Immunization Programs at the population level carried out in several states, allowed deciding the causes that affect poor vaccination coverage in children. In 2010, in the Republic of Moldova, actions have been taken to implement the National Immunization Program (NIP) in collaboration with UNICEF, WHO and the Global Alliance for Vaccines and Immunization (GAVI). Tenders for vaccine acquisition were organized, and 10 vaccines were purchased - rabies immunoglobulin, as well as syringes worth of 9,855,652.85 lei. At the national level, the targets of the National Immunization Program were met: vaccination against tuberculosis in children up to 1 year old, vaccination against hepatitis B in children up to the age of 1 year old, polio vaccination in children up to 1 year old, DTP revaccination in 3-year-old children, diphtheria-tetanus revaccinations in 7-year-old and 15-year-old, ROR revaccination in 7-year-old. **Keywords**: VACCINATIONS, GLOBAL ALLIANCE FOR VACCINES AND IMMUNIZATION, NATIONAL IMMUNIZATION PROGRAMMES.

For the assessment of the effectiveness of different health programs in recent years, population studies as well as data processing by means of statistical methods are used. The results of such actions in assessing the level of protection of the population against preventable contagious diseases by means of vaccination programs serve as a basis for the improvement and immunization services (1, 2). Evaluating the National Immunization Programs at the population level carried out in several states, allowed to identify the causes that explain the poor vaccination coverage in children (for example some studies conducted in the USA) (3, 4). India, Ethiopia, Bangladesh, and Cameroon

showed that the level of vaccination coverage among children aged up to 3 years old is influenced by several factors: ethnicity, living environment, migration, parents' education level, medical staff's presence in the community as well as their professional competence, the materials and the techniques of the immunization service, the family's income, government support programs, shortcomings in the organization of children censuses (4). It has been found that the vaccination coverage in India and Cameroon is influenced by the geographical areas in which these families live, as well as by the order in which children are born. In addition, these studies allow checking the official data concerning the degree to which the vaccination coverage is reported (3, 4).

In the Republic of Moldova, more than 98% of children received the BCG vaccine, the first dose of hepatitis B vaccine (HepB), DTP and polio (Polio 1), which shows the easy access of children to immunization. Vaccination coverage registered a slight decrease for the subsequent doses; thus, 94% of the children received the three recommended doses of HepB, 92% of the children received three recommended doses of the vaccine against diphtheria-tetanus-whooping cough (DTP) and 94% of the children received all the three doses of polio. 91% of the children were vaccinated against measles and 89% were vaccinated against mumps and rubella. The decline in the coverage rates reflects the percentage for vaccination abandon as follows: 6% - DTP, 4% - HepB, and 5% - polio. These numbers represent the proportion of children who received the first dose of a vaccine, but who did not get to receive the third dose, which is acceptable for the case under discussion (5).

National Immunization Program.

In 2010, in the Republic of Moldova, actions have been taken to implement the National Immunization Program (NIP) in collaboration with United Nations International Children's Emergency Fund (UNICEF), World Health Organization (WHO) and the Global Alliance for Vaccines and Immunization (GAVI). Tenders for vaccine acquisition were organized, and 10 vaccines were purchased, rabies immunoglobulin, as well as syringes worth of 9,855,652.85 lei. At the national level, the targets of the National Immunization Program were met for the vaccination against tuberculosis in children up to 1 year old - 97.9%; vaccination against hepatitis B in children up to the age of 1 year old - 96.5%; polio vaccination in children up to 1 year old - 95.0%; DTP revaccination in 3-year-old children - 95.4% (Transnistria - 82.2%); diphtheria-tetanus revaccinations in 7-year-old - 97.6% (Transnistria - 93.4%), and 15-year-old - 96.3 % (Transnistria - 79.6%); ROR revaccination in 7-year-old - 96.3% (Transnistria - 88.9%). The target of the NIP was not met for the vaccination up to 2 years of age against measles, mumps and rubella (MMR) - 74.8%, nor for the vaccination against diphtheria, tetanus and pertussis up to 1 year of age (Transnistria - 77.5%) (6). Starting from 1st November 2010 until the end of the year, 2,237 people (mostly teenage girls) were vaccinated against human papillomavirus infection with "Gardasil".

Adverse events following immunization.

The comparative analysis of the adverse events following immunization (AEFI) shows that there were 90 of such cases in 2010 (71 AEFI - 2009, and 64 AEFI - 2008). Of the total number of adverse events following immunization, there were 55 regional lymphadenitis, 8 ulcers at the injection site, and one cold abscess. After the DTP vaccine was administered, there were 16 AEFI, while after the DTP+Hib vaccine was inoculated, there were 8 AEFI. AEFI in case of DTP and DTP+Hib manifested through

hyperpyrexia, crying, agitation, seizures, fever, local reactions, all of them with a favorable evolution for the patient. Vaccination against rubella had not been done in the Republic of Moldova until 2002, when people aged between 8-29 years were immunized extensively. Data analysis on the degree of immunity against rubella demonstrated the presence of rubella antibodies in the patients' blood in 97.4% of the investigated patients. The proportion of people with minimal protective titer was only 0.3% to 6% of the children proved to be receptive to rubella (5, 6).

The immunizations administered in 2010 as well as the prophylactic and the anti-epidemic measures taken throughout the same year helped to maintain a favorable situation. There were no recorded cases of polio caused by wild virus, diphtheria, measles and rubella. Morbidity due to pertussis was kept at a low level of incidence. Of the 31 cases of pertussis, 23 were recorded in the territories on the right bank of the Dniester (including the town of Chisinau-17 cases), as well as 8 cases in the Transnistrian territory. Out of 31 cases of pertussis, 20 people (64.5%) were not vaccinated against DTP. In children, there were 2 cases of hepatitis B (1 case in 2009). Thanks to the immunizations and the measures taken against epidemics, there was a significant decrease in the incidence of mumps - 3.53% versus 7.12% in 2009. After a period of 6 years which was free from tetanus, there were 2 recorded cases of illness caused by this infection in 2010. These patients had not received complete immunization. One of them died (2, 7).

Vaccination against hepatitis.

Hepatitis viruses B, C and D have an essential epidemiological and clinical significance as over 65% of the world population lives in areas with high prevalence of infection with hepatitis B virus (HBV), and more than 2 billion people have gone through infection. (8, 9). HBV infection is the 9th mortality cause in the world and the first cause of death due to liver cancer in areas such as Southeast Asia and Sub-Saharan Africa. The estimates of the prevalence of HBV infection among the world population indicate the existence of over 360 million people with persistent infection. The trend towards persistent HBV infection is marked even and in the countries where HBV is not so spread (8). The morbidity due to acute and chronic hepatitis leads to extremely serious social and economic consequences. The economic impact caused by these diseases between 1998 and 2005 (as shown by economic damages and treatment expenses supported by the state budget following absence from work) in the country represented 266.5 million lei. Hepatitis viruses B, C and D have an essential epidemiological and clinical significance as over 65% of the world population lives in areas with high prevalence of infection with hepatitis B virus (HBV), and more than 2 billion people have gone through infection (8, 9). HBV infection is the 9th mortality cause in the world and the first cause of death due to liver cancer in areas such as Southeast Asia and Sub-Saharan Africa. The estimate of the prevalence of HBV infection in the general population indicates the existence of over 360 million people with persistent infection. The trend towards persistent HBV infection is marked even and in the countries where HBV is not so frequent (9). Population morbidity due to acute and chronic hepatitis leads to extremely serious social and economic consequences. The economic impact caused by these diseases between 1998 and 2005 (as shown by economic damages and treatment expenses supported by the state budget following absence from work) in the country repre-

sented 266.5 million lei (8, 9).

Over the last 16 years (1992-2007), the following numbers were recorded in Chisinau: 4,344 cases of hepatitis B (HBV), 1,176 cases of viral hepatitis C (HCV) and 417 cases of viral hepatitis D (HVD). Of the total number of patients with HVB, 10% of adults and 90% of children became chronic carriers (1, 7, 10). Since 2001, there has been a trend of decrease of the HVD epidemic process. For example, the incidence of morbidity in 2003 was twice lesser than in 2002 and four times lesser than in 2000-2001. The decisive factor in reducing morbidity was the HBV immunization of the population groups that were at increased epidemiological risk. In 1989, selective vaccination of newborns of HBs Ag-positive mothers was initiated and it was carried out according to the immunization schedule in autumn 1994 (1, 6). In recent years, vaccination has been possible in the case of previously unvaccinated children (birth years 1988-1993). Thus, in Chisinau, 96,885 children aged up to 19 years were subject to immunization (98.9%) in 2007. As a result, there has been a significant decrease in HBV morbidity for 0-2, 3-6 and 7-14-year-old age groups (2, 5).

The Republic of Moldova belongs to highly endemic regions, despite the achievements in the prevention of acute cases of infection with HBV. HVB is still a present-day issue in this country because of the continuous high risk of transmission, which is determined by high level of HBsAg contagiousness (8-12%) among the general population as well as the high prevalence of chronic HBV patients that are the main source of the infection transmission (5, 7). In recent years, the medical community has been increasingly focused on the HIV / HBV co-infection. Probably because of the common routes of transmission of these viruses, of the common endemic regions but also of the fact that HVB is 100 times more contagious than HIV, nearly 70% of those infected with HIV showed HBV-specific markers (1, 5). Thus, death in patients with HIV infection is often caused by a liver condition. According to statistics, hepatitis B infection is the most common cause of death in patients with HIV infection. The seriousness of the problem also results from the fact that, among the people infected with HIV, there are women with children and / or pregnant, and their number is increasing annually (2, 6). The mothers infected with HIV / HBV are certainly the main source of perinatal and habitual infection for their children not only with HIV, but also with HBV.

According to WHO data, in highly endemic regions, the main transmission way of the HBV infection is the perinatal path (70-80% of the infected children). At the same time, about 90% of these children will develop chronic HBV infection which is most likely to occur after10-15 years. According to WHO, hepatitis B represents one of the most widespread infectious pathologies in the world. About 30% of the world population has contracted the infection caused by the HBV (3, 8).

Vaccination against tuberculosis.

In the Republic of Moldova, morbidity and mortality have increased since 2001. In 1993, WHO declared that TB represented an exceptional global situation and took immediate action to avoid the spread of this disease. A high level of illness due to tuberculosis was recorded in Moldova in the late 1990s when economic problems had a negative impact on all areas, medicine included (4, 11, 12).

The funding allocated to the health system plummeted, drugs were scarce and the control of the early diagnosis of diseases was neglected. In this context, the epidemiological analysis became the main way

of developing effective measures in fighting tuberculosis. On an international level, recurrent disease has been noticed, which is due to the demographic explosion, the deterioration of the health of the population in many areas of the globe, the increasing number of homeless people, the people who refuse vaccination and the growing number of immigrants from countries where tuberculosis is endemic (1, 3, 13). Two things contribute to all this: the HIV / AIDS pandemic, which is considered the most important factor that favors tuberculosis and the appearance of tuberculous bacilli which are tuberculostatic resistant. A study conducted by the World Health Organization states that there is a new type of tuberculosis which is spreading in the world and which is highly resistant to drugs, especially in the countries of Eastern Europe, Africa and China (8, 9, 10). The data collected from 81 countries between 2002 and 2006 showed that the new XDR-TB strain, found in 45 countries, is incurable. About 500,000 people worldwide are infected with this form of the disease, which kills around 30% of patients. Most cases of patients with the new form of tuberculosis were diagnosed in Moldova, Azerbaijan, Russia, Ukraine and Uzbekistan (2, 9). Annually, tuberculosis cases in women are increasing and infant morbidity remains at a high level. We are facing multidrug-resistant tuberculosis (from 3 cases in 2001 to 229 cases in 2006). In 2007, 194 patients were diagnosed with tuberculosis, of which 48 patients died. An alarming increase in infant morbidity occurred between 2003 and 2007. What is also alarming is the rise in the number of new cases of MDR TB. Of the total number of patients (46) in the city of Chisinau, 38 (82.6%) were isoniazid resistant, 38 (82.6%) were streptomycin resistant, 32 (69.6%) were rifampicin resistant and 26 (56.5%) were ethambutol resistant. The number of MDR patients was approximately equal in rural areas and in urban areas. Similarly, the number of single-drug resistant patients was almost equal to that of multidrug resistant patients. The rate of morbidity by gender reveals that 27.5% are female patients and 72.5% are male patients. General morbidity of tuberculosis remains high in young pediatric patients and young adults (0-26 years) (2, 5, 6).

Vaccination against mumps.

Mumps represents another important issue for the Republic of Moldova. A study conducted in Chisinau, including 165 patients who had been diagnosed with mumps and hospitalized in various hospitals in the city, reveals that 31% of the patients received the vaccine against mumps in March-April 2008, and the rest did not. The epidemiological investigation shows that 76% of the patients came from areas with outbreaks of mumps-communities (kindergartens, schools, colleges, work). Of the 165 patients, children and adolescents represent 46.7%, while the 20-34 age group represents 53.3%. It has been found that males (73.9%) are more affected than females (26.1%), both in children and adolescents and in young adults. Most patients (89.7%) were hospitalized during the first week of disease, of which 77.7% were admitted to hospital within the first 3 days. Mumps meningitis was diagnosed only in children (2.4%). The mild form of mumps was encountered in 38.8% of patients; the average form was present in 43%, while the severe form appeared in 18.2% (children and young adults with the same frequency). The disease evolved in 97% of cases with parotid gland damage (1, 2, 10).

Following the latest epidemic of mumps in the city of Ungheni, which took place between during 1995-1997 and was due to the small number of immunized people (less than 70% in 1994-1999), the morbidi-

ty due to this disease has shown a downward trend. Reduced morbidity was due to vaccination against mumps (from 90% in 2000 to 97% in 2007), to the implementation of the trivalent vaccine against measles, mumps, rubella (ROR) in 2002, with the two doses at the ages of 12 months and 7 years, respectively. The incidence of mumps was at the average annual level in the country and did not exceed 10 to 15%; it also showed a downward trend, and in 2006 there were only 9 cases of disease. The retrospective analysis of 939 cases of mumps recorded in the district of Ungheni in October 2007-March 2008 showed the following: at the end of March 2008, 52 localities of the district were affected (72.2% of the territory). The distribution of the disease cases by area of origin showed a predominance of the disease in the rural areas 65.7% (617 cases) compared to 322 cases (34.3%) in the urban areas. Most cases of mumps were recorded in pre-university institutions (high school, middle school, kindergarten), mainly in large classes-605 cases (65%) of total number of patients.

The mumps epidemic affected people aged from 1 to 53 years old (3, 6). The average affected age was 16 years. 67.2% of the patients (631 cases) belonged to age group 14-18, and 11.0% (116 cases) belonged to the age group 10-13. The other age groups had little epidemiological significance.

The time interval between the time of immunization and the appearance of the clinical signs of disease had an average value of 13 years, with a minimum time interval of up to 1 year (2 people) and a maximum one of 28 years (1 person). The percentage of sick people who had not received any dose of vaccine against mumps was 14.3% (134 cases). The patients who had been immunized with a single dose of vaccine represented 76% (714 cases), while the patients who had been revaccinated represented 5.2% (49 cases). Most of the infected people who had not received any doses of the vaccine belong to the age group 15-19 (58%), which also includes the patients who had received a single dose of vaccine. Most revaccinated patients belong to the age group 10-14 and make up for over 85% of this group. In terms of the gravity of the clinical picture, mumps cases were distributed as follows: mild cases-235 (25.0%), medium cases-635 (67.6%) and serious cases-69 (7.3%) (1, 3).

The assessment of the knowledge regarding the vaccines has been done by means of questionnaires. For the study, a specific questionnaire with five questions has been especially designed for middle school and high school pupils (grades 7 - 12) in the city of Calarasi, Republic of Moldova (1, 5). Of the total of nearly 300 students, 180 answered the question “What is vaccination?” by saying that it was “a process for protecting the body against infectious diseases”. The others answered that vaccination was “a drug” or “an injection for treating diseases”. The proportion of pertinent responses on the role and benefits of vaccines is satisfactory. However, about 10-15% of respondents indicated other answers: that vaccination was necessary “to be healthy”, “not to catch a cold”, “not to catch a venereal disease”.

It can clearly be noticed that adolescents know too little about the differences between non-transmissible and transmissible diseases. For the question “List the diseases against which vaccines are used”, all the respondents (100%) correctly listed from one to three vaccine-preventable diseases. Tuberculosis was most often indicated (90% of respondents), as well as hepatitis (82.2%), influenza and diphtheria (57.5%); up to 43.1% indicated measles and mumps. When asked to indicate the

place where they received the last dose of vaccine, all the respondents answered that they had received it in medical institutions in the locality where they live. The small number of adolescents who know the name of the last dose of vaccine they received shows their lack of interest in their own health, leaving the medical personnel and / or family to worry about this (10).

CONCLUSIONS

The efficiency of the immunization programs largely depends on the population participation in their implementation. It is important what teenagers and young adults or future parents think about vaccines and vaccination as a preventive measure against some transmissible diseases.

It is necessary to increase personal responsibility of everyone to their own health and it is appropriate for the medical personnel of schools as well as teachers to get involved in the process of training for a healthy life.

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OPEN APPROACH VERSUS MINIMALLY APPROACH FOR THE TREATMENT OF VARICOCELE IN CHILDREN - AN EPIDEMIOLOGICAL STUDY

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OPEN APPROACH VERSUS MINIMALLY APPROACH FOR THE TREATMENT OF VARICOCELE IN CHILDREN-AN EPIDEMIOLOGICAL STUDY **(**Abstract**).** **Aim**: The therapeutic indication of varicocele and the best method of treatment in children are still subjects of extensive debate in the literature. The purpose of the present study is to compare two of the most frequently used methods of treatment, both in terms of patient evolution and in economic terms. **Material and methods:** The observation charts of 69 patients with varicocele, who were hospitalized in the Pediatric Surgery Clinic of the "Saint Mary" Children's Emergency Hospital, have retrospectively been analyzed for a period of approximately 4 years. Three groups of patients have been created depending on the method of treatment - 25 cases operated by laparoscopic approach, 28 cases where the classical approach was used and 12 cases where surgery was denied or delayed. The demographic data, the duration of the surgery, the duration and the cost of hospitalization, the complications and the relapse rate have been compared statistically. **Results and discussion**: Patients mainly came from rural areas (73.8%), with an average age of approximately 14.5 years. The difference in weight of 54.7 / 53.5 kg between the group treated classically and the group treated laparoscopically was insignificant (p=0.76). The total duration of hospitalization and the postoperative duration of hospitalization were slightly higher in the group treated laparoscopically (5.24 / 4.28 days and 2.08 / 1.82, respectively), but the differences were not statistically significant (p=0.13 and 0.29, respectively); yet, the duration of the surgery was significantly higher (p=0.002) in the group treated laparoscopically (73.0 / 57.32 minutes). In terms of relapse, the postoperative progress was comparatively similar - only one patient in both groups relapsed and then was addressed by the other technique. However, regarding the rate of the postoperative occurrence of the hydrocele - a well-known complication following the surgical treatment of varicocele - it has not occurred in any patient treated classically, but it occurred in four patients treated laparoscopically (16%). Both the overall hospitalization costs (1,818/1,999 RON - classical / laparoscopic approach) and the costs divided on medication, sanitary materials used and medical tests were similar. **Conclusions:** The treatment of varicocele by laparoscopic approach began to be practiced in our clinic about four years ago, and although it is still being perfected (long duration of surgery and long duration of hospitalization), the results are encouraging. **Keywords:** CHILD, VARICOCELE, LAPAROSCOPY, CLASSICAL APPROACH.

A varicocele is the most frequent prepubertal andrological pathology which can cause infertility. This condition has been known since the first century, when it was described by Cornelius Celsus as follows: "when the disease has spread over the testicle and its cord, the testicle sinks a little lower, and becomes smaller than its fellow, in as much as its nutrition has become defective" (1). In 1550, Ambroise Pare described varicocele as "a pack of blood vessels well filled with melancholic blood" and, in 1885, Barwell described its surgical treatment, which is the subcutaneous ligation of the spermatic vessels (2). In 1948, Palomo described the retroperitoneal approach for the ligation of the spermatic veins at the inguinal canal exit, with the preservation of the spermatic artery (3). The incidence of varicocele is less than 1% in prepubertal boys, 15-30% in children of 12-18 years of age (4) and approximately 15% in the adult male population. A varicocele may be primitive (idiopathic, essential), as it is usually the one on the left side, which appears after puberty (13-30 years) without a determined cause, and secondary, which occurs due to a condition that produces a barrier to blood flow in the spermatic veins (e.g. right renal carcinoma). Another cause of secondary varicocele is the "nutcracker" syndrome, which is the left renal vein compression by the superior mesenteric artery, and the venous drainage obstruction and stasis at the pampiniform plexus (5). Varicocele may be bilateral in up to 2.5% of cases in adolescents and in up to 10% of cases in adults after the age of 35 - 40 (6). In 95-98% of cases, the condition occurs on the left side in adolescents and, if left untreated, it is the one that causes male infertility in 15-40% of infertile adults (6). Primitive varicocele is favored by several existing anatomical features on the left side, namely: the left spermatic vein is 8-10 cm longer than the right one; the blood flow angle of the left spermatic vein into the left renal vein is a right angle (90 degrees), which favors venous stasis; the colon compresses the left spermatic vein; the left spermatic artery spirals around the vein. The risk factors are: prolonged standing, poor quality of the venous wall, absence of valves inside the veins, hereditary constitutional predisposition and a genetic component, increased abdominal pressure. The pathophysiologic mechanism responsible for the infertility associated with varicocele is not fully elucidated; in addition to certain genetic and molecular causes, many other causes can be mentioned: testicular hypo perfusion and associated hypoxia, oxidative stress, increased temperature, reflux of certain toxic metabolites in the adrenal gland or in the kidneys, hormonal imbalance and toxic exogenous factors (7). Regarding the therapeutic indication of varicocele or the best surgical approach, there are still many controversies in the literature when it comes to children and adolescents. The purpose of the present study is to compare two of the most common methods of treatment, both in terms of patient outcome and in economic terms.

MATERIAL AND METHODS

We conducted a retrospective analytical study on 69 children hospitalized and treated in the Pediatric Surgery Department of the "Sf. Maria" Emergency Children's Hospital of Iaşi, between January 2013 and September 2016. The patients were identified per the disease code entered in the hospital's computerized database, and then the patients' records were extracted from

the hospital archives. 4 patients were excluded from the study for missing data. Demographics, associated disorders, investigations, duration of surgery, duration and cost of hospitalization, complications and relapse rate were statistically compared. Three groups of patients have been created depending on the method of treatment - 25 cases (38.4%) operated by laparoscopic approach, 28 cases (43.0%) where the classical approach was used and 12 cases (18.4%) where surgery was denied or delayed. The data were processed by using the specialized Microsoft Excel data analysis and processing module.

RESULTS

Patients mainly came from rural areas (73.8%), with an average age of approximately 14.5 years (between 8 and 19 years of age). The reason why they went to the doctor was the appearance of a swelling in the scrotum, accompanied by local sensation of heaviness; in 40% of cases, persistent pain was the main symptom. The surgical indication was established on the following criteria: at least clinical second grade varicocele, smaller ipsilateral testis with ultrasound-measured volume and / or persistence of symptoms for more than three months. The decision to intervene surgically and the method of treatment (classically or by using a minimally invasive approach) depended on the doctor's option and experience. For surgeons who are familiar with both methods of treatment, the patient's weight was the one that mattered in the choice of surgical approach. Thus, 18.5% of patients were treated conservatively, 43% were operated on classically and 38.5% of patients were treated by laparoscopic approach.

The difference in weight of 54.7 / 53.5 kg between the group treated classically and the group treated laparoscopically was insignificant (p=0.76). Of the 65 patients, 4 patients (6.1%) had a varicocele on the right, 1 patient (1.5%) had a bilateral varicocele and the rest had a varicocele on the left. 27 children (41.5%) had other associated diseases, which were not related to the disease studied. In terms of imaging tests, 22 of the 28 cases (78.5%) of varicocele treated classically were confirmed by scrotal ultrasound - this investigation highlighted the smaller size of the left testicle as compared to the right one in 11 cases (50.0%); in 19 patients (67.8%), the abdominal ultrasound was also used - this was normal in all cases except for one patient for whom the investigation revealed the presence of bladder stones. For one patient, CT was used due to significant weight loss over the past six months; however, it did not reveal associated abdominal pathology. In the group of patients who were treated laparoscopically, 23 of the 25 patients (92.0%) were also diagnosed by scrotal ultrasound; in 7 patients (30.4%), the affected testicle was smaller than the contralateral one. Of the 25 patients, 23 (92.0%) also underwent abdominal ultrasound, which was normal; one patient with grade III left hydronephrosis and enuresis underwent abdominal CT and one patient with a history of right varicocele and right orhiepididymitis also underwent abdominal MRI.

Of the 12 patients (18.4%) who were not approached surgically, only one patient had grade III varicocele and he refused the surgery; 8 patients were diagnosed with grade I varicocele, and two of them were scheduled for surgery; three patients with subclinical varicocele and six patients with grade I varicocele were proposed long-term

follow-up care. All the patients in this group underwent abdominal ultrasound, which turned out to be normal, and scrotal ultrasound, which showed that one testicle, the affected one, was smaller in 3 patients (25%): one patient who refused the surgery and two patients who were scheduled for surgery.

The total duration of hospitalization (including controls) and the postoperative duration of hospitalization were slightly higher in the group treated laparoscopically (5.2 / 4.3 days and 2.1 / 1.8, respectively), but the differences were not statistically significant (p=0.13 and 0.29, respectively); yet, the duration of the surgery was significantly higher (p=0.002) in the group treated laparoscopically (73.0 / 57.3 minutes). In terms of relapse, the postoperative progress was comparatively similar - only one patient in each group relapsed (4% and 3.5%, respectively) and then was addressed by the other technique. One patient in each group showed postoperative signs of orhiepididymitis in the affected testicle; the patient treated classically was subsequently diagnosed with varicocele recurrence. However, regarding the rate of postoperative occurrence of the hydrocele - a well-known complication following the surgical treatment of varicocele - it appeared on average at 6 months postoperatively in only one patient treated classically (3.5%) and in four of the patients treated laparoscopically (16%) - overall, the postoperative hydrocele rate is 9.4%. In two patients (one in the group treated laparoscopically and one in the group treated classically), the small size of hydrocele was the indication for conservative treatment. One patient was treated by puncture aspiration of hydrocele with favorable results; the other two patients, in whose case the fluid reappeared after having been aspirated, required surgical intervention, i.e. Jaboulay-type hydrocelectomy. Of the 25 patients (38.4%) who were approached laparoscopically, three also received concomitant treatment for other associated diseases-i.e. umbilical hernia in one patient and inguinal hernia in two other patients.

From an economic point of view, both the overall hospitalization costs (1,818/ 1,999 RON, p=0.49 - classical / laparoscopic approach) and the costs divided on medication, sanitary materials used and medical tests were similar. Thus, for the group treated classically, the money was spent as follows: approximately 92 RON on medication, 89 RON on sanitary materials and 62 RON on medical tests, compared to 111 RON (p=0.57), 135 RON (p=0.23) and 86 RON (p=0.32), respectively, in the group treated by minimally invasive approach.

DISCUSSION

A varicocele is a condition that most often (95-98%) occurs on the left side in adolescents; it can be bilateral in up 2.5% of cases in adolescents and in up to 10% of cases in adults (6). In our group of adolescents, the condition was identified on the left side in 93.9% of cases and was bilateral in 1.5% of patients. Varicocele symptoms can be discreet, such as: heaviness in the scrotum, testicular discomfort to continuous pain, difference in size between the two testicles, psychosexual disorders if the pain is persistent. All the patients in our study sought medical attention for swelling with or without heaviness in the scrotum, and almost half of them complained of persistent pain. Quite often, varicocele is asymptomatic and is discovered during routine ultrasounds or during investigations

for infertility in adult couples. Physical effort, exposure to heat and prolonged standing favor the onset of varicocele and can explain the prevalence of the disease in patients from rural areas (73.8%), where children and adolescents are much more physically active and sometimes start work at an early age. The objective physical examination of the patient highlights the increased size of the hemiscrotum; the affected spermatic veins can be visibly dilated, making a specific pattern in the thin scrotal skin. On palpation, this conglomeration of veins is painless and feels soft, mobile, like a "bag of worms". Three clinical grades of varicocele are described by Dubin and Amelar (8): Grade 1 - the varicocele is palpable only during special maneuvers that increase abdominal pressure (Valsalva maneuver); Grade 2 - it is palpable in dorsal decubitus, without other maneuvers; Grade 3 - this type of varicocele is both palpable and visible (the veins are dilated more than 4 mm) and it can be identified by the patient (this degree, but not only this one, is associated with patient's impaired fertility).

Of the radio-imagistic investigations, the scrotal Doppler ultrasound is commonly used to record the spontaneous blood flow speed in the spermatic veins, which usually indicates stagnation of blood in the varicose, dilated veins. Normally, the size of the veins of the pampiniform plexus range from 0.5 to 1.5 mm in diameter; if the dilation of these veins is greater than 2 mm, it suggests the diagnosis of varicocele (9). Testicular ultrasound can also confirm the gonadal hypotrophy, as it happened in 50% and, respectively, 30.4% of our cases; it can also confirm the subclinical varicocele, which is the dilatation of spermatic veins that can be detected only by ultrasound, and cannot be clinically evidenced, not even through the Valsalva maneuver; it is estimated that approximately 25% of patients with varicocele on one side and which can be detected during a clinical examination have subclinical varicocele on the other side (10). Retrograde spermatic phlebography (venography) can be considered the "gold standard" for the diagnosis of varicocele, but just like scintigraphy, it has limited indications due to invasiveness and is used primarily for relapses (11); contact thermography is of historical interest; investigations such as CT or MRI are indicated in the diagnosis of recurrent or secondary varicocele; the spermiogram - a predictive test for an adult patient's ability of fertilization, will be performed before and after the surgery; the spermiogram is usually affected: lower sperm count, with poor mobility. Since our group was made up only of patients of pediatric age, the spermiogram was not carried out for ethical reasons.

The evolution of varicocele is extremely slow. The condition causes discomfort locally and may lead to complications in adults, such as changes in spermatogenesis and infertility because, due to the blood that stagnates in the testicular veins, the local temperature increases and spermatogenesis is affected (both the quality and the quantity of sperm decrease). Therefore, if left untreated, a varicocele can lead to the atrophy of the affected testicle, to lower testosterone levels and, eventually, to infertility (9,10). But since not all adults with varicocele are infertile, the decision to treat a child or an adolescent suffering from varicocele only to prevent possible subsequent infertility is controversial. Unfortunately, in the case of children, there is no agreement on the therapeutic indication of

varicocele and neither on the best surgical approach; the goal of the treatment is the prevention of the testicular function degradation and the gonadal atrophy, as well as the prevention of infertility, but with a low rate of recurrence and appearance of a secondary hydrocele, of nerve injuries or of other complications. The American Society for Reproductive Medicine recommends the treatment of varicocele in adults when it is palpable and at least one of the sperm analysis parameters is abnormal, in case of infertile couples where the woman is normal. In adolescents, the same organization considers that only the patients with varicocele and hypotrophy of the affected testicle should be eligible for surgery (11). Other therapeutic indications in adolescents are: clinical grade 2 or 3 varicocele, testicular discomfort, abnormal semen analysis and venous reflux detected by ultrasound; however, these indications are not universally accepted (4, 8). In our case, the criteria for recommendation of surgical treatment were: at least clinical grade 2 varicocele, smaller ipsilateral testicle measured by ultrasound and / or persistence of symptoms for more than three months (40% of children came in with persistent scrotal pain). There are studies demonstrating the beneficial effect of varicocelectomy in adolescents on the testicular growth, on the normalization of testosterone levels as well as on the improvement of sperm quality. Therefore, the decision to intervene surgically in a teenager with varicocele is left at the discretion of the physician (5,7).

Despite the well-known natural history of varicocele, the optimal method of treatment is still a controversial subject in the literature. There are two main types of treatment for varicocele: varicose vein ligation and sectioning by using a classical or a laparoscopic approach and selective trans venous embolization or varicose vein sclerotization by means of interventional radiology procedures (5); in case of recurrence after one method, the alternative technique may be used. The sectioning of the spermatic veins can be done classically by retroperitoneal surgical approach (Palomo technique), inguinal (Ivanissevich technique), microsurgical (inguinal or subinguinal) (6) or by laparoscopic approach. There are studies that show that the lowest rate of recurrence and complications can be obtained through the microsurgical subinguinal approach, but the technique is more difficult (7). Over the last 20 years, the embolization or the sclerotizing of testicular veins by percutaneous inguinal approach under local anesthesia has had results in terms of fertility that are like the advantage of minimal invasiveness and a low risk of developing postoperative hydrocele (8). Techniques such as NOTES (natural orifice transluminal endoscopic surgical procedures) and LESS (laparo-endoscopic single-site), as well as the robotic-assisted inguinal approach are also attractive, but their role in the treatment of varicocele must be validated through randomized studies, on large groups of patients.

A recent study analyzes the methods of diagnosis, the indications and the treatment alternatives of varicocele in children and adolescents, based on the responses of 131 pediatric urologists (4, 10,11). Comparing the results of this study with our results, we can say that: the decision of surgical treatment is based on the reduced size of the affected testicle (96% of cases), testicular pain (79%), abnormal parameters of the semen analysis (39%), while our decision was based on the clinical grade of varico-

cele, the testicular shrinkage determined by ultrasound and / or the persistence of painful symptoms for more than three months (40% of cases); the average age at the time of surgery was 12.5 years compared to 14.5 years in our study; the preferred method of treatment was the laparoscopic approach (38% of surgeons), followed by the microsurgical sub inguinal approach (28%), the inguinal approach (14%) and the retroperitoneal approach (13%), compared to 38.5% - laparoscopic approach in our case and 43% - retroperitoneal approach (note: the inguinal or the sub inguinal approach is not practiced in our clinic).

The laparoscopic Palomo technique has been the preferred technique for the treatment of the varicocele in children in many pediatric surgery centers since 1988 (4, 9). Laparoscopy has numerous advantages: the patient recovers fast, the surgeon can see the place very well during the surgery, the dilated veins can easily be located, the relapse rate is lower, and there is the possibility to incidentally diagnose and treat possible associated pathologies - three of our patients had their umbilical hernia and inguinal hernia treated during the same surgery. Surgery usually takes less than one hour and the patient requires hospitalization for 24-48 hours postoperatively; these aspects have been confirmed by our study. Thus, in the study presented, the average duration of the surgery was less than one hour for the classical approach and just over one hour for the laparoscopic approach; the latter was calculated by also taking into consideration the three cases that involved complementary surgical procedures for the treatment of associated diseases, which increased the duration of the surgery. Besides the duration of the laparoscopic approach, which was longer, the two surgical techniques proved to be similar in terms of efficacy and cost; here, one must consider the fact that the laparoscopic approach was introduced in our clinic only about four years ago, which means that our pediatric surgeons are still learning in this regard. We expect the future duration of the laparoscopic approach to decrease gradually, as well as the length of postoperative hospitalization (from two days to one day), which will translate into lower hospitalization costs. Thus, the laparoscopic approach will become option number one in our clinic.

Some surgeons perform the mass ligature and sectioning of the spermatic artery and veins as part of the classical approach as well as the laparoscopic approach. There is no evidence that this technique adversely influences further development, since the testicle will still receive arterial blood from the collaterals of the deferential artery. Although the topic is controversial, a comparative study conducted on 122 adolescents who were operated on laparoscopically, with and without protection of the spermatic artery during varicocelectomy, showed significant improvement in semen quality in patients who had their spermatic artery protected (4). Moreover, the protection of lymphatic vessels during varicocelectomy could decrease the rate of postoperative hydrocele (2, 10). The effect of the surgery (the reduction of testicular varicose veins and the improvement of the spermiogram) is visible after two or three months. The relapse rate can reach 20%. In our study, the mass ligature and sectioning of the spermatic artery and veins was performed, regardless of the chosen method of treatment; only one patient in each group relapsed (3.5% and 4%), but this figures can also be the result of an error due to patients' follow-up for a short

period - in the long term, more relapses may occur - or to the fact that patients with a relapse addressed other centers of pediatric surgery. Other possible postoperative complications are: occurrence of testicular hydrocele, permatic artery injury with subsequent testicular atrophy, bleeding and infection. In our study, postoperative hydrocele occurrence rate was 3.5% for the classical approach and 16% for the laparoscopic approach, compared with the rates reported by other studies: 13% and 20%, respectively (2, 11). The reason for the hydrocele occurrence was probably the mass ligature of veins with lymphatic vessels, both in the classical approach and in the laparoscopic one.

CONCLUSIONS

This study found that, since not all adults with varicocele are infertile, it is obvious that not all adolescents with this condition must be operated on. Yet, the criteria regarding *which*, *when* and *how* adolescents with varicocele should be treated have not been clearly defined yet. For children, especially, further studies are needed to formulate clear treatment protocols for this condition. The treatment of varicocele through laparoscopic approach has been practiced in our clinic for about four years, and although it is still being perfected (long duration of surgery and long duration of hospitalization), the results are encouraging.

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THE STUDY OF THE ASSOCIATION OF DECAY RISK WITH MALOCCLUSIONS IN MIXED DENTITION AT CHILDREN FROM NORTHEAST ROMANIA. A TRANSVERSAL RETROSPECTIVE STUDY

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THE STUDY OF THE ASSOCIATION OF DECAY RISK WITH MALOCCLUSIONS IN MIXED DENTITION AT CHILDREN FROM NORTHEAST ROMANIA. A TRANSVERSAL RETROSPECTIVE STUDY (Abstract): **Aim**. The purpose of this study was to investigate if high values of DMFT/dmft indices constitute risk factors for installation in various types of malocclusions in mixed dentition. **Material and Methods** The study consisted of 600 study-cast with mixed dentitions coming from the children in northeastern Romania. WHO (World Health Organization) dental caries criteria were applied to both groups. The existence of an increased caries risk was deducted from the dmft (decay, missed, filled temporary teeth) and DMFT (Decay, Missed, Filled Teeth) indices related to age. Malocclusion was classified in the three planes in accordance with the major symptom. The statistical study was conducted in SPSS 16.0 for Windows. The risk factors we have identified through calculating the odds ratio and relative risk. **Results:** Mean value dmft/DMFT (index in mixed dentition was found 3.64±2.686. The highest values of the dmft/DMFT indices were found in patients with normal overjet (4.73± 2.892) and normal overbite (4.04±2.781). In the transverse plane, the lowest value of DMFT/dmft index was recorded in patients with spacing (2.63±1.915), and the highest values were observed in patients with the combined diagnosis of crowding and lateral cross bite (4.97±3.010) respectively of crowding and spacing (4.65 ±2.792).The presence of malocclusions in the three plans is not influenced by increased DMFT/dmft index (DMFT/dmft>5) unless combined diagnoses of crowding and lateral cross bite, and crowding and spacing where the relative risks are over 2.5. **Conclusions**: The present study concluded that the prevalence of dental caries in children with mixed dentition is higher in patients with normal overjet and overbite. In terms of the risk of developing a certain malocclusion with dental crowding associate with lateral cross bite, respectively dental crowding associate false spacing are present under a high caries activities. **Keywords:** DENTAL CARIES, INDEX DMFT/DMFT, MALOCCLUSIONS, MIXED DENTITION, RISK FACTORS.

Dental decay remains a major public health problem, its management still requires a great deal of the budget provided for healthcare, which is the reason why it still requires continued efforts to find solutions to prevent this disease (1-3). Just as with other chronic diseases with high prevalence in the population, the actual inci-

dence and prevalence data (60-75% for deciduous teeth and 20-40% for permanent teeth at children with mixed dentition) for dental decay were obtained through epidemiological studies (4-11).

In previous attempts to investigate a possible association between dental decay and malocclusions, studies have shown conflicting or inconclusive results (9-11). While some authors have reported a positive association between dental decay and malocclusions (12-20), others could not establish any significant relation (9).

Furthermore, the controversial results were obtained in studies that found a possible relation between malocclusions and various oral hygiene indices (12, 13). The presence of a positive association between malocclusions and periodontal health has been described by Gabris (13).

However, few studies have evaluated the dental and orthodontic statuses simultaneously in an appropriate manner and examined less the possible interactions between them, in terms of dental decay risk. It was only assumed by many authors the hypothesis that dental decay and premature loss of temporary teeth are factors that predispose to malocclusions in mixed and permanent dentition (21). However, a causal relation between dental decay and malocclusions has not been demonstrated with certainty, until now. Clarifying the role of dental decay in the further development of malocclusions can assist in the clinical decision, in setting the priorities in public treatment.

This study's objectives represent the answers to the following questions: 1. Is dental decay in mixed dentition more common in some types of malocclusions? 2. Is the dental decay risk, represented by high values of the DMFT/dmft (Decay, Missed, Filled Teeth) index in mixed dentition, a risk factor for installing certain types of malocclusions?.

In this regard, our study aims to investigate the dental decay risk for the occurrence of malocclusions, calculating the DMFT/dmft indices for mixed dentition in a group of children with malocclusions, from northeastern Romania.

MATERIAL AND METHODS

This transversal retrospective study comprises the random selection of 600 subjects between the ages of 6 and 8 years old, based on documents and medical information (observation sheets, orthopantomography's, intra/extra-oral photographs and study-casts) that have been retrieved from the archives of the Pediatric Dentistry Clinic from Iasi (Romania) and were subsequently analyzed. The selection condition was the permanent first molars and incisors to be erupted (dental age of 8 years old) as well as no orthodontic treatment before the examination. The ethical committee's approval for this study was not necessary because it is a retrospective archive study. As usual protocol, all patients (their parents) sign the informed consent that they agree for patient (children) data to be used in scientific studies. Data were collected by a single examiner, who prior did training and calibration exercises.

The registration criteria of dental decay experience were carried out per WHO (World Health Organization) guidelines using the dmft index for deciduous teeth and the DMFT index for permanent teeth, on both study-casts and intraoral photographs. The premature loss of temporary teeth due to dental decay was considered to exist, when were absent from the dental arch 2 years before the normal exfoliation

and the eruption of the permanent successor tooth.

The increased dental decay risk was assessed per German guides for young teeth (tab. I) (22). These defined the individual dental decay risk of a child, per dmft respectively, DMFT indices, at the child's age. The 1-4 criteria were used for children with primary dentition and the criteria 4-5 for children with mixed dentition. In the present study, we considered an increased dental decay risk, when the index dmft/DMFT>5.

TABLE I
The increased dental decay risk per German guides for young teeth (22)

1	Up to 3 years	Dental decays, dmft>0
2	Up to 4 years	dmft>2
3	Up to 5 years	dmft>4
4	Up to 6-7 years	dmft, DMFT>5 or D/T>0
5	Up to 8-9 years	dmft, DMFT>7 or D/T>2
6	Up to 10-12 years	DMFS>0

The occlusion examination was carried out on study-casts set in static occlusion. All the irregularities in the sagittal, transverse and vertical planes were recorded. According to the extent and prognosis of the orthodontic treatment, the malocclusions were analyzed in all three planes, consistent with the major symptom: in the sagittal plane - normal overjet, class II / 1 incisive malocclusion (increased overjet) class II / 2 incisive malocclusion (retroclined maxillary incisors with minimal or reduced overjet), class III incisive malocclusion (anterior cross bite with or without mandibular overjet); in the vertical plane - normal overbite, anterior open bite, deep bite; in the transverse plane – single tooth cross bite/lateral cross bite, crowding, dental spacing, or associations between them.

The statistical study was conducted in SPSS 16.0 for Windows. We determined the frequency distributions and we determined the elements of descriptive statistics for the numerical parameters: mean value, mean standard error (MSE), standard deviation (SD), minimum and maximum values. We used the *t* test and the Mann-Whitney nonparametric test (U) for independent samples of the comparative study between the groups for numerical variables, having previously checked the hypothesis that variables abide the normal law using the Kolmogorov – Smirnov fitting test. The level of significance $p<0.05$ was considered statistically significant, and the relative risks over 1.5 were considered important. To identify statistically significant differences between qualitative variables, we used the method of contingency tables and the chi-square test, furthermore we have identified the risk factors by calculating the odds ratio (OR) and the relative risks. Intra-examiner agreement was assessed using Kappa coefficients, which were 0.82 for malocclusions and 0.80 for tooth decay.

RESULTS

a. Frequencies by gender, area of origin and anomalies of the study group. Of the 600 analyzed subjects, 291 (48.5%, average age of 7.31±0.6) were females and 309 were males (51.5%, average age of 7.45 ± 0.8) (fig. 1), 318 (53%, average age 7.41 ± 0.55) were from urban areas and 282 from rural areas (47%, average age of 7.37 ± 0.47). In the sagittal plane, the most common were Class II/1 incisive type of malocclusions (40.0%) (fig. 1); in the vertical plane, deep bite (54.0%) (fig. 2); and in the transverse plane, the dental crowding (55.0%) (fig. 3).

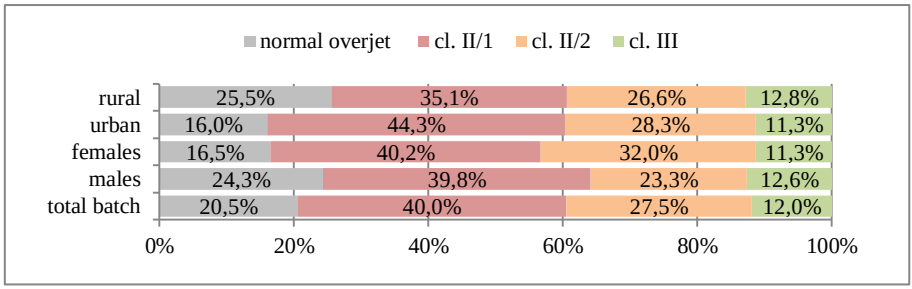

Fig. 1. The frequencies of malocclusions in the sagittal plane

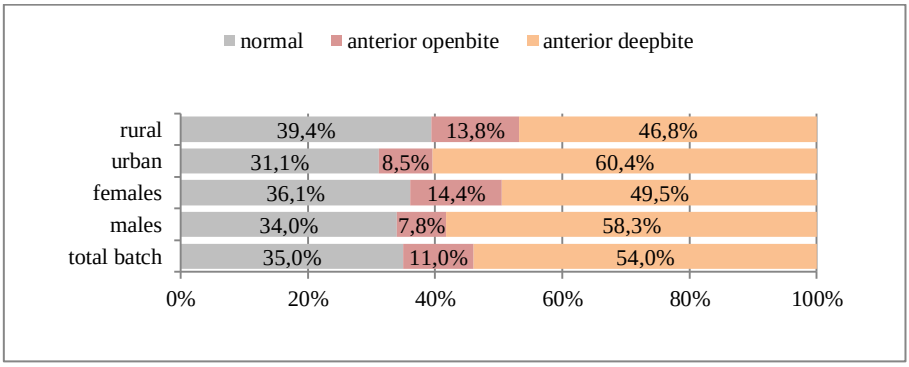

Fig. 2. The frequencies of malocclusions in the vertical plane

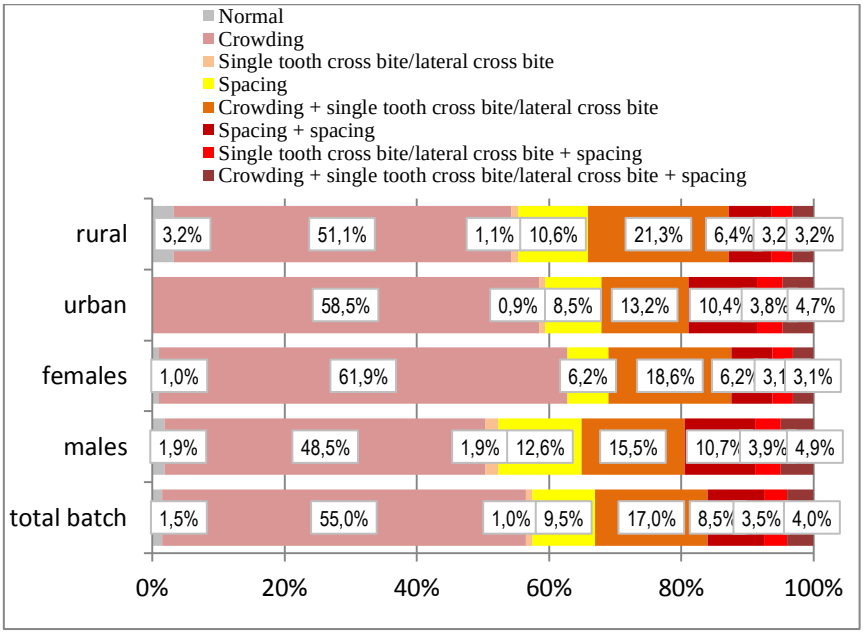

Fig. 3. The frequencies of malocclusions in the transverse plane

b. The characterization of dental status through DMFT/dmft indices of the malocclusion types. We found values ranging between 0-11 for the DMFT/dmft indices and the most common are between 0-5. The overall average of the DMFT/dmft index as quantitative variable is 3.64 ± 2.686 (tab. II).

For the anomalies in the sagittal plane, the mean values of the DMFT/dmft indices are between 2.95±2.314 and 3.66±2.762 and we found statistically significant differences between the mean values of DMFT/dmft index for Class II/1 versus (vs) Class II/2 incisive type (tab. III).

For the anomalies in the vertical plane, the mean values of the DMFT/dmft indices are between 3.41±2.543 and 3.50±2.543 and we found statistically significant differences between the mean values of DMFT/dmft index for patients with deep bite *vs.* patients without deep bite.

For the anomalies in the transverse plane, the mean values of the DMFT/dmft indices are higher (4.97±3.010 and 4.65±2.792) or lower (2.63±1.915), compared to the average values of normal occlusion (3.67±3.279).

We found several statistically significant differences between the mean values of DMFT/dmft index for patients with dental crowding and spacing associated with abnormalities in the transverse plane.

TABLE II

DMFT/dmft indices of the studied group

Variables	Mean	SD	Minimum	Maximum
Total batch	3.64	2.686	0	11
Sex				
Males	3.90	2.679	0	11
Females	3.36	2.669	0	9
Area of origin				
Urban	2.96	2.318	0	8
Rural	4.40	2.865	0	11
Anomalies in the sagittal plane				
Normal overjet	4.73	2.892	0	10
Cl. II/1	3.66	2.762	0	11
Cl. II/2	2.95	2.314	0	7
Cl. III	3.29	2.261	0	9
Anomalies in the vertical plane				
Normal overbite	4.04	2.781	0	10
Anterior open bite	3.50	2.942	0	11
Deep bite	3.41	2.543	0	10
Anomalies in the transversal plane				
Normal	3.67	3.279	1	8
Dental crowding	3.25	2.513	0	10
Single tooth cross bite/lateral cross bite	3.00	1.095	2	4
Dental spacing	2.63	1.915	0	6
Crowding and single tooth cross bite/lateral cross bite	4.97	3.010	0	11
Dental crowding and spacing	4.65	2.792	0	10
Single tooth cross bite/lateral cross bite and spacing	3.86	2.151	0	7
Dental crowding and single tooth cross bite/	3.63	2.975	0	9

TABLE III

The Mann-Whitney test for comparing the DMFT/dmft indices

Groups	DMFT/dmft	
	U test	p value
Anomalies in the sagittal plane		
Normal vs. Cl. II/1	11605.500	0.001*
Normal vs. Cl. II/2	6633.000	0.000*
Normal vs. Cl. III	3100.500	0.000*
Cl. II/1 vs Cl. II/2	17158.500	0.021*
Cl. II/1 vs. Cl. III	8127.000	0.441
Cl. II/2 vs Cl. III	5481.000	0.339
Anomalies in the vertical plane		
Normal vs. anterior open bite	6057.000	0.120
Normal vs. deep bite	29740.500	0.013*
Anterior open bite vs. deep bite	10543.500	0.858
Anomalies in the transverse plane		
Normal vs. crowding	1408.500	0.790
Normal vs. single tooth cross bite/lateral cross bite	22.500	0.580
Normal vs. spacing	238.500	0.732
Normal vs. crowding and single tooth cross bite/lateral cross bite	346.500	0.222
Normal vs. crowding and spacing	198.000	0.511
Normal vs. single tooth cross bite/lateral cross bite and spacing	85.500	0.680
Normal vs. crowding and single tooth cross bite/lateral cross bite and spacing	103.500	0.853
Crowding vs. single tooth cross bite/lateral cross bite	976.500	0.954
Crowding vs. spacing	8271.000	0.142
Crowding vs. crowding and single tooth cross bite/lateral cross bite	11268.000	0.000*
Crowding vs. crowding and false spacing	5881.500	0.000*
Crowding vs. single tooth cross bite/lateral cross bite and spacing	2889.000	0.197
Crowding vs. crowding single tooth cross bite/lateral cross bite and spacing	3739.500	0.646
Single tooth cross bite/lateral cross bite vs. spacing	157.500	0.747
Single tooth cross bite/lateral cross bite vs. crowding and single tooth cross bite/ lateral cross bite	184.500	0.101
Single tooth cross bite/lateral cross bite vs. crowding and spacing	90.000	0.098
Single tooth cross bite/lateral cross bite vs. single tooth cross bite/ lateral cross bite and spacing	40.500	0.176
Single tooth cross bite/lateral cross bite vs. crowding and single tooth cross bite/ lateral cross bite and spacing	58.500	0.474
Spacing vs. crowding and single tooth cross bite/lateral cross bite	1575.000	0.000*
Spacing vs. crowding and spacing	801.000	0.000*
Spacing vs single tooth cross bite/lateral cross bite and spacing	396.000	0.020*
Spacing vs. crowding and single tooth cross bite/lateral cross bite and spacing	535.500	0.117
Crowding and single tooth cross bite/lateral cross bite vs. crowding and spacing	2475.000	0.624
Crowding and single tooth cross bite/lateral cross bite vs single tooth cross bite/ lateral cross bite and spacing	846.000	0.128
Crowding and single tooth cross bite/lateral cross bite vs. crowding and single tooth cross bite/ lateral cross bite and spacing	904.500	0.046*
Crowding and spacing vs. single tooth cross bite/lateral cross bite and spacing	423.000	0.159
Crowding and spacing vs. crowding and single tooth cross bite/lateral cross bite and spacing	454.500	0.071
Single tooth cross bite/lateral cross bite and spacing vs. crowding and single tooth cross bite/ lateral cross bite and spacing	243.000	0.833
* statistically significant p<0.05		

c. High values of DMFT/dmft indices as risk factors for the installation of various types of malocclusions. The presence of malocclusions in three planes is not influenced by increased values of DMFT/dmft indices (DMFT/dmft>5), except for the associated diagnoses of crowding and single tooth cross bite/lateral cross bite respectively crowding and spacing (tab. IV).

The relative risk of patients with DMFT/dmft>5 index is sub unitary for the anomalies in the sagittal plane Class II/1 and Class II/2 incisive type (statistically insignificant) and Class III incisive type (statistically significant, p=0.019). The relative risks of having anomalies in the vertical plane at patients with DMFT/dmft>5 index is statistically insignificant. For certain anomalies in transverse plane the sub unitary risks are statistically significant in the case of patients with crowding (p=0.001) and dental spacing (p=0.001). For the associated diagnostics of dental crowding and single tooth cross bite/lateral cross bite respectively dental crowding and spacing, the relative risk of patients with the DMFT/dmft>5 index is supra-unitary, more than 2.5 and statistically significant (p=0.000).

TABLE IV

The relative risks of developing malocclusions in conditions of high decay experience (DMFT> 5)

Variables	DMFT/dmft>5 (%)	DMFT/dmft≤5 (%)	OR	95% CI		p value
Anomalies in sagittal plane						
Malocclusion Cl. II/1	38.3	40.5	0.945	0.746	1.198	0.637
Malocclusion Cl. II/2	23.4	28.8	0.814	0.584	1.134	0.213
Malocclusion Cl. III	6.4	13.7	0.465	0.237	0.911	0.019*
Malocclusions in vertical plane						
Anterior open bite	8.5	11.8	0.723	0.398	1.313	0.280
Deep bite	46.8	56.2	0.833	0.686	1.011	0.050
Malocclusions in transversal plane						
Crowding	42.6	58.8	0.723	0.588	0.889	0.001*
Single tooth cross bite/lateral cross bite	0.0	1.3	-	-	-	0.172
Spacing	2.1	11.8	0.181	0.057	0.57	0.001*
Crowding and single tooth cross bite/lateral cross bite	31.9	12.4	2.57	1.825	3.619	0.000*
Crowding and spacing	17.0	5.9	2.894	1.726	4.85	0.000*
Single tooth cross bite/lateral cross bite and spacing	2.1	3.9	0.543	0.162	1.815	0.311
Crowding and single tooth cross bite/lateral cross bite and spacing	2.1	4.6	0.465	0.141	1.536	0.195
* statistically significant p<0.05						

DISCUSSION

Previous attempts to demonstrate a direct connection between the presence of dental decays and malocclusions reported inconclusive results (9-11). Assessing the risk of developing a certain malocclusion due to high decay experience during mixed dentition represented the main objective of this article. The relation between different types of malocclusions and high decay

experience was analyzed in our study.

Many epidemiological studies concerning the interrelation of dental decay prevalence and malocclusions at children have been reported in the literature (9-20). Regarding decay experience, many of these studies have considered as benchmark the DMFT>0 (9-11, 14, 16). The Germanic guides consider that for the age of 6-7 years the decay risk is regarded when the DMFT/dmft > 5 index (22). This decay risk was assessed in terms of the installed malocclusion.

In our study, the indices DMFT/dmft presented lower averages for malocclusions in the sagittal plane relative to patients with normal overjet. Thus, it can be stated that the presence of high average values of the DMFT/dmft index for malocclusions in the sagittal plane is not a potential risk for the installation of these anomalies, but acts as a relative protective factor for the occurrence of such anomalies. However, other studies came up with additions like: dental decays were associated with an Angle Class II or III molar relation, in the cases of children with a DMFT>0 index, being two times more likely to be diagnosed with an Angle class II or III malocclusion rather than children without dental decay (23). Stahl et al. (9) found a statistically significant parallelism between the prevalence of dental decays and mandibular overjet in mixed dentition.

In the present study, the DMFT/dmft indices showed lower averages for anomalies in vertical plane compared to patients with normal overbite, also manifesting as a protective factor for the installation of these anomalies. But, additional studies have reached other results, namely: the probability of being diagnosed with an open bite is two times higher at children with dental decays than children without decay (23). Similar results were reported by Peres et al. among Brazilian children (24).

In our study concerning anomalies in the transverse plane, the DMFT/dmft indices presented lower averages at patients with dental spacing and higher averages at patients with dental crowding and single tooth cross bite/lateral cross bite and respectively crowding and regional spacing versus those without this type of anomalies. Once again this validates the theory that there is a close connection between dental decay and the occurrence of dentialveolar incongruence with dental crowding. It is well-known that the mechanism is the premature loss of temporary teeth consecutive to untreated dental decay, which determines subsequent migration of bordering, antagonists and permanent teeth successors. Similar conclusions were obtained by Marquesan et al. (14) in Italy, but concerning temporary dentition, stating that the severity of dental decay was significantly higher among children without anterior maxillary and mandibular dental spacing as well as at children with minimal overjet. The absence of spacing in the maxillary anterior segment was a risk factor for dental decay at preschool children.

In terms of the risk for the occurrence of anomalies in the transverse plane - separately, the high values of DMFT/dmft index acted as a protective factor, decreasing the chances of the installation of these anomalies.

The situation changed, however, concerning the same anomalies, but in the cases of associated diagnoses. Therefore, high values of the DMFT/dmft index presented a statistically significant risk (over 2.5) to develop dental crowding and single tooth cross bite/lateral cross bite respec-

tively dental crowding and spacing. The dental spacing also resulted from the premature loss of temporary teeth because of untreated dental decay (false spacing). Single tooth cross bite/lateral cross bite can be explained by the prolonged lack of masticator stimuli in the lateral area (all due to premature loss of temporary teeth because of untreated tooth decay), which may lead to the underdevelopment of the upper dental arch, this leading to the patient to undergo a change of the lateral occlusion. Proximal untreated decay of deciduous molars or the premature loss of second molars can lead to mesial migration of the permanent first molar and to the changing of the molar relation.

Similar results were obtained from Stahl et al. (9), who found a concordance between the prevalence of dental decays and posterior cross bite.

For the diagnostics of dental spacing with single tooth cross bite/lateral cross bite and respectively dental crowding with single tooth cross bite/lateral cross bite and spacing, statistics showed in our study that the DMFT/dmft>5 index values were correlated with decreased prevalence (subunit risk). These results can be interpreted as inconclusive, since patients who experienced these diagnoses were too few and the risk results were not statistically significant ($p > 0.05$).

Thus, the innovations in this study were represented by findings of high decay experience in mixed dentition (DMFT/dmft>5) which constituted a risk for developing certain malocclusions: dental crowding associated with single tooth cross bite/lateral cross bite and crowding associated with dental spacing).

This study suggests the need to develop national educational programs for the prevention of decay pathology, which constituted a risk factor in the development of dental malocclusions.

CONCLUSIONS

Our study found a high frequency of dental decay in mixed dentition with normal overjet and overbite. The high values of DMFT/dmft indices constituted risk factors in the installation of dental crowding associated with lateral cross bites, respectively dental crowding associated with false spacing. Future studies can be performed on other ethnicities and geographic areas to check the similarity to our study.

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MEDICAL SAFETY

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MEDICAL SAFETY (Abstract): At present, the concept of national security has also acquired a medical dimension, because the political and economic security of a country can be threatened also by the rapidity with which infectious diseases spread in the present global world or by the impact of climatic changes. The present paper intends to illustrate the main threats to/for population health, international organizations and their regulations in the field of medical security, the impact of epidemics on the population, economy and health systems, methods to prevent pandemics, as well as the situations in which the post-communist Romanian health system impacted politically the country image at national and international level. The improvement of the health infrastructure in Romania, motivating the medical personnel by creating better working and payment conditions, as well as the recent experience of other states in the epidemics that occurred in Asia and Africa can all contribute to the successful management of medical emergencies and infectious outbreaks. **Keywords:** MEDICAL SAFETY, INTERNATIONAL ORGANIZATIONS, HEALTH SYSTEM.

Motto:
The greatest test for humanity is the global health crisis. (Bill Gates)

After the ending of the Cold War, health became an interest point on the agenda of the security agencies in western countries (1). The concern for avoiding a nuclear conflict and for protecting the borders made room for an approach focused on the population needs, including medical ones, in defining national security (2).

In the current globalised world, political and economic stability of the states can be threatened by the rapidity with which infectious diseases spread. The pathogens can be easily transformed in weapons, either by terrorists or by certain states. The burden of certain diseases, the most quoted of which is AIDS, can be so severe that it can destabilize an entire region or country at social, political, economic and military level (1). Moreover, the occurrence of infectious outbreaks in various areas of the globe affects dramatically tourism and exports, or even consumption (3).

Medical security concerns protecting the population from the following dangers: infectious diseases, emergent diseases, HIV

virus, malaria, nosocomial infections, the microbial evolution and antibiotic resistance, the intentional release of dangerous chemical and biological materials, the contamination of food, biological weapons and bioterrorism, violence, humanitarian catastrophes, natural disasters, climatic changes, accidental release of chemical and radioactive substances, famine and poverty or, on the contrary, diseases connected to the modern and unhealthy life-style and consumption of processed food (obesity, cardiovascular diseases, diabetes, smoking and alcoholism), economic crisis, intensive agriculture (1, 2, 3).

Another threat to global medical security is represented by counterfeited drugs, because through their inefficiency undermine the credibility of health systems and international organisations, but also stimulate the development of microorganisms resistant to antibiotics (2).

Medical security has to have into consideration the possibility of offering universal access to health insurance. The state will create a legal framework through which the wealthy members of society would finance medical services for the sick and the poor (2). Only in the last century did people fully understand the importance of society and state for the therapeutic act, in which there are more factors involved beside the relationship doctor-patient. That is why, apart from the direct action of the doctor, the entourage and individual life-style, state policies and international organisations have begun to play an increasingly important role in ensuring the healthy state of the population (4).

Furthermore, the state can intervene in stopping the migration of a population scared by an epidemic outbreak, through informative campaigns regarding measures of preventing contamination. Following natural disasters or epidemics, the state can introduce reforms that might prevent the reoccurrence of those: improving the infrastructure and building hospitals resistant to earthquakes, providing free medical materials to population, etc. (2).

The spreading of infectious diseases is enhanced by a growth in population density and mobility, by wars and natural calamities and by the absence of epidemiologic surveillance services (5). A most recent risk is constituted by the current crisis of refugees from Syria, Afghanistan and other conflict areas, which overburdened the social and medical assistance systems in countries as Germany, Austria, Holland, Sweden or Norway.

That is why international cooperation represents an essential element of modern medical assistance. As medical safety was also interpreted by the Western world as a means of protection from the transmissible diseases originated in developing countries, the medical technology exchanges between the rich countries and the poor ones have to be interpreted not in terms of finances, but in terms of security. An example of this kind is represented by the 2007 refusal of Indonesia to provide the strain of avian influenza that appeared in the country, after the country had been refused the delivery of a vaccine obtained from a strain previously provided. This act that endangered the global medical security led to a normalisation of the access to the newest vaccines.

Medicine and infectious diseases permanently remind the states that managed to maintain a stable and social system, without military conflicts, of the danger caused by the lack of wealth transfer toward the less-privileged countries (2).

Military security is closely related and,

to a great extent, a guarantee to medical security. Armed conflicts generated epidemics. Thus, after the 1994 Rwanda crisis, cholera appeared in the Democratic Republic of Congo, and Marburg haemorrhagic fever devastated Angola in 2005. Moreover, ward destroys the medical infrastructure of the countries involved. In this respect, the Geneva Conventions regulate the impartial provision of medical assistance during international conflicts (2).

On the other side, there are situations in which armed forces come to support medicine. For example, as long as Ebola was considered a possible biological weapon, considerable funds were allotted for finding therapies (2). HIV infection was also perceived by the military as a threat to the stability of various countries, because of its rapid spread among the military people. For this reason, in 2000, the United Nations Security Council declared HIV virus a threat to peace and international security (6). The delay in taking the decision to destroy the smallpox sample from the secret laboratories in Russia and USA is again closely connected to military politics (2).

I. The concept of medical security – definition and history

The notion of security includes protection measures that can be taken against various threats to life and life quality (2). The protection of life involves maintaining health, reason for which traditional institutions involved in ensuring national security, such as the army, police and secret services, must be accompanied by health systems. Governments have to ensure the necessary conditions to avoid pandemics, in spite of the risk of corruption scandals due to the involvement of pharmaceutical companies. This is an increasingly important reality, as today insecurity is no longer caused only by the military arsenal of the states or by political conflicts, but also by the rapid circulation of diseases across borders (6).

Together with the great geographical discoveries the contact between civilisations facilitated the import of infectious diseases. 90% of Mexican population disappeared as a consequence of smallpox infection after the Spanish invasion. Smallpox was also used by the British against the American native population, during the colonial conflict. In 2001, in USA, letters contaminated with Anthrax were addressed to members of the American Congress and to journalists. In 2014, the Ebola epidemic in West Africa spread over several countries and in 2015 the respiratory syndrome in the Middle East (MERS) provoked an epidemic in South Korea, which, even if it was efficiently controlled, caused severe damage (5). Having in mind the impact of epidemics across many countries, the notion of collective health, as an extended version of public health, could be introduced when discussing about the concern for the health and wellbeing of the humankind, not only of the citizens of a particular state (2).

However, the upside of globalisation is that, even if it favours the spread of diseases, it might be regarded as a chance to control them, too. International cooperation contributes decisively to the successful prevention of epidemics. In addition, the economic effort of preventing the spread of an infectious disease implies enormous costs, being very difficult to undertake by a single country (1).

The term *medical security* was originally defined in 1994, in the Report for Human Development, elaborated by the United Nations Development Program of UNO

(3). In this report it is shown that the purpose of global security must include, among others, threats from the following areas: economic security, the security of food and environment, personal security, community security, political and *medical*.

In 2003, CHS - *Commission on Human Security* shifted the meaning of the security concept from national to individual level, including its medical aspects, such as protection against diseases and unhealthy lifestyle (3). Thus, a distinction can be made between a state perspective over medical security that regarded health problems as threats to national security and a global one that focuses on health problems at individual level (5).

In the attempt to limit the threats and vulnerabilities of public health caused by globalisation, which favours the appearance of pandemics, a document of the World Health Organisation issued in 2007 recommends eight directions for action: economic stability, international conflicts, climatic changes, biological and chemical threats, the stability of the international health system, HIV virus infection, emergent diseases and strengthening the concept of medical security.

Some of the international organisations that attempt to regulate medical security are World Health Organisation (WHO), Global Health Security Initiative, Commission on Human Security (CHS), established by United Nations Organisations (UNO), Asia-Pacific Economic Cooperation (APEC) and the agency of the European Union, European Centre for Disease Prevention and Control (ECDC) (1,3). These organisations analyse the public health risks represented by infectious diseases, biological, chemical and radioactive agents. Together with these international organisations there are numerous nongovernmental organisations for humanitarian aid as *Red Cross* or *Doctors without Borders*, as well as a new generation of philanthropists: Bill Gates, Warren Buffet or Ted Turner.

There are various documents concerning the control of certain diseases, such as HIV infection – 1308 Resolution of UNO Security Council (1) or biological weapons – Convention for Biological Weapons in 1975 (2). The extended immunisation program initiated by WHO and UNICEF at the end of the 70s by which poor countries were provided free or minimum- priced vaccines, succeeded in fighting tetanus, pertussis, scarlet rash and poliomyelitis in children. Framework Convention on Tobacco Control, elaborated by WHO in 2005 led to cigarettes over-taxation and reduction of smoking. In 1977 the Chemical Weapons Convention took effect (2).

In the mid-90s, WHO initiated Global Outbreak Alert and Response Network (GOARN), meant to provide a rapid response in emergencies and the Euro-Atlantic Disaster Response Coordination Centre (EADRCC) has the same objective (2).

As a reaction to the problems ascertained during SARS epidemic in 2003, in 2005 WHO revised the International Health Regulations. In 2014, the USA launched the Global Health Security Agenda (GHSA) in partnership with various international organisations (5).

The lifestyle in industrialised societies promoted a diet rich in calories but lacking nutritious properties, with a large consumption of meet, alcohol consumption and smoking and the use of personal vehicles. All these have eroded the medical successes following the control of infectious diseases and malnutrition (2). In other words, the developed countries are confronted

with their own threats when it comes to the population's health and wellbeing, only that they are different from the ones in poor countries. That is why the mechanisms of medical security do not only concern protecting the Western world by the viral threats coming from developing countries (1). In the same vein, resistance to antibiotics has come to afflict even the best equipped hospitals in the Western countries (2). Hospital overpopulation in metropolises has as a consequence a slower medical response, even if more proficient (5). The activity of pharmaceutical companies focused more on making profit, competitors and market demand and less on the actual health needs of the population led to the excessive and useless usage of numerous antibiotics which gradually lost their efficiency, developing more resistant strains. This in turn generated excessively high prices to treatments that are necessary, as C hepatitis, and maintained a culture of secrecy regarding medical research, with visible negative effects in doubling, fragmentation and delay of results (2). If in developing countries, the main cause of mortality and morbidity is represented by infectious diseases, in industrialised and urbanised countries the danger comes from an unhealthy lifestyle (3).

II. Measures for maintaining medical security

The first case in which it is known that medical security measures were taken was the plague epidemic in the 14th century, when quarantine was instituted in order to prevent the spreading of bubonic plague across borders (2).

The main actions initiated in case of epidemics or biological weapon attacks are surveillance and emergency intervention. Transparency of information is very important, because timely warning prevents the propagation of infection. An important step in the timely signalling of epidemics was represented by the fact that WHO extended its prerogatives and now can it receive information on potential infectious outbreaks, even in the situation of a single isolated case, not only from governmental sources, but also from nongovernmental organisations (1). Furthermore, the list of diseases considered a threat in the WHO International Health Regulation of 2005 extended over the six initial ones: cholera, plague, recurrent fever, smallpox, typhus and yellow fever, including newer diseases such as HIV, SARS or H5N1 influenza (6).

The efficiency of epidemic control interventions is ensured through the instruction of the medical personnel, improvement of public communication strategies, the existence and functioning of decontamination units and the establishment of vaccines and antiviral medicine reserves.

Global interventions are as useful as those at local level are. WHO can take measures across borders, which include issuing travelling alerts and formulating a list of recommendations, such as those for infection prevention and control (1). The direct involvement of international organisations in the inflicted areas, by sending medical personnel and materials, means that the main measure for controlling the epidemics is no longer borders control. In fact, it is the control of the outbreak in the inflicted area, especially considering that during the latest Ebola epidemic in West Africa the restriction of circulation across borders blocked the access of foreign medical personnel and of international humanitarian aid (1, 2).

At local level, the following measures

are applied: rapid identification of cases (conformed by lab analyses), quarantine (at home or in hospital) and monitoring all contacts (by interviewing, monitoring surveillance cameras and GPS localisation of mobile telephones), closing schools and altering employees' working programs, instructing the primary care givers that tend for the sick persons at home, cancelling public meetings and shows, informing citizens and international partners and involving police forces in the case of uncooperative persons (2, 5).

The organisation of an efficient national health system will guarantee the termination of infectious diseases epidemics produced naturally or following a bioterrorist attack. Such a health system has to isolate the pathogens securely, to have a laboratory network all over the country, equipped with reference samples for various pathogens, an electronic system for reporting infectious diseases, qualified medical personnel and at least a field epidemiologist for every 200,000 inhabitants, as well as a public health centre for emergency operations, capable of offering an emergency response in 120 minutes (5).

Medical security in post-communist Romania

In the 80s, among the institutionalised children in Romania there was a rapid spread of the HIV virus infection. In the communist period, Romania provided false reports to WHO, so that before December 1989 the data indicated the existence of only 13 AIDS cases. At the end of 1990, the Romanian Ministry of Health registered 1168 patients with AIDS, 93.7% of which being children with ages under 13.683 of the cases were institutionalised children that had received treatments against anaemia or malnutrition through blood transfusions or various injections, without a previous correct sterilisation (8, 9).

This episode, in which the sterilisation techniques are the cause for the spread of an extremely dangerous virus, brings into discussion the media scandal around the accusation of diluting the disinfectants provided by HexiPharma Company that attracted the public attention on the alarming dimension of the nosocomial infections phenomenon in Romanian hospitals. In addition, ever since the beginning of the 90s, Romania has been perceived internationally as the country of abandoned and neglected children.

In the summer of 1996, the Southeast of Romania, especially Bucharest, was affected by an encephalitis epidemic determined by the West Nile virus, which caused 17 deaths and 393 cases hospitalised (10). The episode draws attention to the necessity of maintaining the urban infrastructure in optimal conditions, by eliminating the water from building basements and thus stopping the proliferation of mosquitoes.

The absence of a rapid and efficient medical intervention during the fire outbreak from Colectiv, the Bucharest club, caused the fall of the Government led by Victor Ponta, incident that demonstrates the importance of ensuring the medical security of the population in maintaining the political stability in the country. Even though the efficiency of SMURD (Mobile Service of Emergency, Reanimation and Extrication), the intervention unit for providing medical and technical assistance in emergencies, was criticised, the establishment of this service that operates in collaboration with the aviation structures in the Ministry of Administration and Internal Affairs shows that the use of military tech-

nical arsenal in civilian purposes marks an important step in the efforts to improve medical services.

A more subtle threat to medical security in Romania is constituted by the massive migration of the medical personnel toward countries with better performing medical systems. The loss of well-prepared doctors and nurses leads both to a personnel shortage, which block the activity of some hospital sections, as well as to the superficial and rapid recruitment of new medical personnel, without having the guarantee of their competence.

CONCLUSIONS

Certain diseases have become to be regarded not only as public health issues, but also as aspects of national and international security. Medical security represents a relatively new concept, which gradually came to the attention of governments all around the world. The media scandals in which institutions from the Romanian sanitary system were involved drew attention to the importance of ensuring population health for the social, economic and politic stability of the country. A consistent improvement in the medical infrastructure in Romania, increasing the motivation of medical personnel, by creating better working conditions, as well as the recent experiences of other states with the epidemics that affected Asia and Africa could contribute to the successful management of medical emergencies and infectious outbreaks.

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PELVIC ACTINOMYCOSIS MIMICKING A LOCALLY ADVANCED PELVIC MALIGNANCY - CASE REPORT

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PELVIC ACTINOMYCOSIS MIMICKING A LOCALLY ADVANCED PELVIC MALIGNANCY - CASE REPORT (Abstract): We present the case of a former user of an intrauterine contraceptive device (IUD) for 10 years, diagnosed with a bulky, fixed pelvic tumor involving the internal genital organs and the recto sigmoid, causing luminal narrowing of the rectum, interpreted as locally advanced pelvic malignancy, probably of genital origin. Intraoperatively, a high index of suspicion made us collect a sample from the fibrous wall of the tumor mass, large *Actinomyces* colonies were thus identified. Surgery consisted in debridement, removal of a small amount of pus and appendectomy, thus avoiding a mutilating and useless surgery. Specific antibiotic therapy was administered for 3 months, with favorable postoperative and long-term outcomes. Pelvic actinomycosis should always be considered in the differential diagnosis of pelvic tumors in women using an IUD. The association of long-term antibiotic treatment is essential to eradicate the infection and prevent relapses.
Keywords: ACTINOMYCOSIS, INTRAUTERINE DEVICE, PELVIC TUMOR.

Actinomycosis is a chronic infection most commonly caused by strains of *Actinomyces israelii,* a gram-positive, anaerobic, filamentous, non-sporulating bacterium (1,2). It causes a chronic suppurative inflammation with formation of multiple abscesses, sinuses and recurrent fistulas and abundant granulation and dense fibrous reaction tissue. Pelvic actinomycosis is found in about 3% of cases (3). Although the abdominal form of disease can extend directly to the pelvis, pelvic actinomycosis is mainly associated with intrauterine contraceptive devices (IUD) (4). The clinical presentation is mostly nonspecific, mimicking a tumor process (5,6). Moreover, the radiological findings are also nonspecific, making an accurate diagnosis difficult (7). This explains why patients often undergo extensive surgery involving multiple organ resections, with a correct diagnosis being made postoperatively by anaerobic cultures and histopathologic examination. We present the case of a female patient with pelvic actinomycosis diagnosed as locally advanced ovarian cancer in which the diagnosis was made intraoperatively, thus avoiding useless extended resectional surgery.

The diagnostic difficulties and treatment strategies are discussed.

CASE REPORT

A 51-year-old female patient with altered general status was admitted for investigations and treatment of a pelvic tumor mass. The onset of symptoms was insidious, over 4 months, with abdominopelvic pain associated with abnormal vaginal bleeding and a tendency to constipation, low grade fever, anorexia, marked physical asthenia and weight loss of 8 kg. From her personal history we found that the patient had been using an IUD for 10 years, being removed two months earlier. Physical examination on admission revealed a hard, palpable, imprecisely delimited mass in the hypogastrium. Laboratory tests showed leukocytosis (13.800/μL) with mild neutrophilia and anemia (hemoglobin level 10.9 g/dL). C-reactive protein levels were within normal range. Other laboratory tests including tumor markers were all within normal range, except CA 125 which was slightly elevated 19.80/mL. Vaginal examination revealed the presence of a pelvic mass occupying the vaginal vault, encompassing both parameters and an immovable cervix. Uterine curettage showed only an unspecific inflammatory process. Colonoscopy identified rectal extrinsic stenosis of approx 3 cm, at 10 cm from the anal verge.

Pelvic magnetic resonance imaging (MRI) showed a tumoral mass of 74/84/75 mm that included the posterior vaginal wall, extending to the right piriformis and *levator ani* muscles, involving the medial and lower mesorectum (fig. 1).

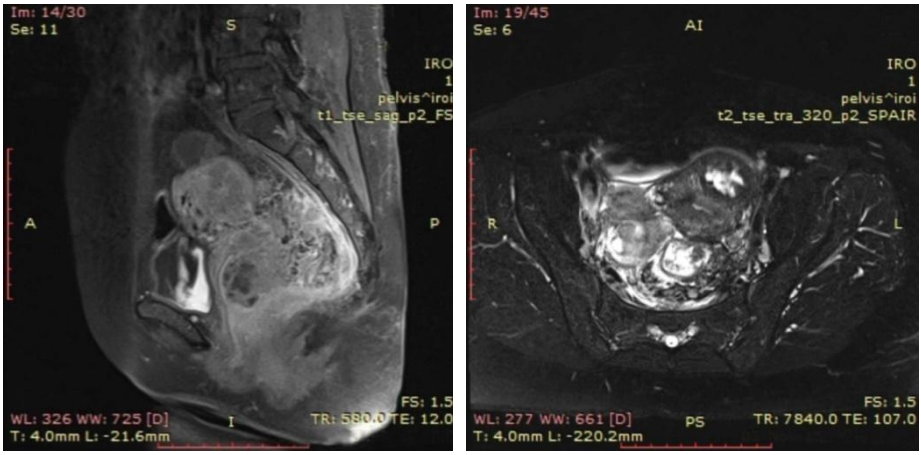

Fig. 1. Pelvic magnetic resonance imagingshowed a tumoral mass of 74x84/75 mm that includes the posterior vaginal wall and extends to the right piriform and levator ani muscles involving also medial and lower mesorectum. A second mass of 52x40/52 mm, located to the right and posterior of right broad ligament is noted.

This tumor was centered by the ampulla recti, with probable invasion of the anterior rectal wall. Another mass of 52x40/52 mm, of unknown origin, located on the right and posterior surface of the right broad ligament was noted. Considering the suspicion of right ovarian tumor, a triple contrast CT was performed identifying gas bubbles in

the uterine cavity. The presence of right obturator and left external iliac and interaortico-cavous enlarged lymph nodes was also noted (fig. 2).

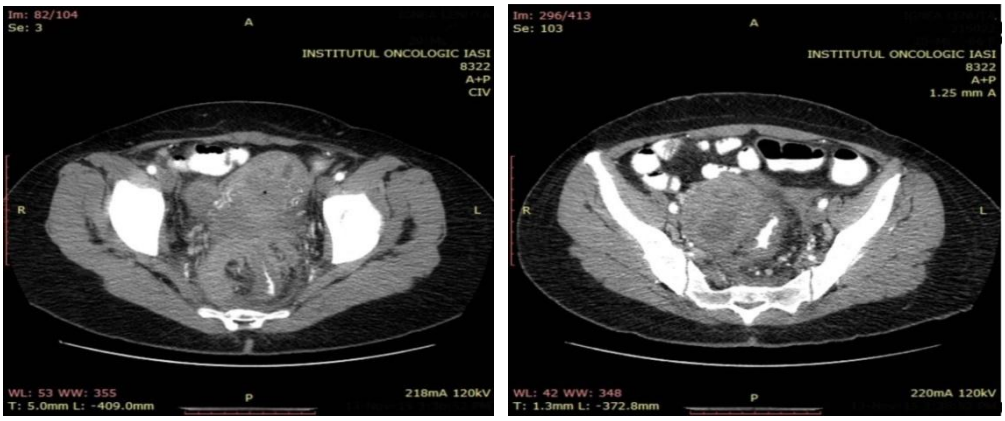

Fig. 2. Pelvic CT scan showing an in homogeneous mass involving internal genital organs without separation from rectum and enlarged pelvic lymphnodes.

A left basal pulmonary nodule of 5 mm with uncertain features was identified. A preoperative diagnosis of locally advanced pelvic malignancy of unknown origin, was made and surgery was indicated. Exploration of the peritoneal cavity revealed a bulky, hard, irregular, fixed, infiltrating abdominopelvic mass involving the uterus, annexes, rectum, small bowel loops and vermiform appendix. During dissection a small amount of pus was exteriorized and collected for cytology and bacteriology exam. At that moment a sample of tumor/abscess wall was removed and sent for fresh-frozen examination which found multiple areas of polymorphous inflammation with abscess formation with large, central, filamentous *Actinomyces* colonies. Surgical debridement, appendectomy, peritoneal lavage and drainage were performed. Pathology established the final diagnosis of pelvic actinomycosis (fig. 3).

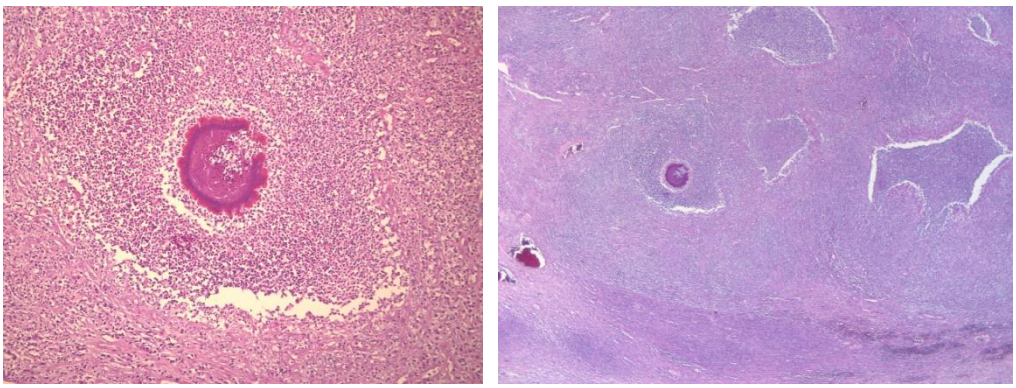

Fig. 3. Abscesses with large, central, filamentous *Actinomyces* colonies (a. HE x25; b. HEx100).

High doses of intravenous penicillin were commenced immediately. The postoperative course was uneventful with the patient being discharged on the 7th postoperative day. The antibiotic therapy was continued with high-dose penicillin for 4 weeks followed by oral penicillin (Augmentin 3g/day) for a further 6 months. A three months' follow-up exam by MRI showed almost normal postoperative appearance of the pelvic region and no clinical complaints (fig. 4).

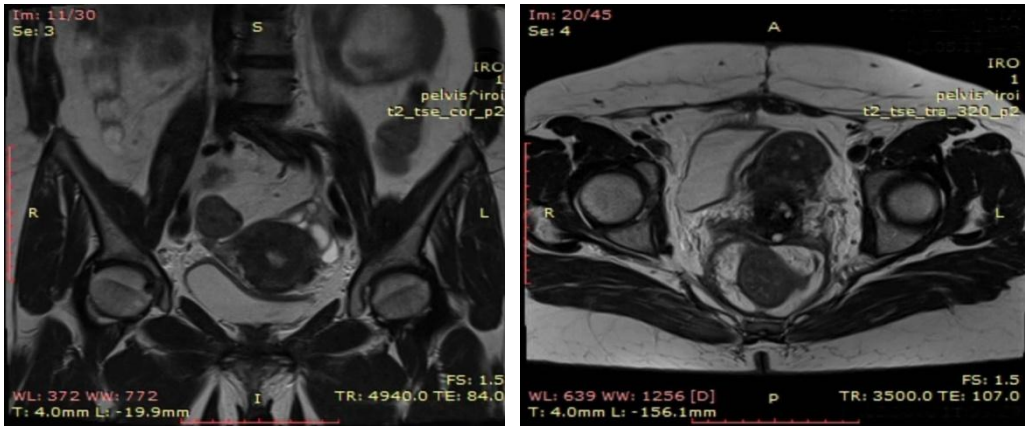

Fig. 4. Three months postoperative – MRI exam almost normal of pelvic region

DISCUSSION

Actinomycosis is a rare infectious disease, its true incidence being difficult to define because many cases with mild infection are probably eradicated through the common use of antibiotics. The abdominopelvic location is responsible for about 20% of human cases (1,2,8). It is generally accepted that actinomycosis has become a less frequent disease, unlike abdominopelvic actinomycosis which has an increasing prevalence due to the increasing use of IUDs. The first case of abdominopelvic actinomycosis associated with IUD was reported by Henderson in 1973. The incidence of *Actinomyces israelii* in IUD users is differently reported by various authors, ranging from 1.6% to 20% (1,4,6) and it increases with duration of IUD use, the mean duration of usage in patients with actinomycosis being about 8 years (7,9). These devices cause prolonged trauma to the endometrium, inducing areas of necrosis in the presence of pre-existing pelvic inflammatory disease, providing a favorable environment for the development of actinomycosis. Disruption of the mucosal barrier of cervical mucus allowing the ascension of germs or detachment of small fragments from the device which could act as foci of infection are other supposed mechanisms (6,7). The main problem related to actinomycosis is the difficulty of preoperative diagnosis, this disease being rightly called the great mimicker (4,7,8). The clinical and imaging features often mimic malignant lesions, inflammatory diseases, tuberculosis, fungal infections, endometriosis (5,9). The most common presenting features, as in our case, are abdominal pain, weight loss and physical weakness associated with abnormal vaginal

bleeding. The tumor related symptoms are often in the foreground with a poorly delimited, fixed and painful pelvic mass accompanied by altered general status, cachexia, and sometimes fever. The biological inflammatory syndrome is constant and the CA 125 is sometimes elevated, as in our case (1,2,4,8). Extension to adjacent organs is possible, especially to the bladder and recto sigmoid with a tendency to fistula formation (9,10). Retroperitoneal involvement is common, causing ureteral and/or vascular compression and sometimes hydro nephrosis or thrombophlebitis (11,12). The lymph nodes are not usually involved, and spread into the peritoneal cavity is exceptional (4,7). The presence of a pelvic tumor mass with frozen pelvis appearance, with rectal stenosis and interaortocaval, left external iliac and right obturator enlarged lymph nodes, created confusion, pleading for the neoplastic nature of the lesion in our case.

Imaging diagnosis of actinomycosis is difficult. Frequently, the seemingly aggressive nature seen on CT or MRI might lead to the suspicion of a malignant disease. Lee describes the main radiological findings in abdominal actinomycosis. Each radiological finding may mimic an abdominopelvic tumor process, therefore a correct differential diagnosis is essential. (5,9,13). The CT scan typically shows a solid, inhomogeneous mass, or a cystic mass with thickened wall surrounded by inflammatory infiltrate in the adjacent fat (5,9). The MRI may help in diagnosis, but has a low specificity (5,13). Recently PET scan has been used to diagnose infectious and inflammatory diseases, but it is unfortunately not specific for actinomycosis. It could however be used to evaluate the efficacy of antibiotic therapy (13).

In terms of histopathology a characteristic chronic inflammatory response is found, with pus production and abscess formation centrally and granulation tissue in the periphery. In this atmosphere of destroyed and damaged PMNs, bacterial colonies of filamentous *Actinomyces* are identified. Confirmation may also be obtained by fine needle aspiration or biopsy under radiologic guidance or during surgical exploration (1,2). Unfortunately, many authors report highly variable rates (1-50%) of isolation of *Actinomyces* species from the histologic material (1,2,4,14, 15,16). In our case, the intraoperative fresh frozen examination identified large filamentous colonies of Actinomyces. The literature also reports the presence of Gram-positive "sulfur granules", with a mycelium-like structure, positive on PAS and Grocott staining's, which are unfortunately non-specific (1,4). The morphologic picture can be attenuated by prior antibiotic treatment thus histological tests should be carried out before the initiation of antibiotic treatment (2,4).

The presence of *Actinomyces* without apparent pelvic infection does not require IUD removal and antibiotic treatment, but many authors believe that a vaginal smear positive for *Actinomyces* is an indication for antibiotic treatment to eradicate the infection or recommend IUD replacement at 4 years (6).

Standard treatment is based on intravenous penicillin G for 2-6 weeks, followed by oral penicillin V (2-4 g/day) for 6-12 months due to the limited penetration into the fibrous tissue and tendency to recurrence (1,8). The risk of developing penicillin resistance is cited as very low. In preg-

nant women, erythromycin can be safe, and in patients who are allergic to penicillin, doxycycline, clindamycin and erythromycin are adequate (14,15,17). In case of concomitant intraabdominal infection with anaerobic bacteria, the combination of amoxicillin and clavulanic acid with metronidazole, or clindamycin can be used. However, the literature shows a high rate of recurrence after antibiotic therapy alone (2,15). Definitive indications for surgical treatment are: abscesses that are not amenable to percutaneous drainage, extensive necrotic tissue, local complications such as compression or stenosis, to rule out a malignant disease, or when medical treatment is inefficient. Complete removal of infected tissue is rarely possible, with a high risk of recurrence. It is very important to make or suspect the diagnosis intraoperatively, because most often a pelvic malignancy is suspected and patients undergo major surgery. (8,14,17).

The prognosis is favorable with long-term antibiotic treatment. Some authors have shown that a combination of complete surgical resection followed by short-term antibiotic treatment is also an effective therapy (1,17). However, severe complications due to local or generalized spread of infection have been reported, justifying the prolonged surveillance (14,15).

CONCLUSIONS

In this case pelvic actinomycosis pelvic, rare infection associated with the use of an IUD over a long period of time, mimiking a locally advanced pelvic malignancy. Therefore, actinomycosis should be included in the differential diagnosis in women with presumed pelvic cancer and a history of long-term use of IUDs. The primary goal of surgery is to obtain a clear evidence for histopathological diagnosis and surgical treatment should be limited to debridement and drainage. Essential to eradicate the infection and prevent recurrences is the association of the specific antibiotic treatment.

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COMBINED EFFECT OF THERMAL LOAD AND MECHANICAL LOAD IN TRANSTIBIAL PROSTHESIS. AN EMPIRICAL STUDY

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COMBINED EFFECT OF THERMAL LOAD AND MECHANICAL LOAD IN TRANSTIBIAL PROSTHESIS. AN EMPIRICAL STUDY (Abstract): Probably the most important factor in evaluating a patient's prosthesis is quality of life. Transtibial amputations, are among the most frequently performed major limb amputations. Many individuals with transtibial amputations successfully achieve rehabilitation at or near their preamputation levels. Discomfort in prosthetic sockets continues to be a critical challenge faced by both prosthetists and amputees. **Material and methods**: This paper proposes a fusion Graphic User Interface that combines two types of information (pressure and temperature). Data from pressure sensors and thermistors (an electrical resistor whose resistance is greatly reduced by heating, used for measurement and control) placed on the stump in transtibial prosthetics are collected in real time using a National Instruments Data Acquisition device. **Results**: All the data stored in files are available for offline processing. The user has the possibility to analyse the signal by zooming or positioning the marker and window on different parts of signal. A complex analysis that involves the pressure and temperature for a location (where both sensors are placed) is available in time domain. **Conclusions**: Blunt-prosthesis interface is characterized by few parameters among the most important are pressure and friction. The action of these parameters during static and dynamic stage is very important because their actions can produce lesions of skin at the level of interface.Despite the advancements in surgical techniques and prostheses, much still needs to be done. We made certain that the sensors were in the same location by pressing on specific cells on the residual limb during various stages of the experimentation. The highest pressures recorded were during the stance phase of walking. The curve that shows the temperature evolution or pressure in one point could differ in different points from patient to patient.
Keywords: BLUNT,GRAFIC INTERFACE, TRANSTIBIAL PROSTHESIS, THERMISTOR, THERMAL LOAD.

The interface skin-liner has a distinctive achievement consisting in a comfortable prosthesis for a person with lower limb amputation. Amputation is a surgery that involves the section of anatomic parts: bones, muscles, blood vessels and nerves. A study of the interface-level pressures presents a particular interest in determining the patient's comfort and quality of life (1,2).In (3) the authors propose placement of six sensors in locations chosen based on experimental criteria to cover pressure

zones for two persons with transtibial amputation. One of the main purposes is to identify areas with maximum pressure variation of these values depending on the patient's activity. A performance investigation of the patient's comfort is proposed in (4). The authors used a four F-Socket sensors array 9811 and a Prosthetic Evaluation Questionnaire which investigates patient satisfaction in relation to the two liners used (4). But from the point of view of registration equipment and pressures of the software, this solution is expensive.

In the last years, few papers discussed the influence of temperature on patient's comfort. Temperature values at skin-prosthetic interface are recorded for different points on stump and the influence of ambient temperature is also discussed. The permanent contact of inorganic materials with skin can cause cutaneous pathology (1).Recent studies reveal the fact that a 2^0 C increase of skin temperature in residual limb can create discomfort (2). Thermal comfort satisfaction is strongly related with ambient temperature that has influence over skin temperature, perspiration and all thermal processes in stump, skin, liner and socket (3).This study reveals that from 38 studies published in PubMed and Web of Knowledge, at least 53% of persons with amputation faced discomfort due to heat, perspiration or both inside their prosthesis, most of them having transtibial amputation. A collection of 16 thermistors and a portable signal acquisition system to record temperature curves is proposed in (4). The thermistors are placed uniformly on the stump and the evaluation of temperature evolution is made using an activity protocol of 30-minute walk bordered by 60 minutes of rest (4). A map of temperatures is presented using thermal contour plots. A new approach to record the temperature on surface of the residual limb is proposed in (5). The protocol uses Gaussian Process for Machine Learning for predictive model that has as input the liner temperature and as output the temperature of the skin located on the residual limb (5).A simple method to describe the temperature on interface skin-prosthesis is proposed in (6). The authors used 16 thermistors placed in a regular grid on stump surface to collect data. One subject was selected for test in 1h protocol rest-walk-rest. Thermal plot contour type was deduced using Matlab in an offline application. An average value of temperature over all 16 thermistors has been calculated in order to deduce the influence of thermal environment on skin-prosthesis interface (6).The experimental studies revealed the fact that a lower temperature at skin level usually reduces the patient's discomfort both due to ambient temperature and wearing of prosthesis. Few researches oriented toward thermal conductivity of materials that are used for construction of prosthesis. Thermal conductivity of materials has been calculated using Fourier's law. One of the most important conclusions of the paper is that the thermal discomfort is reduced in the case of multilayer sockets that use materials having high thermal conductivity (7). A 3-D thermal finite element model is proposed in (8). Thermal characteristics of the tissues (muscle, fat, skin, bone) and prosthetic materials were taken from literature. The skin temperature was predicted using Penne' BHT equation of heat transfer (8). The thermal performance of prosthesis is one of the very important objectives in prosthetic design (9, 10). A socket modification, a cooling channel inserted in socket, is proposed in (9). A socket prototype was created in SolidWorks and implementation was made using a 3D printer

(9). A thermoregulatory system was proposed in (11). The authors verified the system using a phantom model, silicone liner and a thin layer on aluminium as transfer interface between thermal pump and different locations situated on phantom (11).A finite element method is used in (12). The authors propose to use finite element method in modelling temperature distribution at skin surface in order to predict it depending on external conditions. Some authors used the experimental results to design novel prostheses (13) or a complicate system for thermoregulation of temperature inside socket (14).

MATERIAL AND METHODS

A graphical interface is an interface located between the user and electronic devices such as computers, hand-held devices (mp3 players, portable media players), electronic appliances and some office equipment. To present all available information and actions, a GUI provides icons and visual indicators, in contrast to text-based user interfaces, which provide names of commands to be typed in or text navigation. This is intended to facilitate the user's work by eliminating the process of writing the software code. For recording purposes, pressure sensors were used on type CZN-CP6 (pressure sensors) and CZN-CP9 (pressure sensors) self-adhesive wiring diagram to measure resistive input in measuring plate. An analogue digital converter used precise 14-bit resolution, and six sensors were placed in locations determined experimentally in areas with the highest solicitations (and therefore a higher pressure) according to fig. 1.

Ten thermistors of MA100GG type were placed on the sink of the stump in an experiment including a group of three patients with transtibial amputation: A1 (67 Kg, 27 years, male), B3 (30 Kg, 10 years, male) and C4 (76 kg, 32 years) (fig. 2). The thermistors are validated before using the water-bath procedure. The other patients with other diseases, e.g. diabetes, were excluded from the study.

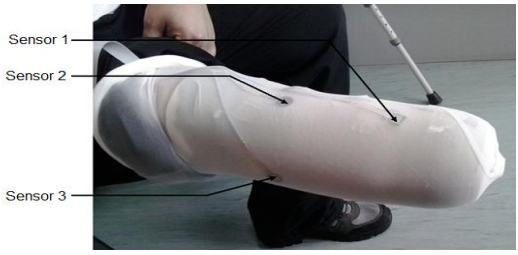

Fig. 1. Pressure sensors

Fig. 2. Thermistor locations

Experiments were conducted by using a protocol that began with a 30-minute walk at fixed speed (a trade off between the speed's options of the two patients) and a 30-minute rest before repeating the experiment. The sensors were placed on the areas known as being the most solicited pressure locations during walk (e.g. lateral side, medial side, distal, etc.). Also, other experiments used a 2D map of the stump split in approximated rectangles with an algorithm for sensors placement in order to identify the most solicited areas by temperature evolution during walk. Finally, all the shortcomings are reflected in the patient's sensation of discomfort. In time, a combination of pressure and temperature can lead to serious affections of skin and teguments

RESULTS

A Graphic User Interface is proposed to analyse offline data collected from pressure sensors and temperature sensors. After authentication, the user can select the two options: Force (pressure) data or Temperature data. Data are collected online using a National Instruments Data Acquisition device and the files are stored in ASCII format. Data acquisition is the process of measuring an electrical or physical phenomenon such as temperature, pressure ith a computer.Records for the same patient (pressure and temperature) are statistically processed. Outliers are removed and replaced by a median value, and the points outside value bordered by ± SD (standard deviation are excluded). The most probable curve is computed from the rest of the points using the LMS (Least Mean Square) method.

The interface has a module that allows calibrating the sensor, the curve being approximated by a polynomial model (a polynomial with maximum degree 7). Practically, the coefficients can be stored individually for each sensor or for all sensors for future purpose. The curve that shows the temperature evolution or pressure in one point could differ in different points from patient to patient.

Probably the most important factor in evaluating a patient's prosthesis is Quality of Life. The most common evaluation of this factor uses questionnaires, the Prosthetic Evaluation Questionnaire being one of the most used in practice (15). Prosthetic Evaluation Questionnaire was developed to provide quantitative measures for patient's satisfaction using 54 qualitative questions. One of the most common materials was used for all patients' prostheses: socket – PP/PE (90% Polypropylene, 10% ethylene), Young's module = 1.5 GPa, Poisson coefficient = 0.3 and liner - soft Pelite (is a polyethylene closed cell foam commonly used as an interface material in prosthetics), with Young's module = 0.38 MPa, Poisson coefficient = 0.39. In this stage of research, we want to measure the degree of comfort using Prosthetic Evaluation Questionnaire for all three patients. Average empiric coefficients were set for fused data, pressure and temperature (a = 0.9 for pressure and 0.1 for temperature). The final Prosthetic Evaluation Questionnaire was found to be 43, 56, and 38 for three patients, which suggests that it is possible for some pairs of socket-liner materials not to be very adequate for some patients. These values are suggested by the fast variation in force versus time (5-6 sec) and a very slow modification in temperature in the same time interval, constant in practice. Further investigation is needed, using new models which study how temperature can affect the patient's discomfort sensation caused by a pressure (force) on the skin at skin-liner interface.

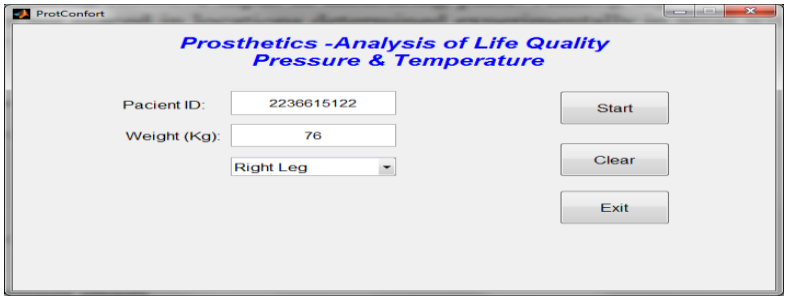

Fig. 3. First page of program after log-in.

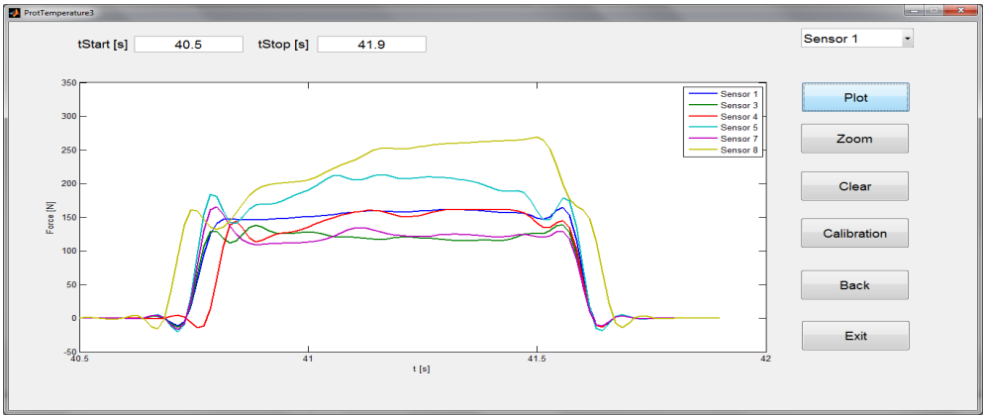

Fig. 4. Record of an episode of movement (walk, last 2 seconds).

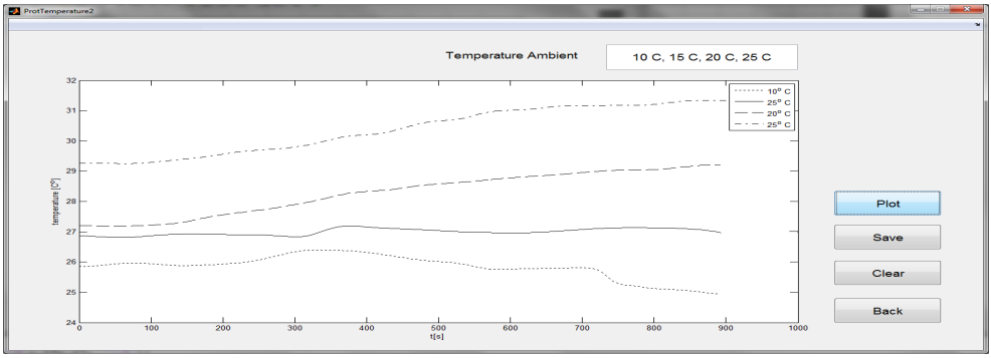

Fig. 5. Wave form temperatures, sensor 3, pos. 5, medial position for ambient temperature 10° C, 15° C, 20° C, and 25° C.

CONCLUSIONS

In this article, Graphic User Interface may contribute towards improvement in the quality of socket design for amputee patients. This interface has been the most common computer modelling method used to try to predict stresses at the socket interface. The contact zone between two

surfaces generates two types of friction, static friction and kinetic friction. The quality and comfort of a prosthetic socket can determine the daily duration for which patients use their artificial limbs. More studies are necessary to find a relationship between patient's comfort sensation and materials used in prosthetic. Beside a custom approach for each patient, statistic studies need to include other factors, such as age or sex. It is clear that a large group of subjects and medical validation are necessary before these preliminary results become applicable.

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DIVALENT CATIONS PROFILE IN PATIENTS WITH LIVER CIRRHOSIS AND VARICEAL BLEEDING

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DIVALENT CATIONS PROFILE IN PATIENTS WITH LIVER CIRRHOSIS AND VARICEAL BLEEDING (Abstract): **Aim**: The objective of this study is to evaluate divalent cations serum levels (magnesium, zinc, copper) in liver cirrhossis and one of its major complication (variceal bleeding). **Material and methods**: A prospective case-control study including 60 patients with liver cirrhosis complicated with variceal bleeding admitted to the Emergency Department, and 35 subjects without liver cirrhosis which served as group control. Serum concentrations of zinc, copper and magnesium were determined using atomic absorption spectrophotometer. **Results**: The concentration of magnesium was significantly lower between patients with liver cirrhosis (11,6 ± 2,77mg/l) vs group control study (17,7 ± 3,86 mg/ l) ($p<0,001$), regarding decreased magnesium levels and the presence of complications as hepatic encephalopathy, ascites (Child C class). Serum concentrations of zinc were decreased (0, 77 ± 0, 35 mg/ l) compared to control group ($p< 0,001$) in patients with liver cirrhosis and variceal bleeding. Serum levels of copper were increased (1, 69 ± 0,722 mg/ l) compared to control group in patients with liver cirrhosis and variceal bleeding and are also correlated with serum albumin levels ($p<0,001$, Pearson coefficient r-0.631). **Conclusions**: Variceal bleeding in patients with liver cirrhosis is associated to important imbalances in blood concentrations of zinc, copper and magnesium. **Keywords**: LIVER CIRRHOSIS, ZINC, COPPER, MAGNESIUM.

Zinc, copper and magnesium are essential trace elements with role in the normal functioning of the liver. Zinc is associated with more than 300 enzymatic systems (1). Zinc augments the natural defense of reactive oxygen radicals by Zn-enzyme Cu-Zn superoxide dismutase (2). Zinc acts as an antioxidant, a membrane and cytosceletal stabilizator, an anti-apoptotic agens, an important co-factor in DNA synthesis, an anti-inflammatory agents etc (3).

Copper is an essential trace element which participates in many enzymatic reactions. Its most important role copper has in redox processes. Reactive copper can participate in liver damage directly or indirectly, through Kupfer cell's stimulation (4). Copper's toxic effects are related to oxidative stress(5).

Magnesium is important for the protein synthesis, enzyme activation, oxidative phosphorilation, renal potassium and hy-

drogen exchange. It is also involved in immunoglobulin synthesis, immune cell adherence, antibody- dependent cytolysis, IgM lymphocyte binding, T helper B-cell adherence and additional responses (6). Only 0.3% of total body magnesium exists in serum. Magnesium deficiency have been reported in various disorders: diabetes mellitus, malabsorption syndromes, chronic alcoholism bronchial asthma, epilepsy, and ischemic heart diseases. There are contrary reports about their serum concentrations in patients with liver cirrhosis**.**

Since zinc, copper, se and magnesium have a possible role in the pathogenesis of cirrhotic complications**,** the aim of this study was to investigate the serum concentrations of mentioned trace elements in patients with liver cirrhosis with variceal bleeding and compared them with concentrations in controls group patients.

MATERIAL AND METHODS

Material. This study is a prospective case-control, in which we determined serum concentrations of Mg, Zn, Cu in patients with liver cirrhosis complicated with variceal bleeding and compared with a control group of patients. The study included patients admitted to the Emergency Departement of "St.Spiridon" Hospital Iasi in the interval April 2013 – September 2014. The diagnosis of liver cirrhosis was established by detailed history of all such patients, positive findings on clinical examination, relevant biochemical tests, ultrasound and endoscopic findings. The diagnosis of variceal bleeding was established on anamnestic data with hematemesis and melena and gastrointestinal endoscopy. Exclusion criteria were malign disease, acute liver failure, renal failure, multiorganic failure, collagen disease, hypertension, heart failure, ischemic heart disease, psychiatric disorders. The control group included subjects with no history of liver disease, heart disease and renal disease, matched with patients with liver cirrhosis for gender and age, who were performed laboratory analysis as part of systematic medical examinations.

Ethical considerations

All the patients enrolled in the study signed an informed consent, through which they were given details on the aim of the study, its methodology, the risks and benefits incurred by the study, as well as information regarding the confidentiality of the results obtained. The study was approved by the local Ethics Committee and was in accordance with the Helsinki Declaration of 1975.

Methods. Blood samples were collected from the cubital vein with all aseptic precautions in glass tubes free from electrolytes. Serum was separated and serum concentrations of magnesium, zinc, copper were measured by spectrophotometer at 510-580 nm. The separation of serum for further analysis was performed by centrifugation at 4000 revs/min for 10 minutes. It was separated by 1 ml of serum in digestion tubes, over which were added 2 ml of concentrated nitric acid and 2 drops of concentrated per chloric acid. The tubes are placed on a plate heated to 130-150 ° C. Once the volume in the tube reached 1 ml, the temperature is brought to 180 ° C. The digestion is continued until per chloric acid will smoke. Subsequently 3 drops of nitric acid and heating is continued until the formation of dry salt. The tube walls are washed out and from top to bottom with deionized water (2-5 ml). Subsequently was added 1 drop of 8% aluminum chloride to remove the interference due to phos-

phate. The tubes were then placed in boiling water for 5 minutes. Then removed and cooled and diluted with 12.5 ml of distilled water. After centrifugation 2 ml of the supernatant will aliquots to each tube. The readings were made with a spectrophotometer absorbance 510-580 nm adjusted to room temperature.

To each patient with cirrhosis and upper gastrointestinal bleeding and to each patient in the control group were determined in addition hematological and biochemical profile.

For analysis data were expressed as mean ± standard deviation. When the data presented the Gaussian distribution, parametric tests were used (Student´s t- test for differentiating of avearages and Pearson´s test correlation coefficient). When data did not have the Gaussian distribution, nonparametric tests were used (Wilcoxon´s test for comparing populations and Spearman´s correlation coefficient) and *p* values less than 0,05 were regarded as statistically significant. Statistical analyses were performed using MedCalc software and SPSS 17.0.

RESULTS

Our study enrolled 60 patients with liver cirrhosis complicated by variceal bleeding presented in the emergency unit with mean age 57.32 ± 9,630 (SD), 41 (68,3%) male and 19 (31,7%) female. The control group included 35 patients with mean age 56.89 ± 11.177 (SD), 20 (57,1%) male and 15 (42,9%) female (tab. I).

In our study we assessed the severity of liver cirrhosis using Child-Pugh-Turcotte (CPT) classification. 30% (18 patients) of patients with liver cirrhosis and variceal bleeding were included in class A, 45% (27 patients) class B and 25% (15 patients) class C. 46,66% patients had alcoholic cirrhosis, 25% viral etiology of cirrhosis and 28,33% with both alcoholic and viral etiology. 73,33% patients was portal hypertension (tab. I).

Hematological and biochemical parameters of patients included in our study statistically significant ($p < 0,001$) (tab. II).

Normal values for divalent cations: $Cu2+$=0.8-1.5mg/l, $Zn2+$ =0.8-1.4mg/l, $Mg2+$ = 16-26 mg/l, $Ca2+$= 8.40-10.20mg/l.

The concentration of magnesium was significantly lower between patients with liver cirrhosis (11,682 mg/l ± 2,77) and group control study (14,614 mg/l ± 4,57) (fig.1). Statistical analysis showed correlation between serum levels of magnesium based on the Child-Pugh class (class C) ($p <0.01$, r-0.803) and the presence of other complications like ascites ($p <0.01$, r-0.557) (tab. III, fig. 2).

Serum concentrations of zinc were decreased (0,6815 mg/l ± 0,40 DS) in patients with liver cirrhosis and variceal bleeding, especially in patients with decreased levels of serum total protein ($p<0,01$, r +0.695) and serum albumin ($p<0,01$, r +0.895). Also, there was a statistically significant correlation between decreased levels of zinc and increased value of MCV (mean cell volume) ($p <0.05$, r – 0.323) and GGT (gamma glutamyl transpeptidase) ($p <0.05$, r – 0.285), this indicates a possible correlation between decreased serum levels of zinc and alcohol intake (tab. IV).

Serum levels of copper were increased (1,7365 mg/l ± 0,60 DS) in patients with liver cirrhosis and variceal bleeding and were statistically correlated with serum levels of total protein (r -.433) and albumin (r -.631) in cirrhotic patients with variceal bleeding ($p < 0,01$) (tab. V).

TABLE I

Demographic data of patients in both study groups.

Parameter	Cirrhosis and variceal bleeding	Control group
Age	57.32 ± 9.630	56.89 ± 11.177
Sex Male	 68,3%	 57,1%
Background Urban	 51,67%	 62,86%
Body Mass Index (BMI)	46,67% normal weight	48,15% normal weight
Smoker	53,33%	54,33%
Alcohol intake	70,27%	37,1%
Diabetes history	29,5%	22,9%
High BP history	41,33%	37,67%
Other diseases - Cardiovascular - Asthma	 21,33% 3,66%	 18,66 5,33%
Etiology - alcoholism - viral infection - alcoholism + viral infection	 46,66% 25% 28,33%	 -
Child-Pugh-Turcotte (CTP) Clasification Class A Class B Class C	 30% 45% 25%	-
Portal hypertension	73,33%	-

TABLE II

Hematological and biochemical parameters.

Parameters	Case	Control	*p*
Hb (g%)	10,115 ± 3,04	12,617 ± 1,59	*p<0,001 (S)*
Ht (%)	30,525 ± 8,00	40,594 ± 5,57	*p<0,001(S)*
PLT (mm3)	120.850 ± 85.327	282628 ± 98723	*p<0,001 (S)*
MCV (fl)	92,553 ±9,87	86,946 ± 6,06	*p<0,05(NS)*
INR (%)	1,4258 ± 0,42712	1,1237 ± 0,2467	*p<0,001(S)*
AST (UI/L)	99,667 ± 95,8853	27,057 ± 14,0335	*p<0,001(S)*
ALT (UI/L)	50,733 ± 44,8798	39,429 ± 16,9155	*p<0,140 (NS)*
GGT (UI/L)	179,317 ± 225,1336	42,486 ± 19,9195	*p<0,001(S)*
BT (mg%)	4,0572 ± 5,74013	1,0211 ± 0,22040	*p<0,05(NS)*
Serum protein (g%)	6,4365 ± 1,17917	7,3257 ± 0,69034	*p<0,001(S)*
Albumin (g%)	3,7042 ± 1,03076	4,3717 ± 0,57693	*p<0,001(S)*
Ammonia (U/L)	114,90 ± 94,759	64,20 ± 23,306	*p<0,001(S)*

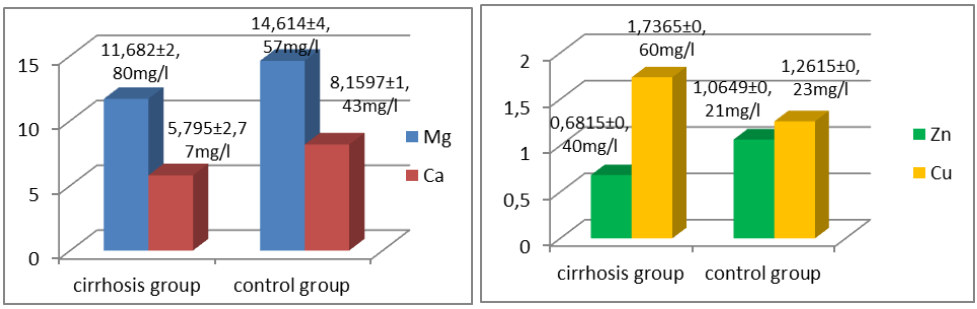

Fig. 1. Plasma concentrations of Mg, Ca, Zn, Cu in liver cirrhosis patients with variceal bleeding and control group.

TABLE III

Correlation between serum levels of magnesium and Child-Pugh classification.

Cirrhosis and variceal bleeding		Mg 2+	Child-Pugh Class	Complications
Mg 2+	Pearson Correlation	1	-,803**	-,557**
	Sig. (2-tailed)		,000	,000
	N	60	60	60
Child-Pugh Class	Pearson Correlation	-,803**	1	,702**
	Sig. (2-tailed)	,000		,000
	N	60	60	60
Complications	Pearson Correlation	-,557**	,702**	1
	Sig. (2-tailed)	,000	,000	
	N	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

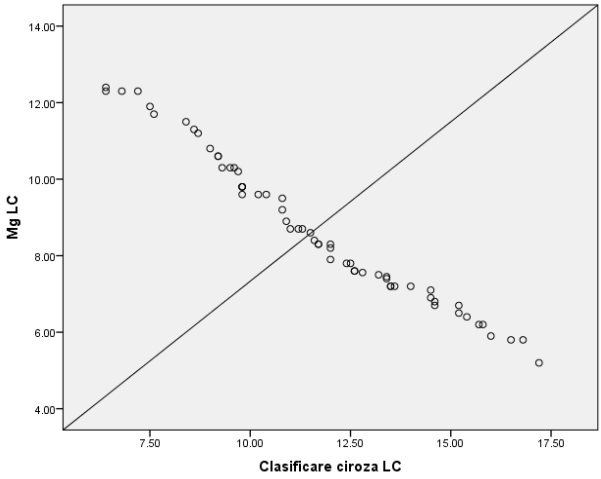

Fig. 2. Correlations between serum level of magnesium and Child-Pugh cirrhosis classification.

TABLE IV

Correlation between serum zinc levels and different biochemical parameters in patients with liver cirrhosis.

Cirrhosis and variceal bleeding		Zn2+	Total protein	Albumin	MCV	GGT
Zn2+	Pearson Correlation	1	.695**	.895**	-.323*	-.285*
	Sig. (2-tailed)		.000	.000	.012	.027
	N	60	60	60	60	60

*. Correlation is significant at the 0.05 level (2-tailed).

** . Correlation is significant at the 0.01 level (2-tailed).

TABLE V

Correlation between copper serum levels and serum levels of zinc, total protein and albumin in liver cirrhosis patients with variceal bleeding.

Cirrhosis and variceal bleeding		Cu2+	Total protein	Albumin	Zn2+	Ammonia
Cu2+	Pearson Correlation	1	-.433**	-.631 **	-.959*	.493*
	Sig. (2-tailed)		.001	.000	.000	.000
	N	60	60	60	60	60

DISCUSSION

Mechanisms linked on ethanol metabolism, especially oxidative stress, redox potentials and acetaldehyde, participate in the emergence of liver damage. Trace elements play an important role in oxidative stress and redox potentials. A possible role of zinc, copper, and magnesium in pathogenesis of liver cirrhosis and its complications is still subject of researches.

Serum Mg levels were statistically significantly lower from the values of the control groups, the results were in contrast to Rahelić study (7). Studies by Kosch (8) showed decreases in Mg concentration in cirrhotic patients with encephalopathy manifests in relation to serum levels of Mg in patients with steatosis or control groups. Our data are in agreement with the results of Rochhi et al. (9) and Kar et al. (10) which showed a significantly lower level of magnesium in patients with cirrhosis. The same results were observed for Suzuki research (11). The study in our group of patients showed the presence of statistical correlation ($p < 0.01$) between decreased serum magnesium based on Child-Pugh class (Class C) results highlighted in the review by Wang (12) but without differences in the studies performed by Rahelić (7), Moscarella (13).

Our research showed statistically significant correlation between decreased serum zinc levels and decreased serum levels of total protein and albumin. Also, we showed statistically significant correlation ($p < 0.05$) between decreased levels of zinc and increased value of MCV and GGT, this indicated a possible correlation between decreased levels of zinc and alcohol intake. Thus, studies of Kugelmans explain the reduction of plasma levels of Zn due to protein restriction, gastrointestinal and urinary losses (14). Research by Celik noted decreased levels of zinc in serum and ascites fluid in cirrhotic patients (15). The reduction of zinc in serum in patients with liver cirrhosis induced by hepatitis B virus

has been reported by Gur et al. (16). Observations of Lin et al. (17) and of Grungreiff (18) show that serum zinc levels were lower in cirrhotic patients compared to subjects in the control group.

The interaction between zinc and copper in their intestinal absorption and their competition for binding sites on the carrier proteins and cellular uptake may be regulators of their homeostasis. Maybe this can explain inverse concentrations of zinc and copper**.** Zinc binds on albumin, transferrin and metalloproteins in the cell, so relative concentrations of these proteins might regulate the serum concentration of zinc (15).

CONCLUSIONS

Our data showed that in patients with liver cirrhosis and variceal bleeding occurs changes in serum concentrations of divalent cations such as magnesium, zinc, copper and these imbalances should be corrected.

The correction of trace elements concentrations might have a beneficial effect on complications and maybe progression of liver cirrhosis. It would be recommendable to provide analysis of trace elements as a routine. We propose that replacements of those trace elements at an early stage of cirrhosis can delay or prevent the severe outcome in this patients.

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NEWS

CLINICAL STUDY CONCERNING THE EVALUATION OF ANTI-INFLAMMATORY EFFETS OF *CURCUMIN GEL* AS AN ADJUNCT TO SCALING AND ROOT PLANING

The complete removal of irritants is not possible using the mechanical therapy alone. Adjunctive use of systemic administration of antibiotics results in the distribution of drug throughout the body, which can give rise to toxicity. *Curcumin* is a constituent of Curcuma longa plant and possess different properties (anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-microbial, anti-hyper-algesic and hypo-cholesterolemic). A study realized by a group of indian researchers aimed to compare the effects of the *Curcumin* (diferuloylmethane) *gel* as an adjunct to subgingival scaling and root planning with the effect achieved using subgingival scaling and root planning alone. In the study were included thirty patients, either male or female, aged between 25-60 years, with chronic localized / generalized periodontitis, with pocket depth of 5-7 mm affecting at least two nonadjacent sites. In the experimental site scaling and root planning was performed, followed by placement of the *Curcumin gel* and periodontal pack application. In the control site, subgingival scaling alone was performed followed by periodontal pack application. The parameters (Plaque index, Gingival index, Probing depth and Clinical attachment loss) were recorded on day 0, 30 and 45 days. A significant reduction in mean was observed in Plaque index, Gingival index, Probing depth and gain in Clinical attachment level were demonstrated in both the groups from baseline to 45 days. Statistical significant reduction was observed in Plaque index at baseline and 30(th) day and Gingival index at 30(th) day. The conclusion of the study indicate that Curcumin can be effectively used along with scaling and root planning (Anuradha BR, Bai YD, Sailaja S, Sudhakar J, Priyanka M, Deepika V. Evaluation of Anti-Inflammatory Effects of Curcumin Gel as an Adjunct to Scaling and Root Planing: A Clinical Study. *J Int Oral Health.* 2015 ;7 (7) : 90-93).

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ECOSOCIAL EPIDEMIOLOGY: MODERN TRENDS IN PREVENTIVE MEDICINE

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ECOSOCIAL EPIDEMIOLOGY: MODERN TRENDS IN PREVENTIVE MEDICINE (Abstract) Each of the structural factors of the environment, national and social, interact with each other within the human ecosystem. The dynamic ecosystem changes and transformations determine the nature of the effect on the structures and functions of human body. Knowledge of these ecosystem-derived effect and their sanogenetic or nonsanogenetic particularities in the major objective of Healthy People Medicine (HPM) based on the interative concept of eco-socio-epidemiology. **Keywords**: ECOSOCIAL, ECOSYSTEM, TRANSFORMATIONS, ECO-SOCIO-EPIDEMIOLOGY

Throughout its existence, man directed efforts towards natural and social environmental change for the benefit of improving his life. In these circumstances, the issue of human and human-environment relationships has become increasingly complex, full of benefits but also of nonsanogenetic factors.

Each of the structural factors of the environment, natural and social, interact with each other within the human ecosystem. In this system, man is subjected to the action of various factors and at the same time he influences them so that the human ecosystem is in constant transformations.

The dynamic ecosystem changes and transformations determine the nature of the effects on the structures and functions of the human body.

Knowledge of these ecosystem-derived effects and their sanogenetic or nonsanogenetic peculiarities is the major objective of *Healthy People Medicine* (HPM) based on the integrative concept of eco-socio-epidemiology.

As humans became more numerous and advanced, the self-regulating mechanisms in the ecosystem began to record important failures, endangering human health and life. This lead to dangerous imbalances in the relationships between humans and their working and living environment and ecosystem structures.

The technical and scientific revolution, as well as civilization, is at first sight responsible for the human health hazards through biological, chemical and physical pollution. In reality, man is the true culprit, as in his relations with the ecosystem structures he ignored the rules governing the interrelations between them, their dynamic but very sensitive equilibrium.

The development of the concept and concerns of preventive medicine and later of HPM required population-based approaches to health and disease problems. Thus, an integrative approach to ecology, epidemiology and sociology became necessary.

HPM promotion cannot be conceived without resorting to ecological, socio-

logical and epidemiological methods and methodologies because health protection requires the detection and neutralization of aggression factors both from the natural and social environment. So HPM includes concerns of medical ecology, sociology and epidemiology. Thus HPM benefits from the contribution of a new discipline, eco-socio-epidemiology, born of interdisciplinarity.

The integrative approach of the three components of global human ecosystem (nature, society and people) is only possible by the associative use of the ecological, sociological and epidemio-logical methods and methodologies. To this is added, as a logical necessity, the cooperation of many other medical and non-medical scientific disciplines.

The activities promoted by *Ecosocial epidemiology* can ensure harmonious and balanced human relations with the ecosystem, with favorable consequences for population health promotion.

The epidemiological processes of diseases affecting human populations occur, develop and present features expressing the nature of human-natural environment and human-social environ-ment relationships.

The epidemiological process consists of factors derived from the natural, social and biological components of the human ecosystem, each representing a distinct category, with a particular role in producing morbid manifestations in population.

Ecosocial epidemiology determines which habitat types are health promoting so that in perspective to ensure human survival. It also highlights the risks at the level of habitats, sub-ecosystems and global human ecosystem through pollution, degradation of the quality of air, water, soil, food and lifestyle, including many non sanogenetic components (nutritional, sedentarism, smoking, alcoholism, drug addiction).

Ecosocial epidemiologic research showed that the changes in the human ecosystem due to technical and scientific revolution are reflected in the emergence of new and diverse sources of aggressive agents for health, ways and means of their spreading into the population and various behaviors of the human body.

In these circumstances, epidemiological processes, especially in non-communicable diseases, have become complex, heavily dependent on natural and social conditions and on changing human biology.

Ecosocial epidemiology plays a fundamental role in HPM as it identifies and capitalizes the factors that can contribute to health promotion, while detecting and neutralizing the non sanogenetic factors derived from the human ecosystem structures.

The complex analysis of the phenomena in the social and natural environment that condition health requires studies on the interconnections between health status and the assembly of human relations with the ecosystem, based on an integrative concept offered by ecology, sociology and epidemiology.

Population health status

Ecosocial epidemiology allows us to know all man-man, man-nature, man-society phenomena and connections, focusing on a global view on population health status as a vital whole integrated in a given society, in particular temporal and spatial conditions.

Integration of phenomena which influence the population health status among the social and natural phenomena is essential for successful disease prevention and control actions and promotion of mental and physical health.

Often, in disease prevention and control the socio-economic factors are decisive, and solving the health problems must overcome economic interests related to correction or even change of some technologies or location of economic units generating risk factors for health. In such situations, social epidemiology brings scientific arguments to support the development of prevention measures. Thus, epidemiology detects and evaluates the risk of some environmental factors, highlighting their effects on populations. It also elucidates the inter relationships between the adaptive capacity of human body and the intervention of some social and natural environmental factors.

As part of its main concerns, epidemiology seeks to discover and explain the incidence of diseases in different human ecosystems. To this end, it provides knowledge of the characteristics of factors that constitute the epidemiological process: factors-host (human-individual variables); factors-agent (disease-bearing variable), factors-natural and social environment (where host factor and agent interact).

The study of the structure and evolution of epidemiological processes by social epidemiology demonstrated the role of social factors. These factors can cause or favor a predisposition to disease, can directly cause a disease state, can transmit the disease causes and can influence the natural progression of disease.

Epidemiological research on the different structures of the human ecosystem reveal or suggest an etiological hypothesis, identify and rank the factors that may cause the emergence and evolution of disease at population level.

CONCLUSIONS

By the large arsenal of methods adequate for population-based studies epidemiology together with ecology and sociology can detect the significant correlations between health or disease status and the factors generated by intrapopulational relationships and between these and the human relationships with the natural and social environment.

In these circumstances, the contribution of *Ecosocial epidemiology* to the development and implementation of health promotion and disease prevention and control programs becomes essential.

These programs provide evidence of the epidemiological, ecological and sociological causal determinism of the health or disease status.

The complex mechanisms of action of biological,physico-chemical, technological, nutritional, educational, psycho-emotional and other factors acting on a determined genetic background are evaluated.

Epidemiology, promoting ecological and sociological concepts in the evaluation of health and disease phenomenon, demonstrates that disease prevention cannot be achieved solely by medical effort, but as a complex process with wide temporal and spatial extension, which should protect individuals from the prenatal period until the end of life.

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NEWS

ADVANCED AGE AND INCIDENCE OF ATRIAL FIBRILLATION IN THE POSTOPERATIVE PERIOD OF AORTIC VALVE REPLACEMENT

Postoperative atrial fibrillation (AF) is the most common complication after cardiac surgery and is associated with higher risks of cerebrovascular accident (CVA), hospital expenses and mortality as well as longer hospital and Intensive Care Unit (ICU) stay. In most cases, it spontaneously reverts to sinus rhythm, without the need for pharmacological intervention. Postoperative AF occurs in approximately 30% to 40% of patients who undergo coronary artery bypass grafting (CABG) and in up to about 60% of patients who undergo concomitant valve surgery. The incidence of this arrhythmia depends on the definitions adopted, the characteristics of the patients, the type of surgery performed, and the monitoring method. AF incidence has been increasing for the past few decades thanks to the higher percentage of elderly patients undergoing cardiac surgery. This arrhythmia occurs typically on the second or third postoperative day, with 70% of the events occurring by the fourth day. However, it can happen at any time after surgery, including after hospital discharge. In fact, AF is the leading cause of early hospital readmission after cardiac surgery. Indications for aortic valve surgery have been increasing due to an increase in population longevity. Even though AF is expected to be more frequent, there are few data on the prevalence of this condition in individuals aged 80 years or older and on its correlation to morbidity and mortality to offer guidance on the possible need for more aggressive prophylaxis during the preoperative period of more elderly patients. In this study was analyzed a sample of elderly patients and described the correlation between age and occurrence of acute postoperative AF after aortic valve stenosis surgery. Secondly, it set out to assess the influence of AF in the incidence of postoperative CVA, hospital length of stay, and hospital mortality (Fernando Pivatto Júnior1, MD; Guaracy Fernandes Teixeira Filho1, MD; João Ricardo Michelin et al. Advanced age and incidence of atrial fibrillation in the postoperative period of aortic valve replacement. *Rev Bras Cir Cardiovasc* 2014; 29(1): 45-50).

Doina Butcovan

ETHICAL MODELS OF PHYSICIAN – PATIENT RELATIONSHIP REVISITED WITH REGARD TO PATIENT AUTONOMY, VALUES AND PATIENT EDUCATION

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ETHICAL MODELS OF PHYSICIAN – PATIENT RELATIONSHIP REVISITED WITH REGARD TO PATIENT AUTONOMY, VALUES AND PATIENT EDUCATION (Abstract): The present paper revisits the ethical models of patient – physician relationship from the perspective of patient autonomy and values. It seems that the four traditional models of physician – patient relationship proposed by Emanuel & Emanuel in 1992 closely link patient values and patient autonomy. On the other hand, their reinterpretation provided by Agarwal & Murinson twenty years later emphasizes the independent expression of values and autonomy in individual patients. Additionally, patient education has been assumed to join patient values and patient autonomy. Moreover, several authors have noted that, over the past few decades, patient autonomy has gradually replaced the paternalistic approach based on the premise that the physician knows what is best for the patient. Neither the paternalistic model of physician – patient relationship, nor the informative model is considered to be satisfactory, as the paternalistic model excludes patient values from decision making, while the informative model excludes physician values from decision making. However, the deliberative model of patient – physician interaction represents an adequate alternative to the two unsatisfactory approaches by promoting shared decision making between the physician and the patient. It has also been suggested that the deliberative model would be ideal for exercising patient autonomy in chronic care and that the ethical role of patient education would be to make the deliberative model applicable to chronic care. In this regard, studies have indicated that the use of decision support interventions might increase the deliberative capacity of chronic patients. **Keywords**: PATIENT AUTONOMY, PHYSICIAN – PATIENT RELATIONSHIP, PATIENT EDUCATION, CHRONIC DISEASES

Autonomy has emerged as a central concept in contemporary health care ethics (1, 2).

In 1981, Miller (3) proposed four senses of autonomy in medical ethics, as follows: autonomy as free action – an action that is voluntary and intentional; autonomy as authenticity – an action is consistent with the person's dispositions, values and life plans; autonomy as effective deliberation – the person is aware of and evaluates both the alternatives and their consequences,

choosing an action based on this evaluation; autonomy as moral reflection – acceptance of the moral values one acts on, as one has reflected on these values and accepts them as his/her own.

Studies have regarded patient autonomy and values to be key variables in the ethical models of patient – physician interaction. Values can be defined as the beliefs or principles of a person, which are used to guide decisions and way of life (4, 5).

It has also been highlighted the importance of good patient – clinician relationships for the ethical principle of respect for autonomy, as supportive relationships can be more facilitative with regard to patient capability for autonomy than concern to allow patient independence (6). Moreover, cancer research has indicated that constructive patient – physician relationships are considered to be essential to minimize disease – related stress and anxiety (7). Additionally, patient education has been increasingly recognized as a method for optimizing treatment of chronic diseases (8).

Thus, the objective of the present paper would be to revisit the ethical models of patient – physician relationship from the perspective of patient autonomy, values and patient education.

PATIENT AUTONOMY, VALUES AND PATIENT EDUCATION IN PATIENT – PHYSICIAN INTERACTION

In 1992, Emanuel & Emanuel (4) outlined the four traditional models of physician – patient relationship. The paternalistic model, where the physician independently decides, assumes that the patient has low health – related values formation, the model being characterized by low patient autonomy and low information disclosure by the physician. In the interpretive model, the physician provides patients with pertinent information and helps them to elucidate their values, but it is the patient who ultimately decides. The deliberative model allows the physician to guide the patient's decision making and the patient to deliberate on the differences between his or her preferences and the ones of the physician. The informative model, where the patient alone takes on decision making responsibilities, presupposes that the patient possesses well known and fixed values, this model being characterized by high patient autonomy and high medical information delivery to the patient (5, 9).

It has been noticed that the models described by Emanuel & Emanuel tightly link patient values and patient autonomy by assuming that, as patient autonomy increases, the strength and formation of patient values should increase as well. However, twenty years after the publication of the paper of Emanuel & Emanuel on the four models of the physician – patient relationship, Agarwal & Murinson (5) proposed a reinterpretation of these traditional models. The reinterpretation draws attention to the independent expression of values and autonomy in individual patients (e.g. the patient with high autonomy, but low formation of health – related values) and adds patient medical knowledge as a new dimension in the patient – physician interaction. In this regard, while the traditional models of patient – physician relationship involve a unidirectional flow of medical information from physician to patient, the reinterpretation of these models also takes into consideration the growing availability of medical information accessible to the general public via the internet.

In addition, patient medical knowledge is supposed to join patient values and patient autonomy.

Furthermore, several papers underline that, over the past few decades, there has been a shift from a paternalistic approach to patients towards an emphasis on patient autonomy (10, 11, 12, 13).

However, it has been suggested that good patient care might involve a closer look at some remaining aspects of paternalism (11). In this regard, it has been revealed that paternalism should still have a role in decision making, if we consider that there are physicians who avoid making difficult medical decisions by hiding behind the respect for patient autonomy (14). On the other hand, studies indicate that the number of physicians who believe in paternalism as a form of beneficence is still significant (15). Moreover, even though patient expectations and desire to be implicated in treatment decisions are increasing, some patients still prefer to leave decision making to their physician (7). Another study has also noted that there are many patients who would prefer a paternalistic approach (16). In addition, there has been outlined that the so-called modern paternalism takes into account patient values and interests, but considers these values as only one factor among others, whereas autonomy refers to patient values as decisive (17). In any case, several authors indicate that the balance between autonomy and paternalism needs reassessment in such a way that beneficence and autonomy would be mutually reinforcing rather than competing (14, 18, 19, 20).

If the four traditional models of the physician – patient relationship would be represented as a single linear axis, the paternalistic model and the informative one would be the two extremes of the axis, neither of the two being satisfactory with regard to decision making approach (4, 5, 10, 21). The informative model, also known in the literature as the independent choice model, reflects a defective understanding of patient autonomy, as it sacrifices physician competence for patient control. In this model, physicians withhold their recommendations and experience and carry out patients' decisions. Such a model presumes that if the patient is to gain power to make autonomous choices, the physician has to lose power (4, 10). Nevertheless, it is suggested that such overemphasis on patient autonomy may suppress beneficent intervention (19).

Therefore, the paternalistic model leaves the patient outside the decision making process, while the informative model leaves the physician outside by reducing the role of the physician to that of a technician providing the chosen service (2, 4, 22).

The deliberative model offers a better alternative to these unsatisfactory approaches, because, in this model, both physician and patient engage in moral deliberation and the patient considers through open dialogue alternative health – related values. Thus, the deliberative model promotes shared decision making and negotiation between the physician, who has a caring attitude, and the patient, being recommended as the best model for the physician – patient relationship and for exercising patient autonomy (2, 4, 9). Shared decision making has also been increasingly regarded as an ideal model of physician – patient interaction, in which both the patient and the physician make active and essential contributions through open dialogue, joint deliberation and mutual understanding (4, 22, 23). An analogous model based on

mutual understanding, described in the scientific literature as an authentic interaction between physician and patient, is the dynamic and genuine dialogue – based model of autonomy, which also reflects a care giving relationship and overcomes the imbalance of power that is present in both paternalistic and informative models with regard to decision making (11). Additionally, the enhanced autonomy model underlines that the physician guides the decisions of the patients in a way that allows patients to exercise autonomy more powerfully if they make decisions that integrate the experience of the physician with their own. Hence, this model promotes, similarly to the deliberative model, to the shared decision making model and to the dialogue – based model, a physician – patient relationship characterized by open dialogue and mutual influence and understanding, where patient and physician share power, negotiate differences and actively exchange ideas (10).

Moreover, in 2014, by revisiting the four traditional models of patient – physician relationship, Reach (9) proposes a model of care in chronic diseases based on patient education, pointing out that the ethical role of patient education would be to make the deliberative model applicable to chronic care and, thus, to give patients the opportunity to exercise autonomy in an ideal situation. In this regard, patient education is referred to as an ethical pathway that links three of the four models of physician – patient relationship, as follows: the physician first gives the facts (the informative model), helps the patients to elucidate their preferences (the interpretive model) and finally gives them the opportunity to choose between their preferences and the ones of the physician, so that the deliberative model can be reached at the end of this pathway.

Studies on decision making in cancer patients have emphasized that the distress experienced during the time of diagnosis might diminish the deliberative capacity of a patient (24). In this regard, it has been pointed out the contribution of decision aids in cancer care, as decision aids are described to be decision support interventions designed to teach patients the deliberative skills needed to negotiate their way through relevant information, to clarify their values and to guide them in the steps of decision making and communicating with others (25, 26).

In order to promote respect for autonomy of patients with chronic life-threatening diseases, it has been revealed that such patients should be encouraged to have a values discussion with their physician and to document the values and goals that should guide future decisions. As values can change, values discussions should be revisited occasionally, especially when patient condition changes significantly. In this regard, it has been emphasized that the physician should recommend the medical means to honor those values and achieve those goals (27).

CONCLUSIONS

To conclude, patient autonomy, patient values and patient education are referred to as central variables in the models of physician – patient interaction.

The deliberative model, the shared decision making model, the dialogue – based model of autonomy and the enhanced autonomy model are recommended to be the best for the physician – patient relationship and for exercising patient autonomy, and, in our opinion, they appear to express, under different names, the same model of

physician – patient relationship. Such a model, which is based on open dialogue, joint deliberation and mutual understanding, would be the balanced alternative between the two extremes represented by the paternalism and the informative model, as it overcomes the imbalance of decision making power between patient and physician.

Moreover, the deliberative model has been suggested to be ideal for exercising patient autonomy in chronic care. Studies have indicated that the use of decision aids could increase the deliberative capacity of chronic patients. In addition, it has been pointed out that, in order to promote respect for autonomy of chronic patients, discussions that elucidate patient values should be occasionally revisited.

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NEWS

FIBROMUSCULAR DYSPLASIA OF CARDIAC CONDUCTION SYSTEM ARTERIES IN TRAUMATIC AND NONNATURAL SUDDEN DEATH VICTIMS

In several reports, luminal narrowing by fibromuscular dysplasia of the sinus and/or AV-node artery was found in examinations of unexplained deaths and, in many cases, was considered as the cause of death. These reported cases were aged between 0 and 49 years; both sexes were affected. However, there are no data on the frequency of fibromuscular proliferations of arterial walls in heart healthy control groups. But, the exact cause of these fibromuscular vessel alterations is still unclear. Possible hypotheses might be either the consequence of the impact of the bloodstream on small vessel branches or a protective mechanism against luminal extension. Since fibromuscular proliferations of arteries of the cardiac conduction system and small coronary arteries occur in 84% in an obviously heart-healthy control group, these findings should be known to every (forensic) pathologist and anatomist. This might reduce the risk of misdiagnosis. In the same time, the reported results demonstrate that microscopically detectable findings of the cardiac conduction system arteries similar to fibromuscular dysplasia do not indicate a defined disease and should not be considered as a cause of death when there are no macroscopic findings in the coronary arteries (Zack F, Kutter G, Blaas V, et al. Fibromuscular dysplasia of cardiac conduction system arteries in traumatic and non-natural sudden death victims aged 0 to 40 years: a histological analysis of 100 cases. Cardiovascular Pathology 2014, 23: 12–16).

Doina Butcovan

IMIDAZOLINE RECEPTOR ANTAGONISTS IDAZOXAN AND EFAROXAN ENHANCE LOCOMOTOR FUNCTIONS IN RATS

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IMIDAZOLINE RECEPTOR ANTAGONISTS IDAZOXAN AND EFAROXAN ENHANCE LOCOMOTOR FUNCTIONS IN RATS (Abstract) Discovered in 1984, imidazoline receptors (I_1, I_2, I_3) are located centrally and peripherally being involved in various physiologic and pathophysiologic processes in the body. Experimental and clinical investigations have suggested the interrelations between imidazoline, adrenergic, dopaminergic, glutamatergic and opioid systems, which may explain the influence of different substances acting on imidazoline receptors in cognitive disorders, behavioral disturbances and motor diseases pathways. **Aim:** To investigate the effects of two imidazoline receptor antagonists on locomotor activity and endurance capacity in rats. **Material and methods:** The experiment was carried out with white male Wistar rats (200-250g) divided into 3 groups of 7 animals each, treated intraperitonealy with the same volume of solution as follows: Group I (Control): distilled water 0.3ml/100 g body weight; Group II (IDZ): idazoxan 3 mg/kbw; Group III (EFR): efaroxan 1 mg/kbw. Exercise capacity was evaluated using a locomotor PanLAB treadmill test. The data were presented as mean +/- standard deviation and significance was tested by SPSS Statistics for Windows version 17.0 and ANOVA method. Experimental protocol was implemented according to recommendations of the Gr.T. Popa" University Committee for Research and Ethical Issues. **Results:** Intraperitoneal administration of idazoxan and efaroxan resulted in a significant increase in running distance compared with the control group ($p<0.05$). At the same time a reduction in the number and time of electric shocks delivered to motivate the animal to keep running was observed. In this experimental behavioral model the effects of idazoxan on the evaluated parameters were more intense than those of efaroxan. **Conclusions:** In our experimental conditions we demonstrated the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve fatigue resistance during forced running in rats. **Keywords:** IDAZOXAN, EFAROXAN, TREADMILL, LOCOMOTOR.

Imidazoline receptors were identified for the first time in 1984, three types being known today: I_1, I_2 and I_3. I_2 imidazoline receptors have been subdivided into two subtypes according to their affinity for amiloride: I_2A subtype is amiloride-

sensitive and I_2B subtype is amiloride-insensitive (1).

Literature data revealed that imidazoline receptors are located centrally and peripherally, being involved in numerous physiological and pathophysiological processes, but the mechanisms are not yet fully understood (2).

Until now four endogenous imidazoline receptors ligands have been characterized, of which agmatine (decarboxylated arginine) is the most known and studied. The others are: two varieties of beta-carbolines: harmine and harmaline and imidazoleacetic acid robotide (3).

Imidazoline receptors have a great affinity for binding to various compounds with imidazolinic structure. Most of the ligands with affinity for imidazoline receptors also bind to alpha-2 adrenergic receptors, these two types of imidazoline receptors being difficult to differentiate (4). Modern methods, such as autoradiography and radio-ligation techniques, offer only indirect evidence regarding the presence of distinct binding sites for alpha-2 and imidazoline receptors (2).

Numerous experimental studies and clinical trials have showed the involvement of imidazoline receptors in different physiologic and pathophysiologic conditions in the body.

- I_1 imidazoline receptors mediate sympathetic inhibitory actions of imidazoline derivatives associated with the decrease of blood pressure (5);
- I_2 imidazoline receptors modulate the level of central monoamines and activate hypothalamic adrenal pituitary axis with a potential antidepressant activity (6);
- I_3 imidazoline receptors regulate insulin secretion in the beta Langerhans pancreatic cells (7).

The potent neurotransmitter agmatine is involved in the mediation of stress response, analgesia, drug addiction, withdrawal syndrome, and in the modulation of seizures development and in neuroprotection (1, 6).

Various experimental and clinical studies suggested the interrelations between imidazolinic, adrenergic, dopaminergic, glutamatergic and opioid systems; thus they might explain the influence of different substances acting on imidazoline receptors on cognitive disorders, behaviour disturbances and in motor diseases pathways. (8, 9) The results reported in the literature are inconsistent and some of them controversial.

The investigations regarding the involvement of substances acting on imidazoline receptors in behaviour, memory, and locomotor activity might lead to new data on the pathophysiological mechanisms of spontaneous behaviour alterations and cognitive disturbances (6, 9).

Idazoxan is a 2-(2,3-dihydro-1,4-benzodioxine-2-yl)-4,5-dihydro-1H-imidazole derivative that selectively blocks alpha-2 adrenergic and imidazoline receptors. Various studies have been performed to investigate its effects in depression, schizophrenia and different types of psychosis, but the results are not yet validated (10, 11). Some recent researches demonstrated the neuroprotective activity of idazoxan with a reduction of blood brain barrier damage during experimental autoimmune encephalomyelitis in mouse (12).

Efaroxan, 2-(2-Ethyl-2,3-dihydro-1-benzofuran-2-yl)-4,5-dihydro-1H-imidazole derivative is an alpha-2 adrenergic receptor antagonist and also an imidazoline receptor ligand (2, 5). In the last few years investigations on the involvement of this imidazo-

line receptor antagonist in the regulation of blood pressure, pancreatic insulin secretion and also in depressive conditions have been conducted (2, 5, 13).

The aim of this study was to investigate the effects of imidazoline receptor antagonists idazoxan and efaroxan on locomotor activity and endurance capacity in rats.

MATERIAL AND METHODS

The experiment was carried on white Wistar rats (200-250g). Animals were housed in plastic cages in an animal room maintained at 23 ± 1°C on a 12-hour dark cycle (light period, 07:00 – 19:00). Standard laboratory food and tap water were freely available, except during the time of the experiments.

Rats were divided randomly into four groups 7 animals each, treated intraperitoneally with a single dose as follows: Group I (Control): distilled water 0.3 ml/100g weight; Group II (IDZ): idazoxan 3 mg/kbw; Group III (EFR): efaroxan 1 mg/kbw.

Idazoxan and efaroxan (purchased from Sigma Aldrich Chemical Co, Germany) were diluted in distilled water and prepared extemporaneously.

Exercise capacity was assessed using a PanLAB treadmill for locomotor-fatigue. This apparatus consists of a rolling belt (driven by a servo-controlled motor) with an adaptable speed and angle profiles, enabling forced exercise training and accurate testing of fatigue in laboratory animals (13, 14). Treadmill System is a fully computerized automatically controlled device designed to assess rodent exercise capacity when forced to run. It is provided with a grid that administers an electric shock (power 700 V Dc at a maximum current of 0.4 mA) that forces the animal to run when it stops and two counters to record the parameters necessary to investigate the physical performance. One counter records the distances and the other the number of electrical shocks received by the animals during the experiment.

In our experiment, rats were trained at a speed of 40 cm/second on a 5 degree tilting of the belt, having run as forced exercise on motorized treadmill 10 minutes sessions.

The locomotors performance of rats was quantitatively evaluated by recording the following parameters: distance run, number of electrical shocks and time of delivering the stimulatory shocks (16).

In this behavioral model of forced exercise, the decreasing of time interval of delivering the electric shocks or the reduction of the number of shocks necessary to motivate the animal corresponds to the endurance-enhancing effect induced by the investigated substance (16, 17). Opposite to this, the increase of time interval for stimulating shock delivery or the number of electric shocks may be correlated with the diminution of physical endurance (17).

On the other hand, the increasing of distance run on the rolling belt may be related to an enhancing of endurance to effort and also to a stimulation of motor activity after substances administration (18, 19). In contrast, the reduction of distance run at the same time interval of the experiment signifies the alteration of animal physical performance in the forced locomotion test (18).

The data were presented as +/- standard deviation and significance was tested using the *SPSS Statistics 17.0* for windows software. Analysis of variance (ANOVA one-way method) and *post hoc* Newman-Keuls and Tukey independent tests were used to determine statistical differences between

the investigated groups. P-values less than 0.05 were considered statistically significant compared to control group.

Experimental protocols were implemented according to the recommendations of the"Gr. T. Popa" University Committee for Research and Ethical Issues. Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the experiment (20).

RESULTS AND DISCUSSION

The treadmill running test is one of the most frequently used behavioral valuable non-invasive method for assessing the effects of imidazoline receptor antagonists on the locomotor activity and endurance capacity of laboratory animals during physical effort. In addition, the treadmill test was used to clarify the movement motivation of rats (21).

Using the behavioral experimental model of forced locomotion we obtained three types of information regarding the animal physical ability. The following parameters recorded during the 10 minute sessions (distance run, number and delivery time of electric shocks) represent essential tools used to estimate the endurance capacity, and an important modality to corroborate and validate the biological significance of data (14, 22).

During habituation to monitorized treadmill, rats treated with idazoxan (3 mg/kbw) showed a significant increase ($p<0.05$) of distance run (248.17±27.25 meters) compared to the control group (205.75±39.60 meters) (fig. 1).

Intraperitoneal administration of efaroxan 1 mg/kbw lead to an increase in distance run (220.00±19.09 meters), statistically significant ($p<0.05$) compared to the control group (205.75±39.60 meters) in the same behavioral model (fig. 1).

The mean distance run by rats treated with efaroxan was shorter than of rats treated with idazoxan, in the same time interval in the experiment (tab. I).

These results proved the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve fatigue resistance during forced running on this behavioral experimental model.

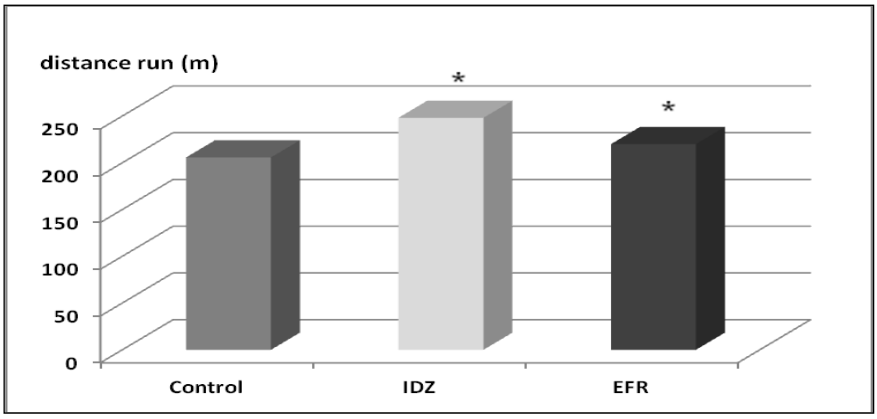

Fig. 1. The effects of idazoxan and efaroxan on distance run in the treadmill exercise test. Each value is the mean ± SEM of distance run (meters) for seven rats. *$p<0.05$, **$p<0.01$ *vs.* control group.

TABLE I
Descriptive statistics – distance run

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	205.75	39.60	14.00	116.00	233.00	
IDZ	8	248.17	27.25	9.23	163.00	252.00	**0.031***
EFR	8	220.00	19.09	6.75	179.00	239.00	**0.046***

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

TABLE II
Descriptive statistics – number of electric shocks

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	96.88	79.90	28.25	26.00	273.00	
IDZ	8	78.36	61.65	31.35	11.00	216.00	**0.039***
EFR	8	85.38	84.59	29.91	6.00	279.00	**0.047***

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

The administration of idazoxan (3 mg/kbw) was associated with a significant reduction ($p<0.05$) in the number of electric shocks (78.36±61.65) and also in delivery time of shocks (25.28±19.23 seconds) compared to control group (96.88±79.90, 32.64±25.57 seconds respectively) (fig. 2.).

The treatment with efaroxan 1 mg/kbw resulted in a significant ($p<0.05$) decreasing of both electric shocks number (85.38±84.59) and delivery time of shocks (28.59±25.35 seconds) compared to control group (96.88±79.90, 32.64±25.57 seconds respectively) (fig. 2, 3).

The effects of idazoxan on these parameters were more intense than those of efaroxan in the forced exercise test (tab. II, III).

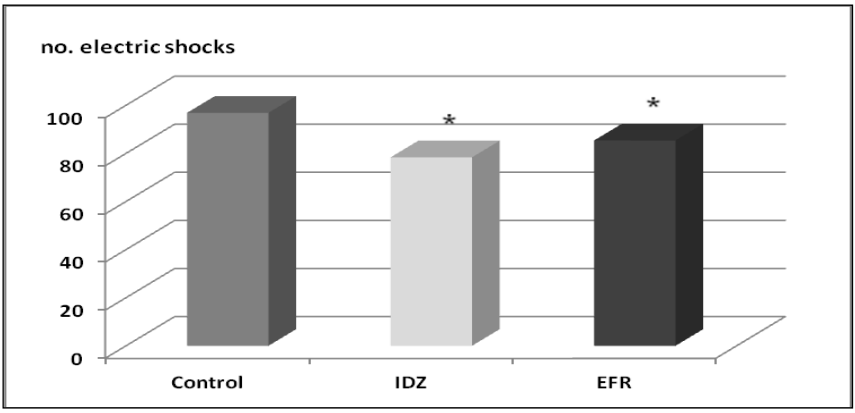

Fig 2. The effects of idazoxan and efaroxan on the number of electrical shocks in treadmill exercise test. Each value is the mean ± SEM of number electric shocks for seven rats. *$p<0.05$, **$p<0.01$ *vs.* control group.

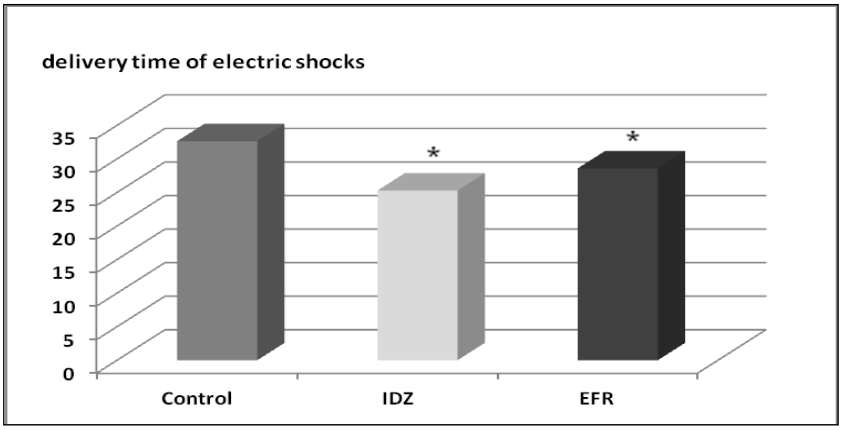

Fig 3. The effects of idazoxan and efaroxan on the delivery time of electric shocks in treadmill exercise test. Each value is the mean ± SEM of distance run (meters) for seven rats. *p<0.05, **p<0.01 *vs.* control group.

TABLE III
Descriptive statistics – delivery time of electric shocks

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	32.64	25.57	9.04	8.40	74.80	
IDZ	8	27.93	24.83	6.32	7.90	74.40	0,29
EFR	8	28.59	25.35	8.96	9.00	76.00	0,45

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

CONCLUSIONS

Our experimental investigation demonstrated clear beneficial effects of both imidazoline receptor antagonists idazoxan and efaroxan on endurance exercise performance.

The intraperitoneal administration of idazoxan and efaroxan resulted in an improvement of physical performance and also of endurance capacity in the forced locomotion experimental model in rats.

These findings prove the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve locomotor abilities and to delay fatigue in rats during forced running on this behavioral experimental model. The effects of idazoxan on the recovery of locomotor activity of rats were more intense than those of efaroxan in the treadmill test.

Much more, using this behavioral experimental model, we demonstrated the valuable effect of idazoxan and efaroxan on the improvement of depression-like behavior in rats.

ACKNOWLEDGEMENTS

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NEWS

IL-10 AND ET-1 AS BIOMARKERS OF RHEUMATIC VALVE DISEASE

Rheumatic fever (RF) represents a serious public health problem. It is a rheumatic and inflammatory disease of autoimmune origin, which occurs in response to an infection by group A streptococcus. On a global scale, this agent is responsible for approximately 15.6 million annual cases of rheumatic heart disease, with 282,000 new cases and 233,000 deaths each year. From this perspective, health systems face higher expenses with clinical exams, surgeries and frequent hospitalizations due to congestive heart failure. The pathogenesis of RF involves a complex network of genetic, environmental and immunological interactions. Genetic factors predispose individuals to developing autoimmune reactions. Cytokines are proteic molecules, glycosylated or not, that send a range of stimulatory, modulatory or inhibitory signals to the various cells of the immune system. Studies indicate that the inflammatory response in acute RF on cardiac tissues is generated by antigenic mimicry of the protein M leading to an abundant infiltration of CD4+ T cells. This leads to production of inflammatory cytokines (e.g., TNF-α, IL-2, and IL-10), which have a decisive influence on the immune response of patients with rheumatic fever. It is also known that increased levels of Th1 inflammatory cytokines (TNF-α and IFN-γ) and lower levels of Th2 and regulatory cytokine IL-4 lead to maintenance and progression of rheumatic valvulopathy. Endothelin is a highly potent vasoconstrictor peptide. This peptide is composed of 21 amino acids and has three isoforms. The three isoforms are called endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). Endothelin-1 is the subtype predominantly produced by cardiac endothelium. Some studies show gene expression of endothelin in heart valves of patients who underwent surgical valve replacement. Cardiac involvement in acute RF characterizes the most serious and most important of all manifestations of the disease because of the possibility of progressing to chronic rheumatic valvular disease or death. The most common rheumatic mitral valvulopathy is a dual unbalanced dysfunction, i.e, insufficiency and stenosis in different stages of development, which may lead to an indication of surgical repair or replacement of the damaged valve in children and young people in productive age. In this study were compared the levels of some interleukins (TNF-alpha, IL-4 and IL-10) among different patients with RF. In addition, was assessed gene expression of endothelin-1 in native replaced mitral valves (Leão SC, MD; Menezes Lima MR, Menezes do Nascimento H, et al. IL-10 and ET-1 as biomarkers of rheumatic valve disease. Rev Bras Cir Cardiovasc 2014; 29 (1): 25-30).

Doina Butcovan

GC-MS ANALYSIS OF *MALUS DOMESTICA* BARK AND LEAF EXTRACTS IN PESTICIDE NON-EXPOSED AND EXPOSED MATRICES

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GC-MS ANALYSIS OF *MALUS DOMESTICA* BARK AND LEAF EXTRACTS IN PESTICIDE NON-EXPOSED AND EXPOSED MATRICES (Abstract): **Aim:** Research on phytochemical constituents' existent in *Malus domestica* leaves and bark is a nowadays topic of interest especially due to the presence of various secondary metabolites with potential pharmacological characteristics. Quantifying the residual levels of singular pesticides, e.g. tebuconazole, and/or of chemicals in clearly stated combinational formulations is also of great interest especially due to all negative effects induced by pesticides on human health. **Material and methods:** The strategy within the present study corroborates field activities (i.e. clear treatment scheme, leaf and bark samples collection from the *Golden Delicious* and *Jonathan* apple varieties from an orchard in Miroslava, Iasi, Romania, before and after treatments spread) with sample analysis by gas chromatographic methods. Ultrasound-assisted techniques and clean-up procedures were used during the preparative steps. All methanol (MeOH) extracts were analyzed using a gas chromatograph, Agilent 7890A, in tandem with a mass spectrometer, Agilent 5975C, Agilent Technologies, United States of America. **Results:** Analysis of the chromatographic profiles obtained for the investigated pesticide non-exposed matrices revealed the existence of many potential therapeutic bioactive compounds with positive benefits on human health (e.g., lupeol, friedelin, α-tocopherol known also as vitamin E, mellein). Chromatographic run profiles of methanol extracts associated to sample matrices collected at different periods within the treatment scheme revealed the existence of the chromatographic peak corresponding to tebuconazole at a retention time of 13.75 minutes. Pesticides existent in the in-use clearly stated combinational formulations have been as well identified. **Conclusions:** A preliminary characterization of the secondary metabolites existent in *Golden Delicious* and *Jonathan* apple leaves and bark was achieved for the first time for samples collected from an orchard in Miroslava, Iasi, Northeastern Romania. In pesticide non-exposed matrix compounds of pharmacological interest have been identified. Clear evidence has been obtained about the existence of tebuconazole and of other pesticides from the in-use clearly stated combinational formulations, in all samples collected in a well-established time span from the treatment spread. Samples containing residual pesticides might induce possible negative consequences for human health. **Keywords:** TEBUCONAZOLE, *JONATHAN*, *GOLDEN DELICIOUS*, LEAF, BARK.

In temperate geographic areas apple tree is one of the most widely cultivated fruit trees (1). Apples, as rich sources of phytochemicals, are the fruits extensively used worldwide (2).

Minerals such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, copper, manganese, zinc are present in important amounts in apple leaves (3). Apple leaves and bark are often assigned as potential sources of antioxidant compounds (4,5,6). Flavonoids and other phenols from plants, such as phenolic acids, stilbene, tannins, lignans and lignin, are particularly common in leaves, flowers and woody tissues, such as stems and bark (7).

Nowadays, tebuconazole (TEB) which is a systemic triazole fungicide is intensively used to combat foliar diseases in fruit trees (8). This triazole fungicide group inhibits lanosterol 14∝-demethylase (CYP51) activity by disrupting pathogen's membrane and by inhibiting pest's cells growth. It has been already shown that tebuconazole is highly efficient against pathogenic fungi from various plants and that it is widely used for controlling white rot disease. Important quantities of apple are exported from China (9) to Europe (10), and studies performed in field on Chinese crops have shown that tebuconazole is an excellent fungicide for controlling white apple rot (9). There are studies suggesting that tebuconazole is highly efficient in combating mold and rust in fruit, and that once these tebuconazole-containing fruits are consumed they can exert serious health consequences (11).

MATERIAL AND METHODS

Treatment and sampling. Clearly stated combinational pesticide formulations, containing mainly tebuconazole, were used during sample treatments. The interest products have been sequential sprayed (4 times) in full agreement with the producer's recommendation (Bayer) and the samples (leaves, bark) have been collected by following an own developed strategy in the April 9, 2016 – October 6, 2016. No significant precipitation events have been recorded during treatments and sampling steps, Iasi region facing mainly drought throughout the period of interest.

Sample preparation. Samples collected from two varieties of apple, i.e. *Golden Delicious* and *Jonathan*, were subjected to several preparative steps for further analysis by GC-MS technique. Accurately weighted amounts from each matrix of interest, whether or not previously subjected to field treatment with tebuconazole and clearly stated combinational pesticide formulations, each in normal and double dose, were passed through ultrasound assisted extraction process in two different solvents.

To increase extraction efficiency and the requested accuracy for a specific study on pesticide dynamics in the field, each sampled matrix was well shredded with a scalpel. This step was immediately followed by mass weighing on an analytical balance Adam PW254 with an accuracy of 10^{-4} g. For each matrix ~ 0.2 g of sample was weighed (i.e. *Golden Delicious* and *Jonathan* matrices, two modes of treatment, singular tebuconazole and with clearly stated combinational pesticide formulations, each at normal dose and double dose, at each collection point).

Each weighed sample mass placed in an Eppendorf tube was treated with 3 mL of solvent. For the ultrasound assisted extraction procedure two solvents were initially used, i.e. methanol (MeOH) and acetoni-

trile (ACN). Later, MeOH was preferred due to its higher extraction efficacy. After adding the solvent, the samples were subjected to the ultrasound-assisted extraction process in an Elmasonic S70H ultrasonic bath for 45 minutes (3 cycles × 15 minutes), which has been proven to suffice a good extraction yield and not to aggressively degrade the chemical composition of the extract (12). After this step, the Eppendorff units containing the samples extracted into methanol (MeOH) were coated with aluminum foil and allowed to stand in the dark for 24 hours. Immediately after, the Eppendorff units containing the samples were centrifuged for 5 minutes at 5000 rpm at a temperature of 40°C using a Hettich Universal 320R centrifuge. Each generated supernatant was transferred with dedicated Pasteur pipette into new Eppendorf tubes. The obtained extracts were subjected to specific drying procedures using the principle of solid phase extraction (SPE). Homemade cartridges filled with anhydrous sodium sulphate (Na_2SO_4 anh.) and glass wool as stopper were used to dry each obtained extract. The extracts thus generated were passed through Pall Acrodisc PSF, G×F/GHP filters of 0.2 μm porosity. Volumes in the 1-2 mL range were transferred into brown vials and then stored in the refrigerator (4^oC) until the analysis.

Sample analysis. Analysis of the entire set of samples was performed on the GC-MS system at the CERNESIM Center, Integrated Center of Environmental Science Studies in the Northeastern Region, at the "Alexandru Ioan Cuza" University of Iasi, Faculty of Chemistry, Analytical Chemistry Laboratory. Samples were manually injected with a micro syringe into a gas chromatograph, 7890A GC model from Agilent Technologies, equipped with flame ionization detector (FID) and a mass spectrometer 5975C MS model from Agilent Technologies, USA.

For sample analysis the optimized working conditions of the equipment were: 1) helium as mobile phase; 2) capillary column HP-5ms type (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness) as a stationary phase; 3) 270 °C operating temperature of the injector; 4) 0.003 mL injected volume under spitless conditions; 5) temperature gradient with the following steps: 60 ˚C for 1 minute, ramp with 30 ˚C/min. up to 220 ˚C (0 min.), followed by a ramp of 2 ˚C/min to 240 ˚C, constantly 4 minutes, followed by a ramp of 10 ˚C/min to 290 ˚C (constant 10 min.) 6) 230 °C MSD temperature; 7) 150 ˚C quadrupole unit temperature; 8) full scan mode for acquisition of mass spectra; 9) scanned mass range m/z 60-650 atomic mass units; 10) ChemStation, Agilent Technologies soft keys and NIST database for data analysis.

RESULTS

Gas chromatography tandem mass spectrometry (GC-MS) has been used for the analysis of the non-exposed and exposed pesticide matrices. In the phytochemical constituents of both *Golden Delicious* and *Jonathan* matrices, tebuconazole and pesticides present in clearly stated combinational pesticide formulations have been identified upon the sample state, i.e., pesticides non-exposed and exposed matrices.

Attempts have been made to derive chromatographic fingerprints of the investigated samples following the GC-MS analysis of *Golden Delicious* and *Jonathan* leaf and bark extracts from pesticide non-exposed and exposed matrices.

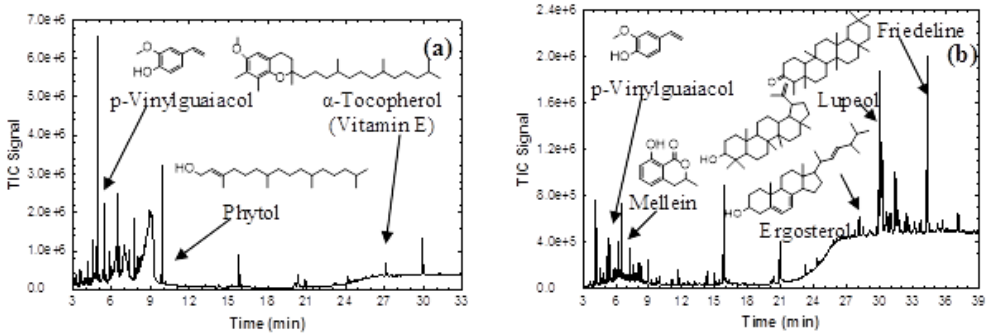

Fig. 1.a,b: Total ion chromatogram (TIC) profiles for pesticides non-exposed *Golden Delicious* matrices: (a) – leaves and (b) – bark methanol extracts.

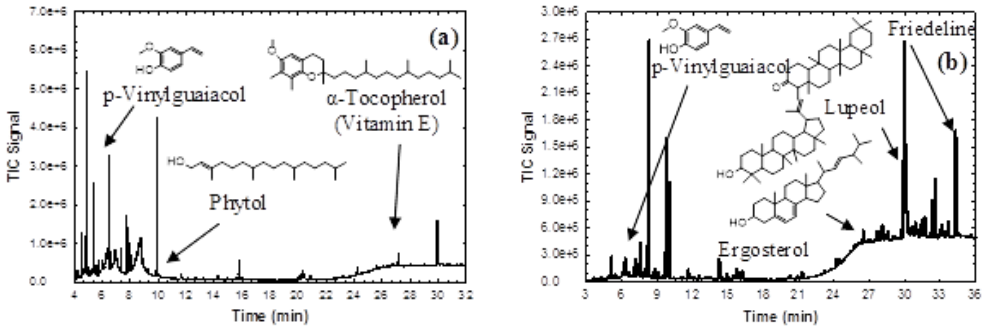

Fig. 2.a,b: Total ion chromatogram (TIC) profiles for pesticides non-exposed *Jonathan* matrices: (a) – leaves and (b) – bark methanol extracts.

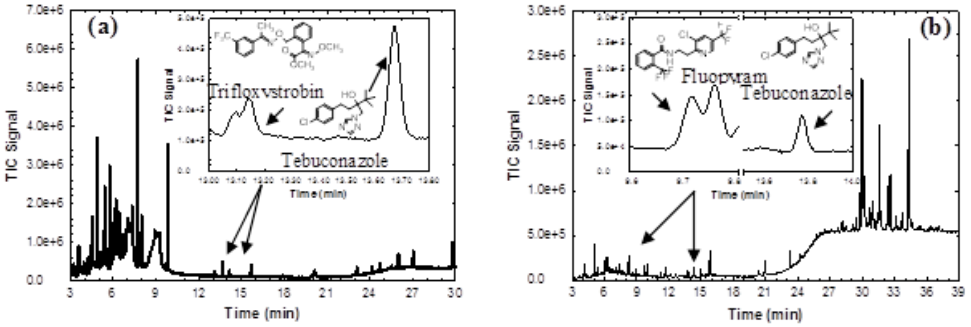

Fig. 3.a,b: Total ion chromatogram frame of methanol extract from pesticides exposed *Golden Delicious* variety: (a) - leaves, (b) - bark. The inset figures highlight in details the chromatographic peaks associated with tebuconazole, trifloxystrobin and fluopyram.

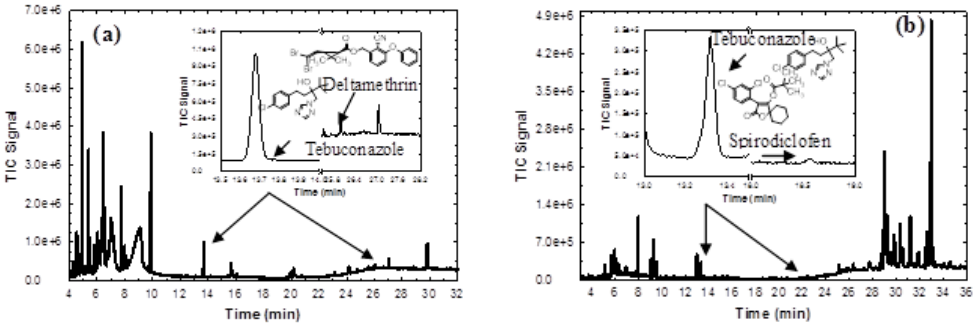

Fig. 4.a,b: Total ion chromatogram frame of methanol extract from pesticides exposed *Jonathan* variety: (a) - leaves, (b) - bark. The inset figures highlight in details the chromatographic peaks associated with tebuconazole, deltamethrin and spirodiclofen.

DISCUSSION

In the present study attempts have been made to derive chromatographic fingerprints of the investigated samples following the GC-MS analysis of *Golden Delicious* and *Jonathan* leaf and bark extracts from pesticide non-exposed and exposed matrices. Figures 1.a,b show some of the main phytochemical constituents (phytol, p-vinyl guaiacol, α-tocopherol/vitamin E) identified in pesticide non-exposed *Golden Delicious* apple leaf (fig.1.a.) and, bark, respectively (fig.1.b.) matrices where compounds such as p-viny guaiacol, lupeol, friedelin, ergosterol, mellein have been identified.

Analysis of the obtained chromatographic profiles highlighted the existence of an important number of bioactive compounds in methanol extracts from both leaves and bark of *Golden Delicious* variety (pesticide non-exposed and exposed matrices) and leaves/bark extracts of *Jonathan* apple variety from pesticide non-exposed and exposed matrices. For sample GC-MS analysis total ion chromatogram (TIC) acquisition mode was preferred to single ion monitoring (SIM) mode and this procedure allowed us to identify a larger array of chemicals. In the investigated pesticide non-exposed matrices several important phytochemical constituents such as methoxyphenol compounds, terpene, tocopherols (α-tocopherol or Vitamin E), phytol, sterols, etc. have been identified.

Chromatographic profiles presented in Figures. 1. a, b and 2. a, b, respectively, clearly highlight features of methanol (MeOH) leave (fig.1.a and fig.2a) and bark extracts (fig. 1.b and fig. 2.b) from pesticide non-exposed *Golden Delicious* and *Jonathan* apple varieties. Regarding the metabolites present in the investigated matrices quite similar information was generated by the investigated samples. However, the abundance of secondary metabolites identified in the analyzed matrices varied from one apple sort to another, i.e. *Golden Delicious* and *Jonathan*. Abundances were determined based on the relationship between the area of each integrated chromatographic pick and the total area of all existent and identified chromatographic peaks (via the NIST database) in a processed signal.

Phytol was identified as one of the most abundant secondary metabolite in both

Golden Delicious and *Jonathan* leaf extracts. Phytol is a secondary metabolite obtained during chlorophylls degradation. It plays an important role in tocopherol synthesis, which is one of the most abundant chloroplast liposoluble antioxidant groups. Analysis of the entire data-base generated by the present study allowed us suggesting the idea about the existence of different metabolic processes in the investigated trees compartments, in full agreement with information from the literature. Phytol is a widespread diterpenoid compound, and recent studies have shown that phytol can act as an excellent immunostimulant with antioxidant, anti-inflammatory and anti-allergic properties (13). Moreover, phytol exhibits also antimicrobial activity against *Mycobacterium tuberculosis* (14) and *Staphylococcus aureus* (15).

α-Tocopherol known also as vitamin E is another important secondary metabolite identified in leaves but not in bark extracts. Vitamin E is the name of all known varieties, referring to a group of compounds exhibiting biological activity, and an example of such a compound is α-tocopherol. It is an active chemical ingredient with one of the highest biological activity in most biological systems. However, the results of recent research studies indicate that there are other vitamin E compounds that can be even more efficient in specific biological mechanisms. Due to its solubility in fat, α-tocopherol has a unique function of protecting cell membranes made of fatty acids against damage by the action of Reactive Oxygen Species (ROS) and is also involved in the photosynthesis process (16).

Another important metabolite identified in leaf and bark extracts of pesticides non-exposed and exposed *Golden Delicious* and *Jonathan* matrices is p-vinyl guaiacol (PVG). It is an important metabolite generated during ferulic acid degradation and is an abundant phytochemical compound found in plant cell wall compartments. The presence of p-vinylguaiacol in the investigated matrices could suggest its potential importance as a phenolic compound existent in various plants' compartments usually known for their high content of antioxidant potential components (17).

In pesticides non-exposed leaves and bark methanol extracts from *Golden Delicious* and *Jonathan* apple varieties, compounds of pharmacological interest, i.e. lupeol, friedelin, α-tocopherol/vitamin E, methoxyphenols, mellein, have also been identified. Introspection of the recorded chromatograms revealed that good separation resolution was achieved for most identified compounds. Although now the information is only of a qualitative nature, the results can then be exploited in establishing strategies for appropriate scientific approaches to elucidate aspects related to the role and implications induced by the presence of pharmacologically relevant metabolites in the investigated matrices.

Lupeol is another secondary metabolite identified in *Golden Delicious* (fig. 1.b) and *Jonathan* (fig. 2.b) bark. It is a triterpene known mainly as fagarsterol and is a compound for which there are suggestions that it would have beneficial effects as a therapeutic and preventive agent for various diseases. Over the past 15 years, much improvement has been made regarding development of this very important molecule for clinical use in the treatment of various disorders. Various studies, focusing on investigating lupeol's action mechanism or its anti-inflammatory potential, have also proven that effective therapeutic doses do not present toxicity to normal cells and

tissues (18). In a research study on lupeol's ester effects it has been shown that this compound posess higher anti-inflammatory properties than indomethacin, with stronger effects in reducing arthritis foot inflammation in rats (19).

In *Golden Delicious* (fig. 1.b) and *Jonathan* (fig. 2.b) bark matrices friedelin was another important secondary metabolite identified. The results of various research studies reported in the literature have indicated that friedelin is very efficient in the treatment of inflammatory diseases, and that it can act as a potential drug with analgesic and antipyretic effect. Synthesis process inhibition and inhibition in the release of inflammatory mediators are considered as important action mechanism for this compound. Due to friedelin's remarkable biological activity presently is strongly believed that it would be imperiously necessary to carry out further research to develop a drug containing this active ingredient (20). In another research study it has been shown that friedelin has also antidiarrheal activity due to its anti-secretory and intestinal anti-motility properties (21).

In the methanol extract of *Golden Delicious* bark (Fig. 1.b) mellein appears as another identified compound. It is an active compound assigned as hepatitis C inhibitor. Mellein compounds are well-known dihydroisocoumarins that are widely distributed in fungi. According to the details in Pontius et al., 2008 (22), mellein was firstly described as a metabolite from *Aspergillus melleus* in 1933. The *Aspergillus ochraceus* extract was found to inhibit the final polyprotein processing step in the hepatitis C virus replication time interval and this fractional bioassay process resulted in the isolation of mellein as an active component of the extract (23).

Vitamin E was identified in *Golden Delicious* (fig. 1.a) and *Jonathan* (fig. 2.a) leaves extracts. Vitamin E antioxidant properties are well known, but in recent years the research interests switch to the potential of this vitamin to be used in pediatrics. It seems that vitamin E supplementation during pregnancy is required especially for α-tocopherol concentration in the colostrum (24) while after a high-risk pregnancy it would be necessary (25).

Analysis of the chromatograms presented in Figure 3.a,b and Figure 4.a,b, respectively, presenting TIC signals of *Golden Delicious* and *Jonathan* methanol (MeOH) leave (fig. 3.a and fig. 4.a) and bark (fig. 3.b and fig. 4.b) extracts clearly revealed the presence of the pesticides of interest in the investigated matrices. Use of an optimized analysis method for the investigation of the matrices of interest by the GC-MS technique has resulted in the identification of tebuconazole at a retention time of 13.75 minutes. In the chromatograms associated with pesticide exposed matrices it was also possible to detect other pesticides present in the in-use clearly stated combinational pesticides formulations. In any case, while for the chromatographic peak associated with tebuconazole the analytical aspects were very clear, it seems that for other compounds the method should be optimized.

Analysis of the chromatographic details specific for *Golden Delicious* bark extracts in pesticide non-exposed and exposed matrices (fig. 1.b and fig. 3.b) allowed us to identify the fact that peak intensity associated with mellein (fungus) gradually diminished after pesticides treatments spread. It is believed that such behavior clearly underlines the potential aggressive effect of treatment components on various promising phytochemical constituent existent in

plant compartments.

CONCLUSIONS

In our study methanol extracts from *Golden Delicious* and *Jonathan* leave, and bark matrices collected from an orchard in Iasi, northeastern Romania were obtained, and a systematic investigation of the existing secondary metabolites was conducted for the first time. Compounds of pharmacological interest, i.e. lupeol, friedelin, α-tocopherol/vitamin E, mellein, have been identified with output creating premises for subsequent scientific approaches in the topic of interest. In the pesticide exposed matrices subjected to GC-MS analyses, there was clear evidence of the presence of tebuconazole and other pesticides in the in-use clearly stated combinational pesticides formulations, containing trifloxistrobin, deltamethrin, fluopiram and spirodiclofen. By monitoring the behavior of mellein metabolite (fungus), mainly existent in the bark of *Golden Delicious* variety, it was possible to highlight the potential aggressive action of the in-use clearly stated combinational pesticides formulations on promising phytochemical constituent existent in various plant compartments.

ACKNOWLEDGEMENTS

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DESCRIPTIVE STUDY ON ACUTE GASTROENTERITIS IN 3,883 PATIENTS ADMITTED IN "SF. MARIA" CHILDREN HOSPITAL FROM IASI, ROMANIA, BETWEEN 2008 AND 2013

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DESCRIPTIVE STUDY ON ACUTE GASTROENTERITIS IN 3883 PATIENTS ADMITTED IN "SF. MARIA" CHILDREN HOSPITAL FROM IASI, ROMANIA, BETWEEN 2008 AND 2013 (Abstract): The **aim** of study was to assess some population characteristics in children of acute gastroenteritis (AGE) admitted in a regional pediatric hospital. **Material and methods**: For the present transversal descriptive study we considered a group of subjects hospitalized for AGE in 2008-2013 (2013 – only 6 months) in "Sf. Maria" Emergency Hospital for Children Iasi, Romania. Consultations in the Emergency Unit of this hospital did not take into consideration. **Results**: Number of AGE cases admitted to “St. Maria” Emergency Hospital for Children Iasi between 2008 and 2013 has seen a steady decline. Male subjects are better represented (54.1%); largest age group 1-4 years, with 1,827 cases, immediately followed by group 0-1 year (1789 cases); together, the two age groups account for 93.1% of admissions for AGE. Distribution of cases by seasons showed balanced values, with higher percentages in summer (29.7%) and spring (25.5%). The number of cases is relatively constant and lower in winter, affecting predominantly younger groups (0-5 years) compared to the summer when cases aged 5 to 14 years were admitted more frequent. Most cases presented at admission vomiting (41.2%) and fever (40.7%); the presence of bacterial flora was identified in 82.2% of stool specimens, and blood in 13.4% of cases. **Conclusions**: A higher number of education measures should be implemented in urban and rural areas in order to decrease the contamination with pathogenic agents involved in infectious acute gastroenteritis in children. **Keywords:** ACUTE GASTROENTERITIS, PEDIATRICS, DIARRHEA, EPIDEMIOLOGY

Acute diarrheal disease (acute gastroenteritis - AGE) has a high prevalence worldwide and the information came as a result of epidemiological surveillance are of great practical importance. In order to intervene appropriately in situations affecting public health, surveillance systems need to provide correct and complete data about AGE often under diagnosed and underreported, particularly parasitic and viral etiologies (1, 2).

“Sf. Maria” Emergency Hospital for Children Iasi is the largest children's hospital in Moldova, assisting the Iasi County

and the other seven districts of Moldova, Romania. The number of children hospitalized annually range from 28,680 in 2012 to almost 34,000 (2009) of which 5.2% are cases with a diagnosis of AGE (primary or secondary).

MATERIAL AND METHODS

For the present transversal descriptive study we considered a group of subjects hospitalized for AGE in 2008-2013 (2013 – only 6 months) in "Sf. Maria" Emergency Hospital for Children Iasi, Romania. Consultations in the Emergency Unit of this hospital did not take into consideration.

The criterion for inclusion in the study was the main diagnosis of AGE at discharge, so that only 3,883 cases (2.2% of all admissions) were selected from all cases of AGE recorded between 2008-2013 (8,986 cases - 5.2% of all admissions).

The following variables on the characteristics were studied: distribution by years, gender, age group, area of residence, number of hospitalization days, by seasons, and some clinical aspects.

Database was performed in MS-Excel 2010 software. Statistical analysis was performed with *SPSS 20.00*. For comparison, we used the χ^2 test (chi-square). The value of p (significance level) which reflects the probability that the difference between the analyzed variables are random, was considered greater than 0.05.

RESULTS

Of the total admissions between 2008 and 2013 (174,490 cases), 8986 cases (5.2%) were diagnosed with AGE, and 3,883 of those (2.2% of all hospitalized patients) were selected for our study, suffering from AGE as main diagnosis.

Number of cases with AGE, admitted to "Sf. Maria" Emergency Hospital for Children Iasi has seen a steady decline, higher in 2010, 2011, 2012 (from 1057 and 1016 cases in 2008 and 2009 to 672 in 2010, 571 in 2011, and 448 in 2012) (fig. 1).

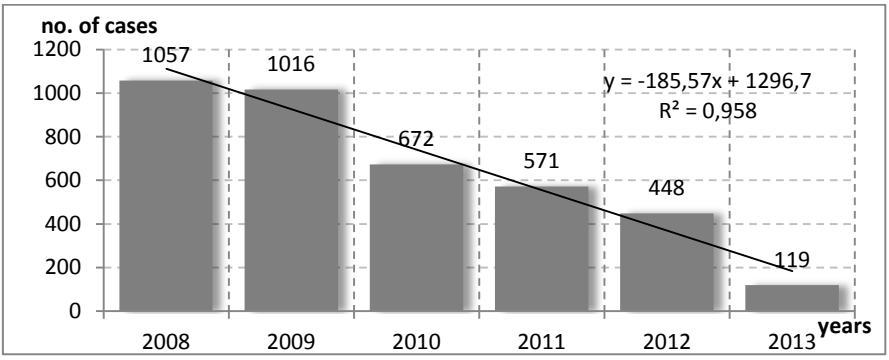

Fig. 1 Distribution by years

Distribution of cases by sex has a higher value in males (54.1%), and the best represented age group was 1-4 years old, with 1827 cases, immediately followed by group 0-1 year (1789 cases); together, the two age groups accounted for 93.1% of admissions for AGE. The mean age of the entire group of 3383 children was 1.29 years. In males and children from rural areas, the mean age is below that of the entire group of study (1.26 or 0.99) (tab. I).

TABLE I
Mean age of cases by sex and residence area

Age	Total of group	Comparative values – by sex		Comparative values – by residence area	
		Female	Male	Rural	Urban
Mean	**1.29**	**1.32**	**1.26**	**0.99**	**1.50**
Standard error of the mean	0.036	0.055	0.047	0.054	0.047
Standard deviation	2.226	2.308	2.154	2.148	2.256
Minimum	0	0	0	0	0
Maximum	14	14	14	14	14

In the 0-1 age group, most cases were recorded in 2008 then the frequency of admissions decreased, reaching 56 cases in 2013. From group 1-4 years, 5-9 and 10-14, most children were admitted in 2009 (517, 53 and 25 cases, respectively) and the fewest in 2013 (49, 7, and 7 cases, respectively) (fig. 2).

Distribution by age and sex showed that only in the 10-14 age group, the girls were sick more often than boys (55.7% *vs* 44.3%; in the other age group, AGE affected predominantly males, but statistically not significant (p=0.071) (fig. 3).

Most children with AGE were from urban areas, regardless of age, this distribution was statistically significant (p <0.0001) (fig. 4).

Distribution of AGE cases by seasons was relatively uniform, only in summer, there were more cases (29.7% of total), with a cumulative maximum in July (403 cases) and a minimum of 250 cases in November, with a moderate statistical significance (p=0.038) (tab. II).

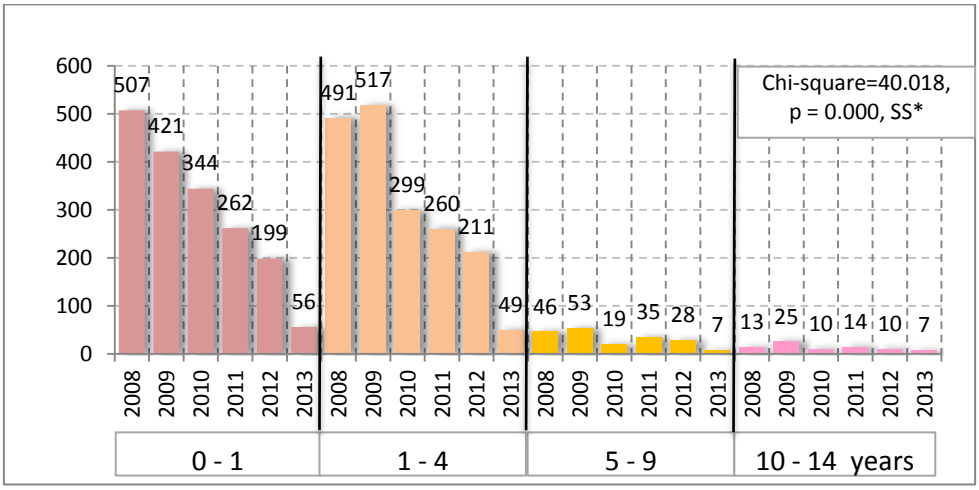

Fig. 2 Distribution of cases by age group and year of study. *SS – statistically significant

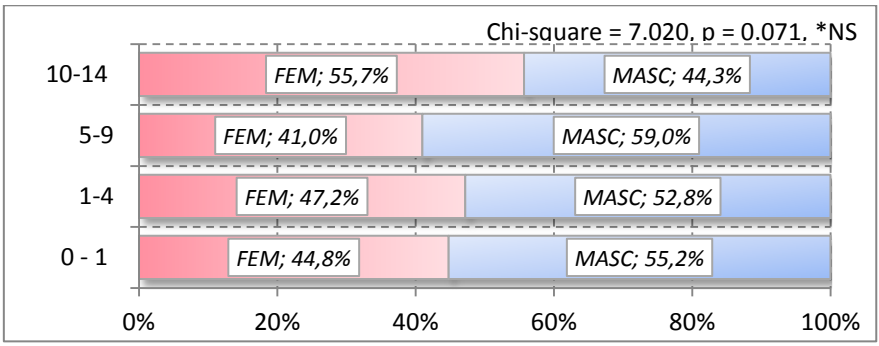

Fig. 3 Distribution of cases by age group and sex . *NS – statistically not significant

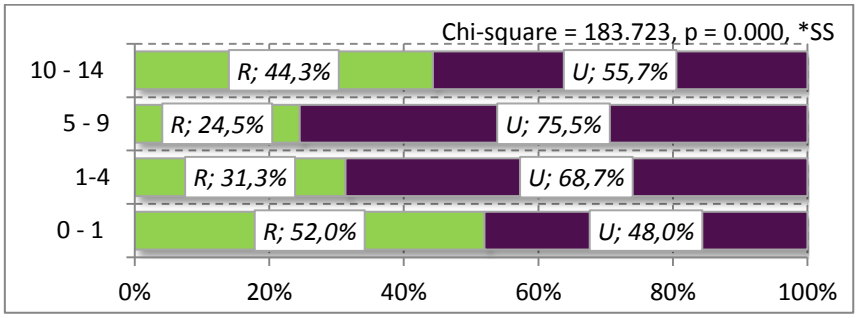

Fig. 4 Distribution of cases by age group and residence area. *SS - statistically significant

TABLE II

Test chi-square – distribution of age group and seasons

Chi-Square Tests			
	Value	df	Asymp. Sig.(2-sided)
Pearson Chi-Square	48.768[a]	33	.038, *SS
Likelihood Ratio	49.480	33	.033
Linear-by-Linear Association	9.553	1	.002
N of Valid Cases	3883		

a. Test distribution is normal/*SS - statistically significant

In all age groups, the fewer cases were recorded in the winter, and most of the summer, except for children 10-15 years old, who presented more AGE admissions in fall season (31.6%) than in summer (24.1%), that situation might be due to start school in September (fig. 5).

The reported frequency of clinical signs at admission (other than diarrhea which was the criterion for inclusion) was: vomiting (41.2%), fever (40.7%), loss of appetite (17.1%), and abdominal pain (9.9%). Results of cytograms from stool specimens on admission showed bacterial flora present in 82.2% of cases, the mucus in 48.8%, PMN leukocytes in 27.7%, and enterocytes in 14.6%. 13.4% of samples were positive for blood, and this was highly significant statistic in the age group 0-1 years and 1-4 years (tab. III).

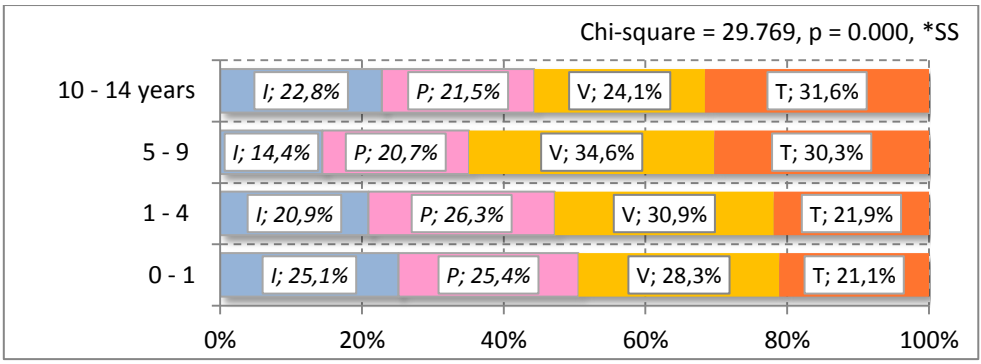

Fig. 5 Distribution of cases by age group and season.
*SS - statistically significant

TABLE III

Distribution of cases by age group and the presence of blood in stool specimens (cross tabulation)

BLOOD		Age group (years)				Total
		0 - 1	1 - 4	5 - 9	10 - 14	
YES	N	284	225	9	3	**521**
	% row	54.5	43.2	1.7	0.6	**100.0**
	% column	15.9	12.3	4.8	3.8	**13.4**
NO	N	1505	1602	179	76	**3362**
	% row	44.8	47.7	5.3	2.3	**100.0**
	% column	84.1	87.7	95.2	96.2	**86.6**
TOTAL	N	1789	1827	188	79	**3883**
	% row	46.1	47.1	4.8	2.0	**100.0**
	% column	100.0	100.0	100.0	100.0	**100.0**

Number of hospitalization days had a mean of 5.04 for children of 0-1 age group, with a maximum of 30 and a minumum of 1 day. The mean was 3.92 days in cases from 1-4 age group, 3.17 in cases of 5-9 years old and 3.41 in 10-14 age group, being statistically significant ($p<0.0001$) at Kruskal-Wallis test and Kolmogorov-Smirnov test (tab. IV, fig. 6).

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	29.556[a]	3	0.000, *SS
Likelihood Ratio	34.723	3	0.000
N of Valid Cases	3883		

a. Test distribution is normal / * SS – statistically significant

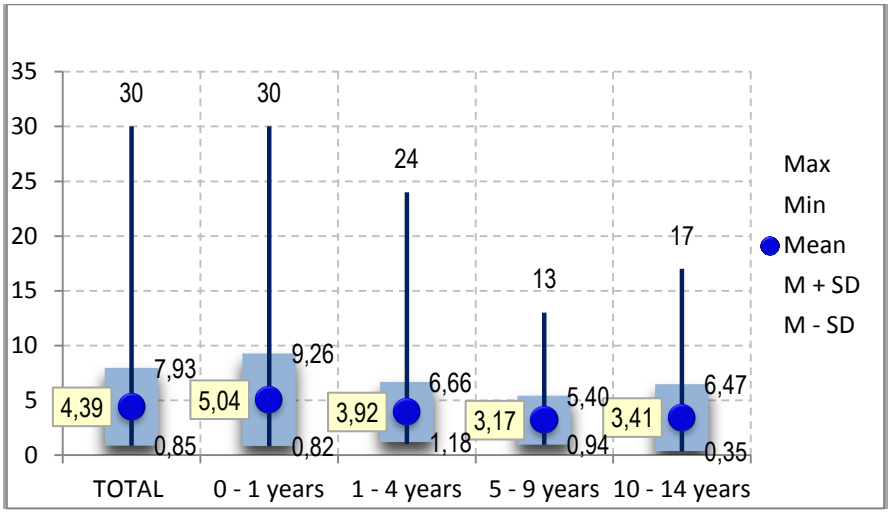

Fig. 6 Distribution of cases by age group and number of hospitalization days

TABLE IV

Distribution of cases by age group and number of hospitalization days.

*** SS – statistically significant**

No. of hospitalization days Kruskal-Wallis test Chi-square = 87.097 p = 0.000 *SS	**Total**	**Comparative values by age groups (years)**			
		0-1	**1-4**	**5-9**	**10-14**
Mean	4.39	5.04	3.92	3.17	3.41
Standard error of mean	0.057	0.100	0.064	.163	0.344
Standard deviation	3.544	4.222	2.739	2.233	3.057
Minimum	1	1	1	1	1
Maximum	30	30	24	13	17

One-Sample Kolmogorov-Smirnov Test		
		No. of hospitalization days
N		3,883
Normal Parameters[a,b]	Mean	4.39
	Std. Deviation	3.544
Most Extreme Differences	Absolute	0.169
	Positive	0.152
	Negative	-0.169
Kolmogorov-Smirnov Z		10.560
Asymp. Sig. (2-tailed)		0.000
a. Test distribution is Normal. b. Calculated from data.		

DISCUSSION

Acute gastroenteritis is a common cause of significant morbidity and mortality worldwide, despite the successful introduction of oral rehydration solutions in the 1980s (3, 4). In financial developed regions, AGE is rarely fatal, but in others, more than 1.5 million children die annually. In Europe, it was estimated that 16% of children presentation in emergency rooms are for AGE with any etiology, so that AGE becomes the second most common cause of hospital admission after acute respiratory tract infections (3, 5). AGE accounted for 7-10% of all admissions in children under 5 years old in the developed countries (3).

Wiegering V. et al, in a study conducted in a University Hospital (Würzburg) reported a different number of hospitalization days according to etiologic spectrum, from 4.9 +/- 0.1 days for viral etiology at 5.7 +/- 0.4, respectively bacterial etiology *Salmonella* infection (6). Clinical signs with statistical significance in viral and bacterial AGE were bloody diarrhea and fever in a study conducted over a period of 14 years in Spain, by Alcalde et al. (7).

CONCLUSIONS

Number of AGE cases admitted to "Sf. Maria" Emergency Hospital for Children Iasi between 2008 and 2013 has seen a steady decline. Male subjects are better represented (54.1%); largest age group 1-4 years old, with 1,827 cases, immediately followed by group 0-1 year (1,789 cases); together, the two age groups account for 93.1% of admissions for AGE. In fact, studies of AGE in children could be performed just by the 0-5 age group, but in order to assess the number of hospitalizations and their frequency should be considered the whole group of 0-14. The mean age of the entire group of 3,383 children is 1.29 years, which reflects a concentration of cases in younger age groups.

Distribution of cases by seasons showed balanced values, with higher percentages in summer (29.7%) and spring (25.5%). The number of cases is relatively constant and lower in winter, affecting predominantly younger groups (0-5 years) compared to the summer when cases aged 5 to 14 years were admitted more frequent.

The study was considered only cases with acute diarrhea on admission. Most cases presented at admission vomiting (41.2%) and fever (40.7%).

The presence of bacterial flora was identified in 82.2% of stool specimens, and blood in 13.4% of cases.

Bacterial etiology can be suspected in most cases.

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NEWS

IN VITRO EVALUATION OF ANTIBACTERIAL EFFICACY OF PINEAPPLE EXTRACT (BROMELAIN) ON PERIODONTAL PATHOGENS

Periodontitis represents an inflammatory disease resulting in the destruction of periodontal tissues. Various treatment modalities (mechanical and surgical therapy) have been tried. Antimicrobial agents have been used as a monotherapy and as an adjunct with mechanical debridement. As antibacterial agents have been used different plant extracts. Pineapple extract (Bromelian) is one such agent. The aim of the study was to assess the antibacterial efficacy of Bromelain on aerobic and anaerobic periodontal microorganisms. Minimum inhibitory concentration (MIC) of Bromelain was tested on isolated strains of *Streptococcus mutans*, *Enterococcus fecalis, Aggregatibacter actinomycetemcomitans* (Aa), and *Porphyromonas gingivalis* (Pg) using serial dilution broth method. The results of the study showed that *S. mutans* showed sensitivity at the lowest concentration of 2 mg/ml as compared to *E fecalis* (31.25 mg/ml) while *P gingivalis* showed sensitivity at the lowest concentration of 4.15 mg/ml as compared to *Aggregatibacter actinomycetemcomitans* (16.6 mg/ml). In conclusion Bromelain exerts an antibacterial effect against potent periodontal pathogens; hence, it may be used as an antibacterial agent. To validate this result further trial has to be conducted. (Praveen NC, Rajesh A, Madan M, Chaurasia VR, Hiremath NV, Sharma AM. In vitro Evaluation of Antibacterial Efficacy of Pineapple Extract (Bromelain) on Periodontal Pathogens. *J Int Oral Health*, 2014; 6 (5): 96-98).

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CLINICAL AND EPIDEMIOLOGICAL ASSESSMENT CONCERNING HYBRID REVASCULARIZATION TECHNIQUES IN THE TREATMENT OF MULTILEVEL ARTERIAL OCCLUSIVE DISEASE

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CLINICAL AND EPIDEMIOLOGICAL ASSESSMENT CONCERNING HYBRID REVASCULARIZATION TECHNIQUES IN THE TREATMENT OF MULTILEVEL PERIPHERAL ARTERIAL OCCLUSIVE DISEASE (Abstract): **Aim** of the study was to report a novel hybrid technique for multilevel arterial lesions of the lower extremities and to evaluate the clinical outcomes. In patients with multilevel arterial disease, the combined (hybrid) treatment, consisting of endovascular intervention and classical surgical intervention on the same vascular axis seems to be the most indicated treatment in order to obtain an adequate inflow and outflow. **Material and methods**: we have performed a non-randomized study during a 44-month period (January 2010 – September 2013) in a number of 94 patients treated by hybrid revascularization techniques. All the patients included in the study have been post-surgically surveyed at well established intervals (1, 3, 6, 9, 12, 24 and 36 months) by: clinical examination, laboratory tests, Duplex ultrasound, and, as needed, CT or MR Angiography. **Results**: the 6 months primary patency in each studied group (corresponding to the years of 2010, 2011 and 2012) was 58.69%, 68.42%, and 62.06%, respectively; the 12 months primary patency was 45.65%, 57.89%, and 34.48%, respectively. Clinical improvement has been noticed in 83 patients (88.29%). There have been registered 19 amputations (20.21% of the cases): 11 majors (thigh and below the knee), representing 11.7% of the total number of cases and 8 minors (toe or transmetatarsal), representing 8.51% of the total number of cases. The amputation-free survival period ranged between 7 days and 24 months, with an average of 7.66 months. **Conclusions**: the hybrid techniques are a feasible option for the multilevel arterial disease, with favorable patency and limb salvage rates. **Keywords**: MULTILEVEL ARTERIAL DISEASE, HIBRID REVASCULARIZATION

Multilevel involvement is typically observed in peripheral arterial occlusive disease (PAOD). A staged approach to multilevel occlusive disease was the standard for numerous years, consisting of balloon angioplasty of the iliac artery followed by interval infrainguinal surgery (1, 2). This approach was a rational strategy at a time when open and endovascular surgical techniques were only performed in separate settings.

Multilevel revascularization, using a combination of endovascular and open techniques, was first reported in the early

1990s (3). Hybrid surgery is ideal for multilevel lesions, as it allows the application of minimally invasive treatment on complex anatomy in high-risk patients. Usually, the procedures are performed simultaneously, although individual patient anatomy plays a part in the decision of whether to perform the two procedures simultaneously or not. The aim of the treatment is to obtain an improvement of both the inflow and the outflow (4, 5).

The rate of hybrid interventions has progressively increased during the last decade and it is estimated to be around 5-20% of all vascular reconstructions (6).

In the hybrid technique, the endovascular stage can be an inflow procedure, an outflow procedure, a combination of the two, or a bypass revision.

The open techniques consisted of an autologous or synthetic bypass graft and/or endarterectomy.

MATERIAL AND METHODS

We have performed a non-randomized study in two centers. The data was gathered retrospectively for a 20-month period (January 2010 – September 2011), and prospectively for a 24-month period (September 2011 – September 2013) for a number of 94 patients treated by hybrid revascularization techniques at the Cardiovascular and Thoracic Surgery Clinic, "Imelda" Hospital, Bonheiden, Belgium.

The including criteria for the patients were: aged 18 and over, multilevel peripheral arterial disease in any of the Leriche-Fontaine stages, with uni- or bilateral pathology, and had an indication of hybrid revascularization by both endovascular therapy (either balloon angioplasty, stenting or endoprosthesis insertion), and an open technique (bypass or endarterectomy, with or without angioplasty). The study included 94 patients (female 35.1%, male 64.9%, mean age 70,68 years).

The patients were divided into two groups: 1 – included patients who had the hybrid techniques done in one procedure; 2 – included patients treated in two succesive procedures.

The second group was divided into two sub-groups of patients: one for which the open revascularization technique was done first, followed by an endovascular procedure; and the second, for which the initial procedure was the endovascular one, followed by the open technique.

Peripheral arterial disease was localized at a minimum of two levels on the same vascular axis: aortic, iliac, inguinal (involving the common, deep femoral artery and the origin of the superficial femoral artery), or infrainguinal (supra- or infragenicular).

All patients were pre-operatively evaluated by physical examination, blood tests, Duplex scan, CT or MR Angiography. Also, the risk factors and associated co morbidities were noted.

Postoperative surveillance at well established time intervals (1, 3, 6, 9, 12, 24 and 36 months), consisted of: blood tests and Duplex scans, clinical examination (improvement of the claudication distance, amelioration of rest pain, healing of trophic lesions).

We evaluated the primary patency, assisted primary patency and secondary patency of all procedures, initial technical success, complication rate, morbidity and mortality associated with each technique, amputation-free survival rate, symptomatic improvement, and the risk factors that influenced the postoperative outcome and recovery period.

The *statistic analysis* and *epidemiologi-*

cal data were performed in *SPSS 15.0* (SPSS Inc., Chicago, IL, USA). A p value of less than 0.005 was considered statistically significant.

During the study, the primary and secondary patency were analyzed using the Kaplan-Meier estimator.

RESULTS

Thirty-three patients (35.10%) were revascularized by simultaneous hybrid procedures and 61 patients (64.89%) by succesive hybrid procedures: the open technique of 42 patients (44.68%) was followed by an endovascular procedure and in 19 patients (20.21%) the endovascular technique was followed by an open procedure (fig. 1).

The majority of patients treated by simultaneous hybrid procedures were classified as stage II Leriche-Fontaine (fig. 2).

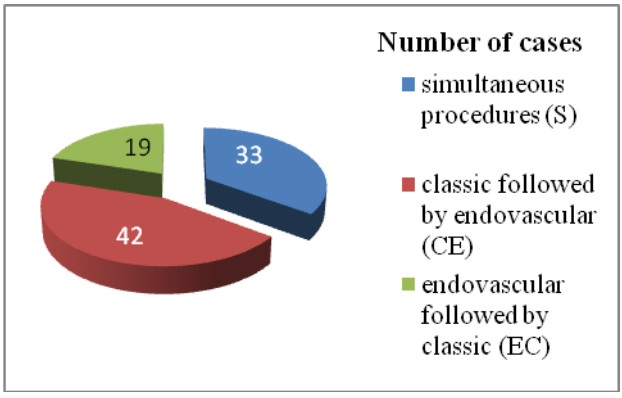

Fig. 1. The distribution of cases according to the revascularization procedure sequence

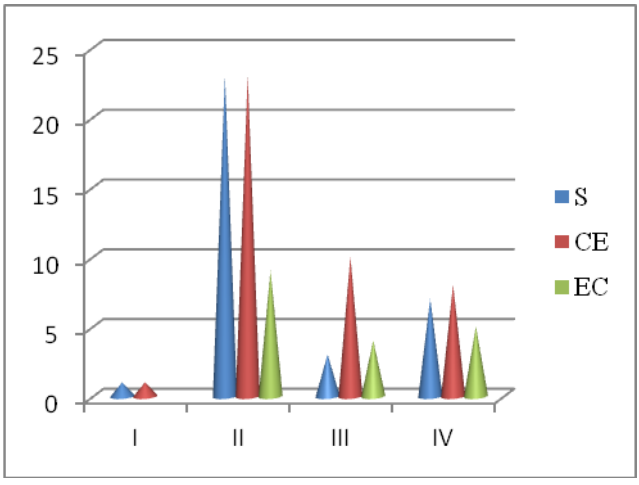

Fig. 2. The distribution of hybrid procedures according to the disease stage.

For the studied period of time, there were 49 bypass procedures performed, 37 common femoral artery endarterectomies and angioplasties, 23 desobstructions using the Fogarty catheter, 97 thrombolysis procedures, 164 endovascular angioplasties

and 100 endovascular angioplasties followed by stenting.

Elective procedures were performed for 61.7% of the patients and emergency procedures for the rest of 38.3% of the patients, with acute or subacute ischemia. Emergency procedures consisted of thrombolysis, bypass, or native artery thrombectomies, with or without a bypass or supra/subjacent percutaneous angioplasties (PTA) on the same arterial axis.

The initial technical success for all procedures was 100%.

We had 19 early artery or bypass occlusions (less than 30 days) and one case with a postoperative local haematoma which required a reintervention for haemostasis and evacuation.

We had one severe graft infection (for which a myoplasty was performed, with favorable postoperative recovery) and 78 late occlusions (after more than 30 days), which entailed follow-up procedures for reestablishing the patency.

We had 7 intra-stent stenoses at the level of the superficial femoral artery, which were solved by angioplasty and restenting or femoro-popliteal bypass.

There were19 (20.21%) amputations recorded :11 majors (thigh or below-knee), representing a rate of 11.7% of the total number of cases and 8 minors (toe or transmetatarsal), representing a rate of 8.51% of all cases. The major amputations were 10 unilateral and one bilateral (successive). Limb salvage rate for the patients in stage III and IV Leriche-Fontaine was 73.17%. Clinical improvements was noticed in 83 (88.29%) patients. After six months primary patency in each studied group (corresponding to the years of 2010, 2011, and 2012) was 58.69%, 68.42%, and 62.06% respectively, comparable with those published in the literature. After twelve months primary patency was 45.65% in the 2010 studied group, 57.89% in the 2011 group of patients, and 34.48% for the 2012 group (fig. 3, 4, 5).

We did not find statistically significant differences between the averages of the primary patency of various disease stages. The mortality rate for the entire period studied was 0% .

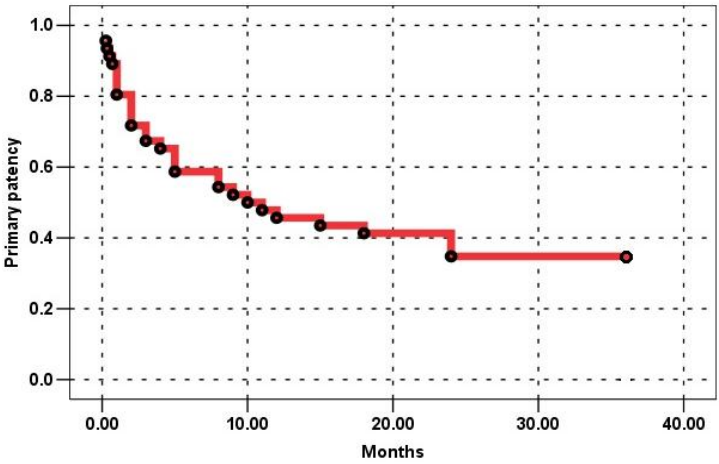

Fig. 3. The Kaplan-Meyer diagram of primary patency for the 2010 patient group

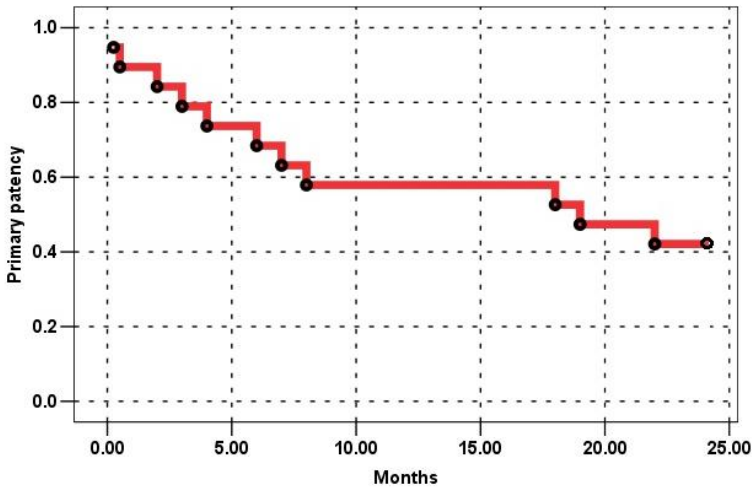

Fig. 4. The Kaplan-Meyer diagram of primary patency for the 2011 patient group

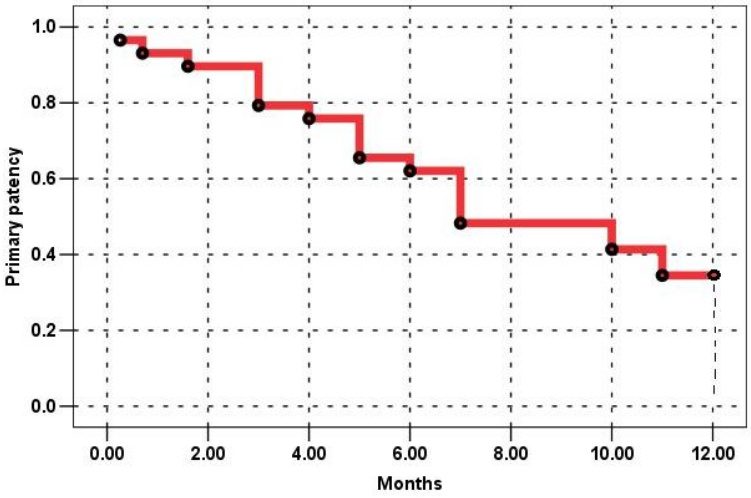

Fig. 5. The Kaplan-Meyer diagram of primary patency for the 2012 patient group

In regards to the influence of the hybrid procedure type on the primary patency (PP), we found that in the case of succesive endovascular and open procedures, the average PP is significantly higher compared to the PP of open technique followed by an endovascular procedure (Fig. 6).

The statistic analysis identified the only risk factors which significantly influenced the primary patency in the studied group: *obesity* and *renal insufficiency*.

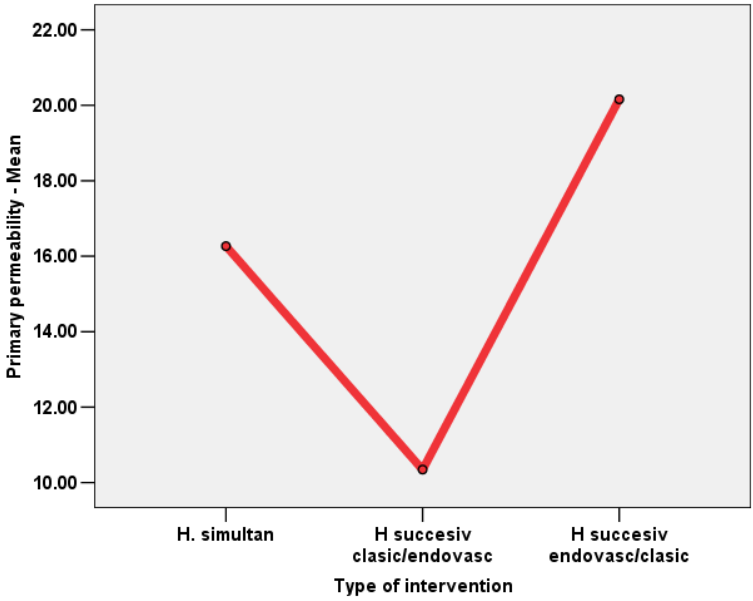

Fig. 6. The influence of the type of hybrid procedure on the primary patency

DISCUSSION

Implementing hybrid techniques in the vascular surgery practice and the possibility of performing them in hybrid operating rooms offer therapeutic options that come to meet the needs of the patients with multilevel lesions and high surgical risk.

For the cases treated in a simultaneous hybrid manner, the main role was played by the common femoral artery (CFA), and its suitability for supra- or subjacent endovascular angioplasty. Endarterectomy, with or without patch angioplasty has been performed on this artery in 28 cases.

The most common endovascular procedure user was baloon angioplasty followed by stenting. Combined open and endovascular revascularization may be performed with either a staged or simultaneous approach. Endovascular surgery has been enthusiastically embraced by a large number of vascular surgeons, leading to increased experience with these types of procedures. In numerous hospitals, hybrid operating rooms have been constructed, making it possible to use both open and endovascular techniques. It is both easy and comfortable to perform simultaneous hybrid surgery in this environment. The hybrid approach has several advantages (7). Firstly, there is no delay in providing complete revascularization to the ischemic limb. Secondly, the length of hospital stay is reduced and finally, puncture-site complications are eliminated since the target artery is accessed through the surgical field and the access site becomes the location of proximal anastomosis.

Cotroneo *et al* (8) reported on 2-year results of hybrid revascularization. The technical success rate was 100% whilst the primary patency rate was 86.2% at 6 months and 79.1% at 24 months. Hybrid surgery has a theoretical advantage com-

pared with open or endovascular revascularization performed separately, in terms of patency rate. With hybrid surgery, inflow or outflow arteries may be revascularized, which may affect the patency rate.

Antoniou *et al* (9) also reported on 3-year results of hybrid revascularization. The technical success rate was 100%. Primary patency rate was 71%.

The annual number of hybrid procedures is increasing. Aho and Venermo (10) reported that this number ranges between 4, in 2004, and 73, in 2011. Out of this total number of hybrid procedures, the proportion performed by vascular surgeons increased from 0% in 2004 to 86.3% in 2011. Relatively young vascular surgeons have adopted endovascular surgery and became familiar with it. According to a survey conducted by the Society for Vascular Surgery (11), younger vascular surgeons (aged <50 years) frequently reported that >50% of their workload is endovascular, compared with older vascular surgeons (aged ≥50 years) ($P<0.001$). The endovascular skills of vascular surgeons have improved through education and simulator-based endovascular skills training (12, 13, 14, 15).

CONCLUSIONS

Hybrid interventions have become a well-accepted strategy for revascularization in patients with critical limb ischemia due to multilevel arterial occlusive disease. Technical success and short- and long-term limb salvage outcomes of hybrid interventions have been shown to be at least comparable to the conventional endovascular and surgical revascularization procedures. Hybrid revascularization offers the efficiency and convenience of a single-stage therapy without added risks for those with lower extremity ischemia.

In our study hybrid procedures are a feasible option for multilevel peripheral arterial occlusive disease, with favorable patency and limb salvage rates. The observations of the current study indicate that femoral endarterectomy plays an important role in hybrid surgery.

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NEWS

ROLE OF OSTEOPROTEGERIN CGA HAPLOTYPE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Osteoprotegerin (OPG) has been associated with increased risk of atherosclerotic disease in the general population and have been described several polymorphisms in the OPG gene with functional effects on cardiovascular disease in non-rheumatic individuals. A group of researchers from Spain aimed to analyze the effect of three of functional OPG polymorphisms on the risk of cardiovascular disease in a large and well-characterized cohort of patients with rheumatoid arthritis. Three OPG gene variants (rs3134063, rs2073618 and rs3134069) were genotyped by TaqMan assays in 2027 patients with rheumatoid arthritis. Anti-cyclic citrullinated peptide (anti-CCP) antibody testing was positive in 997 of 1714 tested. Also, 18.3% of the whole series had experienced cardiovascular events, including 5.4% with cerebrovascular accidents. The relationship between OPG variants and cardiovascular events was assessed using Cox regression.No association between OPG gene variants and cardiovascular disease was observed in the whole group of rheumatoid arthritis patients or in anti-CCP positive patients. Nevertheless, a protective effect of CGA haplotype on the risk of cardiovascular disease in general, and specifically in the risk of cerebrovascular complications after adjusting for sex, age at disease diagnosis and traditional cardiovascular risk factors was disclosed in anti-CCP negative patients. They concluded that their results indicated a protective effect of the OPG CGA haplotype on cardiovascular risk, mainly due to a protective effect against cerebrovascular events in anti-CCP negative rheumatoid arthritis patients. (Genre F, López-Mejías R, García-Bermúdez M et al. Osteoprotegerin CGA Haplotype Protection against Cerebrovascular Complications in Anti-CCP Negative Patients with Rheumatoid Arthritis. *PLoS One.* 2014 Sep 3; PMID:25184828).

Simona Tucaliuc

EPIDEMIOLOGICAL AND LABORATORY FEATURES OF CHRONIC HEPATITIS B CASES IN THE INTERVAL 2010-2013

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EPIDEMIOLOGICAL AND LABORATORY FEATURES OF CHRONIC HEPATITIS B CASES IN THE INTERVAL 2010-2013 (Abstract): HBV virus infection is an important public health problem because of its huge transmission potential, and severe evolution to cirrhosis or liver cancer. **Aim**: Analysis of the epidemiological and laboratory features of chronic hepatitis B virus infection. **Material and methods**: The patients with chronic hepatitis B admitted to the "Sf. Parascheva" University Hospital for Infectious Diseases in the interval: January 1st, 2010 – December 31st were analyzed. **Results**: Patients age was 18 to 66 years with a prevalence of middle-aged males. Most patients came from urban areas. Alanine aminotransferase (ALAT) levels were elevated, without significant differences between HBeAg-positive and HBeAg-negative patients, the elevated ALAT levels being associated with the increased prevalence of fibrosis. HBeAg-positive patients had viral loads above the threshold of 2,000/l in 34 cases (89.5%), and below 2,000 IU/l in only 10.5% of cases, and the majority (88%) of HBeAg-negative patients presented high viral load levels. The prevalence of stage F2-F4 liver fibrosis was 63.4% in the HBeAg-negative patients with viremia > 25,000 IU/l compared to 55.2% in the HBeAg-positive patients. The correlation between the level of viral load and fibrosis shows that there are significant differences between viremia and the status of HBeAg-positive or negative patients. Increased viral load was correlated with increasing prevalence of fibrosis, significant in HBeAg-negative patients, and the increasing fibrosis prevalence was correlated with low viral load. **Conclusions:** The correlation between viral load and fibrosis shows that there are significant differences between viral load and the status of HBAg-positive or negative patients. **Keywords**: VIRAL LOAD, FIBROSIS, SEROLOGICAL MARKERS

Infection with hepatitis B virus (HBV) is a major public health problem due to the great number of people affected nationally and worldwide. Although in most adults acute symptomatic hepatitis B virus infection is clinically curable, the factors contributing to infection persistence and chronic evolution are not fully known yet. The current importance of acute hepatitis caused by HBV is due to its worldwide spread, high potential of chronicization, especially in newborns, and slow, possibly severe progression to cirrhosis or hepatocellular carcinoma; despite vaccination, the

disease is the 10th leading cause of death worldwide. This paper presents the results of an analysis of epidemiological and laboratory features of chronic hepatitis B cases admitted to the Iasi "Sf. Parascheva" University Hospital for Infectious Diseases during a four year interval, 2010-2013.

MATERIAL AND METHODS

The medical records of 401 adult patients (over 18 years) diagnosed with chronic hepatitis B admitted to the Iasi "Sf. Parascheva" University Hospital for Infectious Diseases between January 1st, 2010 and December 31st, 2013 were reviewed in view of describing the demographic, epidemiologic and virologic characteristics. The following laboratory variables (blood counts, liver samples, HBV-DNA and viral load determinations) were analyzed and correlations were made between the serological and biochemical markers according to area of residence (urban/rural), age, and sex of patients. Serological tests were done to determine HBsAg, HBeAg, total anti-HBe, anti-HBe, and antibodies to HDV in view of detecting HBV-HDV co-infection, and quantitative virologic tests were used as a guide mark for assessing progression severity in relation with the fibrosis grade. The patients in which HBsAg was present for over 6 months, regardless of hepatocytolysis level and viral load were considered to have chronic infection. Statistical analysis data was performed with the help of *SPSS* software, using confidence intervals, statistical significance tests, variations, dispersion, and correlation.

Descriptive analysis of the cases showed that the annual number of chronic hepatitis B cases was 150 (37.40%) in 2010, 88 (21.94%) in 2011, and 93 (23.19%) in 2013, with a male predominance (64.83%). The mean age of the study group was 41.61 years (range 18-66 years), most patients belonging to the 51-60 (26.93%) and 31-40 (25.68%) age groups (fig. 1).

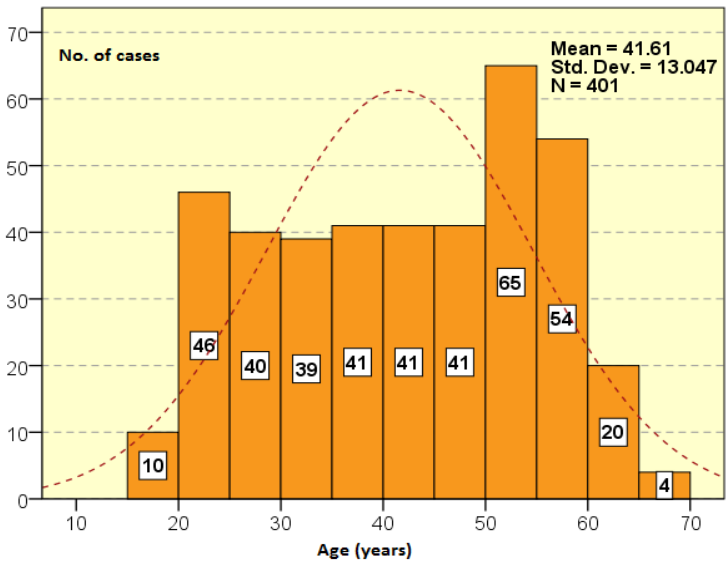

Fig. 1. Age distribution of study cases

The fewest cases were recorded among elderly and young adults, indicating the prevalence of middle-aged patients. Distribution by area of residence showed the prevalence of urban areas (284 cases - 70.8%); sex ratio was in favor of males, with 272 cases (64.83%). Serum transaminase levels ranged from normal to 520 IU, 35% of patients having ALAT levels within normal ranges. Transaminase levels were high, without significant differences between HBeAg-positive and HBeAg-negative patients (average 118 IU/l), slightly higher in HBeAg-positive patients. In HBeAg-positive cases, transaminase levels ranged from 75 to 290 IU/l, average 118.5 IU/l, with a standard deviation of 324.36, while in HBeAg-negative cases transaminase levels were lower (< 150 IU/l), standard deviation 184.23, but without significant statistical differences. Increased ALAT level was associated with increasing prevalence of fibrosis, in both HBeAg-positive and HBeAg-negative patients. Serological marker analysis showed that 38 patients were HBeAg-positive (9.5%) and 363 patients (90.5%) were HVeAg-negative. Of all patients, 87.57% had a viral load > 2000 IU/L. Thirty-four (89.5%) HBeAg-positive patients had viral loads above the threshold of 2000IU/l and only 4 (10.5%) below 2000 IU/l. Most HBeAg-negative patients (88%) presented high viral load, in only 12% being below 2,000 IU/L (tab. I).

The prevalence of HBeAg-negative patients with viral load > 25,000 IU/l was 59.3%, compared to 63.4% of those with viral load <25,000 IU/l. There was a relationship between HBeAg antigen status and fibrosis. The fibrosis grade was correlated with viral load value (tab. II).

Fibrosis stages F2-F4 was found in 19 (50%) HBeAg-positive patients and 215 (59.3%) HBeAg-negative patients.

The prevalence of F2-F4 fibrosis in HBeAg-negative patients with viral load > 25000IU/la was 63.4% compared to 55.8% in those with viral load below this level. The prevalence of F2-F4 fibrosis in HBeAg-positive patients with viral load > 25000IU/la was 55.2%, compared to 33.5% in those with viral load below this level.

To cover all data variations in the study groups we present a Box Plot chart (fig. 2)

TABLE I

Association AgHBe – Viral load / (2000IU/l)

HbeAg			Viremia IU (2,000)		Total
			< 2,000 No/%	2,000 + No/%	No/%
HBeAg	Positive	Count	4	34	38
		% within HBeAg	10.5%	89.5%	100.0%
	Negative	Count	43	315	358
		% within HBeAg	12.0%	88.0%	100.0%
Total		Count	47	349	396
		% within HBeAg	11.9%	88.1%	100.0%

TABLE II:
Prevalence of HBeAg in the studied group according with fibrosis grade

			Fibrosis					Total
			F0 No./%	F1 No./%	F2 No./%	F3 No./%	F4 No./%	No./%
HBeAg	Positive	Count	0	19	16	3	0	38
		% within HBeAg	0.0%	50.0%	42.1%	7.9%	0.0%	100.0%
	Negative	Count	6	142	131	67	17	363
		% within HBeAg	1.7%	39.1%	36.1%	18.5%	4.7%	100.0%
Total		Count	6	161	147	70	17	401
		% within HBeAg	1.5%	40.1%	36.7%	17.5%	4.2%	100.0%

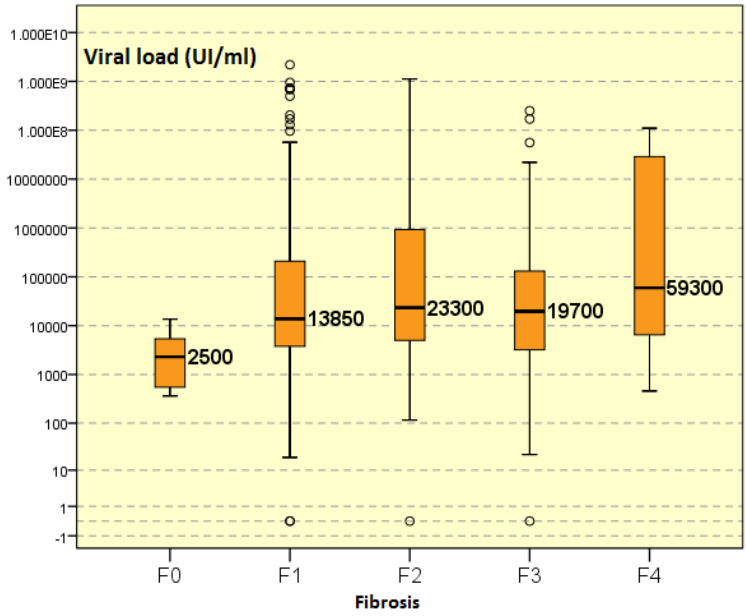

Fig. 2: Box Plot analysis for association between fibrosis and viral load.

We used the logarithmic scale because viral load had very wide variations. By Kruskal Wallis test we found statistically significant differences (p=0.027). Patients with advanced fibrosis (F4) had a significantly higher plasma viral load compared to those with early fibrosis (F0). Twenty-one patients (9.98%) had an associated hepatitis D virus infection. Of these, 17 (81%) had detectable HBV-DNA, and 9 of the (52.9%), viral load > 2000; in the remaining cases viral load was below this level. Fibrosis grade varied as follows: 9 patients (42.9%) had F1 fibrosis, 6 (28.7%) F2 fibrosis, 5 (23.8%) F3 fibrosis and one case (4.8%) F4 fibrosis. A small percentage of patients (4.98%) presented liver cirrhosis.

DISCUSSION

Previous studies (1) have demonstrated the presence in our area of Mediterranean strains characterized by the absence of HBeAg in parallel with the presence of HBV/DNA, which by the mutations in the pre-core region induce this particular profile. The presence of HBeAg in only 38 patients of our study group (9.5%), along with viral loads above the threshold of 25,000/l in 88% of the patients seem to provide evidence of this mutation which in the future needs to be certified by molecular biology studies and its genotype identified, because it is widely recognized that infections caused by genotype C are predictive for a more severe progression, with active replication and higher risk of progression to cirrhosis compared with genotype B (2, 3, 4). The prognosis of HBeAg-negative patients was better than that of HBeAg-positive patients (5).

Immune status at the time of acute infection influences the risk of chronicization. In people with impaired immune status (e.g., malignant diseases, Down syndrome, hemodialysis, transplant, HIV-positive) the risk of chronicization is significantly higher. Determination of viral load (HBV-DNA) was an important factor for assessing the stage of the disease and for monitoring the therapeutic response in chronic hepatitis B (6). Under similar conditions of exposure to HBV activity, the male sex shows a stronger tendency for HBV infection progression to persistent infection.

HBeAg-positive patients, although presenting high rates of viral replication can develop pre-core mutations (1, 3, 4). HBV viral load influences the development of liver fibrosis (7, 8). The correlation between the level of viremia and fibrosis showed that there were significant differences in viral load between patients with F0 and F4 fibrosis (on average 2500 IU/ml *vs.* 59300 IU/ml). Previously published studies show that HBV level monitoring is a better predictive indicator of infectivity than monitoring HBeAg status alone (7, 8, 9). In our study the mean values of fibrosis, F1, F2, F3 and F4 were: 6.5, 7.1, 9.1 and 19.8 kPa, respectively, similar to those obtained by Marcellin (10).

CONCLUSIONS

Chronic hepatitis B virus infection was diagnosed more frequently in young adult males. Hepatocytolisis syndrome was present with an average value of 115 IU/L. ALAT level elevation was associated with increasing prevalence of fibrosis in both HBeAg positive and HBeAg negative patients. Only a minority of patients were HBeAg-positive at the time of diagnosis. 9.98% of patients were co-infected with HDV. The correlation between the level of viral load and fibrosis showed that viral load of patients with F0 fibrosis was significantly lower than of those with F4 fibrosis. HBV viral load influenced the development of liver fibrosis. The correlation between the level of viral load and fibrosis showed significant differences between viral load and the status of HBeAg-positive or negative patients. Increased viral load levels were correlated with an increased prevalence of fibrosis, significant in HBeAg-negative patients, and in HBeAg-positive patients increased fibrosis was correlated with low viral load. The risk to of advanced fibrosis begins to increase when ALAT value is 5-times higher than normal, but it is not influenced by the high viral load value. In the future, a better monitoring of the HBV-induced chronic hepatitis cases,

alone or in association with VHD is needed in view of establishment and implementing new strategies for the prevention and treatment of HBV infection.

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YOUNG PATIENTS' PERCEPTION ON DIFFERENT SURGICAL MANAGEMENT OF THE DISTO-OCLUSAL GINGIVAL OPERCULUM IN SECOND MANDIBULAR MOLARS

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YOUNG PATIENTS' PERCEPTION ON DIFFERENT SURGICAL MANAGEMENT OF THE DISTO-OCLUSAL GINGIVAL OPERCULUM IN SECOND MANDIBULAR MOLARS (Abstract) **Aim**: This baseline study aims to find out the young patients' perception on different surgical management of the disto-oclusal gingival operculum in second mandibular molars: the conventional technique and the laser assisted one. **Material and Methods:** The current study included a batch of 20 patients who needed surgical bilateral removal of the disto-oclusal operculum in second mandibular molars order for them to receive conservative treatment. Each patient benefited of two different surgical techniques (laser assisted and conventional). Patients' perception towards these procedures was evaluated with a simple questionnaire. **Results:** The questionnaires were evaluated by a specialized team and the results were illustrated in the specific charts. On one hand the conventional technique wasn't perceived as very comfortable and on the other hand the laser assisted technique was very well perceived by the patients. **Conclusions:** The overall perception of young patients towards laser assisted periodontal surgery was very high and this is a means of reducing operating time and anxiety caused by the surgery itself. **Keywords**: DISTO-OCLUSAL OPERCULUM, PERCEPTION, DIODE LASER.

After Caranzza et al (1) the space between the crown of the tooth and the overlying gingival flap called operculum is an ideal area for the accumulation of food debris and bacterial growth. Although in some cases there are no clinical signs or symptoms, the gingival flap is often chronically inflamed and infected and has varying degrees of ulceration along its inner surface.

In general the acute inflammatory involvement is a constant possibility and may be exacerbated by trauma, occlusion, or a foreign body trapped underneath the tissue flap.

This is a reason why the surgical management of this soft tissue in young patients should be a contemporary preoccupation both for the periodontist and for the pediatric dentist.This baseline study aims to find out the young patients' perception on different surgical management of the disto-oclusal gingival operculum in second mandibular molars: the conventional technique and the laser assisted one.

MATERIAL AND METHODS

The current research included a sample population of 20 patients with ages between 13 and 16 years old. The patients were selected from the Department of Pediatric Dentistry and the Department of Periodontology in the University of Medicine and Pharmacy "Grigore T. Popa" from Iasi.

All the patients needed bilateral surgical removal of disto-oclusal operculum on the second mandibular molar (fig. 1, 2). This periodontal surgical procedure was needed in order to treat the cause of local inflammation and to provide proper access to the pediatric dentist for the restorative procedures on these teeth.

Every patient was surgically treated bilaterally but using different techniques. In one quadrant the patient received conventional surgical treatment and in the opposite quadrant the surgery was done using a diode laser with a 940 nm wave length and a 1.5 W energy. The surgeries were done separately, first the conventional technique and afterwards the laser assisted surgery. For safety reasons both surgeries were done under local anesthesia (fig. 3).

After the healing period of both surgeries the youngsters were asked to complete an easy questionnaire evaluating their perception regarding the two different periodontal surgical procedures and the postoperative recovery. The questionnaire was containing five questions both for the first and for the second surgery (fig. 4).

Fig. 1. Initial clinical aspect

Fig. 2. Marking of the incision line

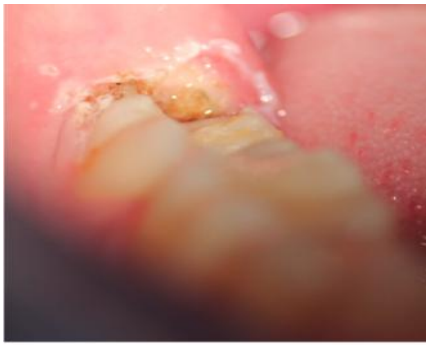

Fig. 3. Immediate post op aspect

Fig. 4. Clinical aspect 7 days after the surgery

RESULTS AND DISCUSSION

The questionnaires were evaluated by a specialized team and the results were illustrated with the help of the specific charts.

On one hand the conventional surgical technique (fig. 5) proved not to be very comfortable for the young patients and also was perceived as a source of great anxiety. Other authors claimed as well in their works that traditional gingivectomy procedures have been a challenge for dentists who confront issues of patient cooperation and discomfort (2).

On the other hand 90% of the patients reported that they felt well and excellent during the laser assisted surgery (fig. 6) which is a significantly better perception of the surgery compared to the conventional one. Other published research showed that laser soft-tissue surgery is very well accepted by children (2).

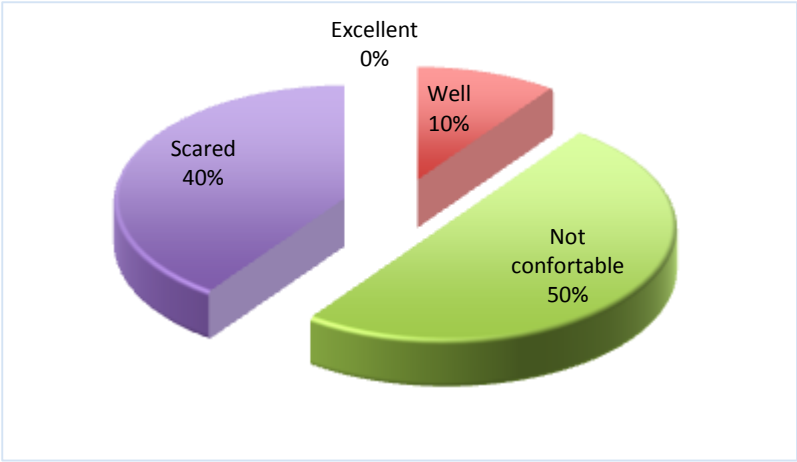

Fig. 5. Perception of comfort after the conventional surgery

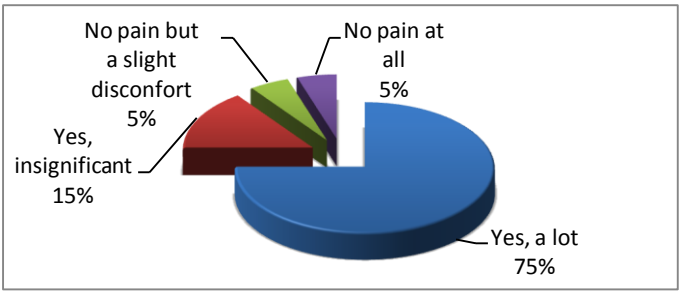

Fig.7. Perception of pain after the conventional surgery

A large majority of 75% of the patients reported that the felt a lot of pain (fig. 7) during their post operative period after the classical surgery due to the inflamed area that was removed and the scalpel cut.

The recovery period after the laser sur-

gery was perceived far better by the patients where most of them didn't experience pain but only a slight discomfort (fig. 8). The ones that reported that they felt pain said that the intensity of it was insignificant. The absence of pain can be justified by fundamental research which proved that the morphological differences identified at the gingival epithelium level and subjacent *lamina propria* support the value of laser therapy, stimulating an improved healing of the damaged tissues (3).

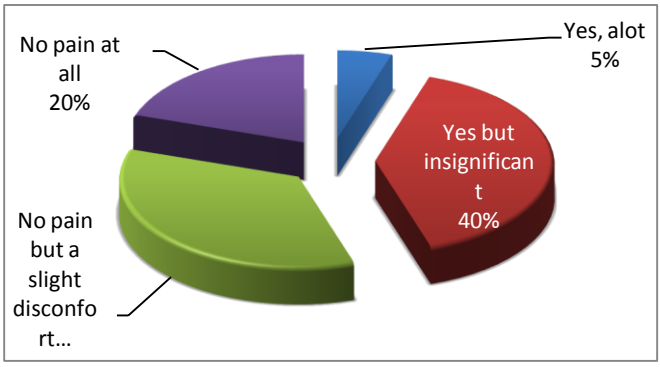

Fig. 8. Perception of pain after the second surgery

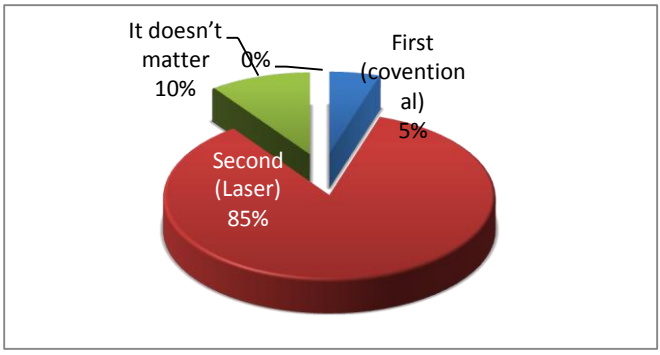

Fig. 9. Preferred treatment if the patients had to choose

If the patients had to choose between the two techniques it was shown (fig. 9) that a significant percentage of 85% would choose the laser surgery rather than the classical technique.

We do not subscribe to other authors' opinion regarding the low level of appropriateness of using diode lasers in different dental specialties (4) and we encourage this minimally invasive approach.

From the training point of view, although a diode laser was the most common type of laser used, of the eight postgraduate orthodontic programs in USA and Canada (22 percent) not offering laser training, four indicated having no plans to begin using lasers or training on their use (5).

We should focus more on implementing

the lasers in the postgraduate departments of the universities especially because of the high level of patients' perception.

CONCLUSIONS

This study confirmed that the conventional technique took the periodontist more time than the laser assisted one and this was due also to the poor cooperation of the young patients when they saw the surgical instruments. The laser assisted technique was faster and with a better cooperation from the patient's side.

The overall perception of young patients towards laser assisted periodontal surgery was very high and this is a means of reducing operating time and anxiety caused by the surgery itself.

It can be concluded that a tight cooperation should be established between the periodontist and the pediatric dentist in order to achieve optimal results both from the clinical and from the patient's perception point of view.

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NEWS

MICROGLIA: SCAPEGOAT, SABOTEUR, OR SOMETHING ELSE ?

Microglia is normally composed of cells which are distributed in brain and also in spinal cord, displaying a distinct immune potential. They can be mobilized and efficiently involved in a specific cellular immune monitoring activity, in case of tissular injuries or specific neurological diseases. The common opinion that the activation of microglia is neurotoxic is widely accepted, but it seems that the most dramatic instance of activation is represented by prion disease. Every failure to destroy prions can transform microglia in a real saboteur which spread the disease.On the other hand, aged or sick microglial cells can not perform an adequate synaptic remodeling-these dysfunctions contribute to neurodevelopmental or psychiatric disorders. Experimental studies sustain now that microglia has distinct actions in distinct circumstances.The understanding of its role in health and disease requires new tools to explore microglia, based on fact that the CNS and the immune system are intimately connected. (Aguzzi A, Barres BA, Bennett ML, *Science* 2013, 339(6116): 56-61)

Carmen Zamfir

COMMUNICATION IN DENTAL MEDICINE: IMPORTANCE IN MOTIVATING ELDERLY DENTAL PATIENTS

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COMMUNICATION IN DENTAL MEDICINE: IMPORTANCE IN MOTIVATING ELDERLY DENTAL PATIENTS (Abstract). Dental services for elderly patients are characterized by a series of particularities related to the vulnerability of this age group, which is affected by various co morbidities, and the diminished physical, cognitive and financial capacities. Finding ways to keep elderly patients coming to a dental office is possible by improving the dentist-patient relationship and implicitly the quality of care by increasing the self-esteem of the elderly and their place in society, by increasing the role of oral health in the quality of life, and here we refer to the pleasure of eating, the pleasant physical aspect and normal diction. The present paper presents the psychological aspects that interfere in the communication process between the dentist and the elderly patient and the changes motivation undergoes when people are in pain. These data can sometimes change the reticent attitude of the dentist towards the elderly patient which is often considered to be a risk patient.
Keywords: DENTIST-PATIENT DIALOGUE, MEDICAL CONSULTATION, COMMUNICATION, MOTIVATION, ELDERLY

Ageing is a continuous, gradual process which begins at birth and continues over the lifespan. It represents the irreversible inherent progressive alteration of many body functions which cannot be eliminated and which increases the individual vulnerability. Ageing presents both positive and negative aspects. These is not a specific age to clearly mark the beginning of old age, but the phenomena related to ageing are widely presented in the literature dealing with this subject, although the mechanism by which the human body is getting older is not clearly known, yet.

Generally, the ageing processes affect differently the structure and the functions of various organs and tissues. The modifications refer to either the degradation of anatomic structures accompanied by the survival of others which are provided with a compensatory activity, as it is the case of nephrons or muscle fibers, or by the continuous loss of neurons, followed by the gradual decrease of sensitive-sensory and behavioral abilities.

The particularities of the third age have lead to the necessity of organizing departments of Gerontostomatology within the schools of dentistry. This way, dentists will become familiar with the specific features of the dental services for seniors. Moreover, it appears that dental care for other age

groups has a certain impact on the elderly patient too; it is considered that if teeth are properly cared from childhood the better the oral hygiene will be later on in life, because the lack of dental hygiene affects teeth more than the process of ageing (1).

The fall out of teeth is more frequent at the level of the upper jaw, consequence of dental caries or periodontal disease. Water fluoridation and better oral hygiene, reducing the frequency of caries and buccodental infections can avoid or delay edentation in the elderly. The process of dental degradation affects both the enamel, which becomes pigmented and gradually lost through attrition, and the dentine, whose odontoblastic activity reduces the pulp cavity up to complete obstruction. To these, the weakened periodontal ligaments, gingival atrophy and bone resorption at the level of the alveolar area are added.

The oral mucosa presents a certain degree of atrophy and dryness due to the low quantity of mucin and saliva. The filiform and circumvallate papillae on the surface of atrophic tongue decrease taste sensibility by almost 30% by the age of 70.

If we refer to the elderly, during the process of oral care, the patients are not the only ones who must be motivated, but the dentists as well since their attitude towards the elderly often reflects the negative stereotypes society has in their respects (2). This attitude is represented by the refuse to treat the elderly patient, seen as a high risk patient due to the numerous co morbidities, or because the various dental procedures have to do more with removing than with preserving, extraction, with its mutilating effects, being chosen over treatment. In the young population, edentation is the oral affection with the highest impact on self-esteem, and it plays an important role in patient motivation. If dentists were familiarized with the physical and psychological features of ageing, they would no longer consider third age patients as part of the less important cases.

The dentist has the moral and professional duty to create, from the first contact and during the entire process of examination and care, the best climate for cooperation, based on transmitting a feeling of confidence regarding the positive evolution of the disease, patient's healing and his family and social reintegration. Self-esteem (behavior and attitude) stands at the base of the doctor-patient relationship and it represents the first condition for therapeutic success.

For humans, communication represents the main mean of socializing, his personality being structured in his own communication area.

The purpose of communication is that of making the interlocutor feel, think or act in a certain way. The purpose exists even if the partners of communication are not conscious of it (3).

Within the doctor-patient communication relationship, the doctor must be able to recognize and properly decipher the patient's verbal and nonverbal messages; to use the proper verbal and nonverbal communication means for transmitting information to the patient, depending on the nature of the message and context (severity of the disease, type of patient, emergency etc.) (4).

The essence of communication consists in eliminating patient's and doctor's uncertainty regarding the nature of the disease; clinical uncertainties (when there is clear uncertainty regarding the diagnosis and its prognosis) and functional uncertainties (when the doctor knows both the diagnosis

and the prognosis, but he keeps the patient and his family in a state of uncertainty for not being forced to reveal unpleasant information to the patient).

Dental medicine must become an active part in knowing the oral involutive physiological and pathological processes for being capable of creating geriatric prevention programs.

PATIENT MOTIVATION

Patient motivation is one of the least understood aspects of treatment but also one of most importance.

The components of the motivational system are numerous and vary in their origin, way of satisfaction, and functions. Human motivation includes needs, reasons, interests, convictions, tendencies, intentions, desires, and aspirations. The elements indicating a state of physiological or psychological unbalance are the tendencies. They are experienced as states of agitation, inner alert, tension (5).

If an obstacle prevents a need from being satisfied, then a state of tension appears; this is called frustration. The answers to frustration are different, the most frequent one being represented by aggressiveness. Frustration is unavoidable in psychic life; when moderated, it has positive effects on the development of personality. Thus, the impossibility of fulfilling love due to social status differences can stimulate the fight for climbing the social hierarchy. Given the unavoidable nature of frustration, balanced personalities manifest tolerance to frustration, supporting, within certain limits, states of intense tension without developing pathological behaviors.

Quite often, patient motivation is wrongly understood as the technique which changes patient behavior and not a change in patient perception of treatment importance and impact. The main idea is to reach that phase in which after becoming aware, the patient manages to motivate himself; this can be achieved if the clinician uses and stresses the right words in order to achieve a dramatic impact on patient's psychic.

One of the difficulties encountered in motivating the patient is that certain words mean different things depending on the person, and many times patients in need of motivation are those who do not need many instructions. What they need is not motivation, but the reason for which the doctor has given them those instructions.

THE BASIC STRUCTURE OF MOTIVATION

For achieving the desired result of a certain action, it is compulsory for that action to be determined by a reason. The patient must be aware that the action is right for achieving the desired result. Although the patient may be aware of these things, inner feelings may interfere with the motivational process, attitudes which influence the decision of taking action or not, so that in the end the decision will depend on inner or environmental factors.

Failure of motivation can rely on the existence of various aspects with weak structure and which can be disintegrated (6). For example, patients who do not want to change the way they perform their oral hygiene but who pretend to do it.

The positive influence on the patient could be obtained by advising him about the correct oral hygiene, identifying the reasons for which the patient is reticent, and the transformation of the doctor into a psychologist who understands the patient.

The doctor, as a good psychologist, will

get close to the patient in the latter's best interest. The doctor's affective openness towards the patient eliminates anxiety and uncertainty and humanizes the medical act.

Each patient has his own personal reasons for performing a correct brushing, and the doctor can make sure the patient maintains a correct oral hygiene. The confidence in diagnosis can be reached using a large variety of methods, from the simplest to the most complex, but in general, the diagnosis is set after a good relationship is created between the doctor and his patient.

The internal influences which affect motivation include the patient's feelings and attitude. If the patient has tried and managed to improve his oral hygiene, then the patient is more confident in the concept of oral hygiene. If the patient realizes that he plays an important part in any type of communication with the doctor, he will provide positive feed-back. Generally speaking, the patient's attitude is the result of other frequently underestimated experiences and which can influence motivation.

External influences are probably the most important, and the patient's motivation in maintaining the action or modifying the behavior depends of life style, inner pressure and the influence of the external environment.

There are three key concepts which will be taken into consideration for the best result possible: communication, environment and attitude.

Communication plays an important role in motivating the patient. It represents the ability of communicating with the patient for stimulating behavioral changes. If the patient initiates a change in his behavior, he must be helped by the clinician to obtain the expected effect. In fewer words, communication requires knowing personal attitudes and also the ability of listening to others.

The importance of the environment - apart from the feelings of both parties there is the physical situation of the patient in the dental chair frequently in decubitus, a position seen as uncomfortable and stressful. A consequence of this uncomfortable position is the fact that the patient is no longer interested in listening and understanding the advices received.

The position at the same level will give the possibility to achieve a positive result in motivating the patient. The non-verbal language will be considered as well: body language, smell, noise.

Attitude. Improving the motivational aspect is a job of the entire medical team, the purpose being sometimes difficult or even impossible to achieve. One monitoring method could be represented by the use of forms and indices systems. Thus, the patient is informed about the necessity of these measurements and the need of maintaining their values. After recording the indices, they are presented to the patient at each visit having thus the possibility to analyze the patient's effort and the results achieved.

In case the patient does not respect the indications provided, in time he will notice the appearance of complications and he will be interested in remedying the situation. Because the indices used are highly sensitive, the patient will notice the situation improves and he will be more confident with the treatment and the positive solution of his disorder.

The "self-assessment" *concept* implies encouraging the patient to monitor himself at home.

State of mind is an important element as far as quality of life is considered. Elderly

people, although they don't recognize it, are unhappy because they have lost their social status, are no longer important and respected. Elderly people realize that with the passage of time they lose the importance they once had in their social relations, due to the fact that they are no longer that active in fulfilling their role in society. Slowly, they lose the role of parents, they withdraw, suffer from chronic diseases and they must deal with the conditions imposes by their new status of pensioners.

Sadness can easily be considered a characteristic of old age. Nevertheless, this does not mean it has to be ignored. Many times elderly people are so sad that they no longer take care of themselves and thus they hurry their end; some of them even decide to commit suicide. Deficitary oral health for the elderly patient can be due to the anxiety caused by painful medical interventions, uncertainty regarding their physical aspect but also to the social isolation in which elderly people often live (7).

Another aspect of the elderly patient's motivation in improving the communication with the dentist is represented by the need of maintaining the integrity of his oral health, determined by social and psychological factors. At more advanced ages, when the work volume decreases, eating becomes one of the few pleasures of life (8). At the same time, contemporary society promotes youth, prolonged also through plastic surgery. Apart from the aesthetic consequences, oral rehabilitation facilitates a clear pronunciation, far from the nasal incomprehensible speech which is maliciously associated to old age. That is why, the number of remaining teeth, the use of prostheses or not, the level of education, social status and rural or urban native environment influence the frequency with which elderly people come to the dentist's office.

CONCLUSIONS

Motivation is an inner process and not an imperative which can be imposed from the outside, and communication creates the bridge between the two, doctor and patient.

Unlike the young patient, elderly patients tend to avoid discussing with the doctor subjects related to their emotional states as they consider a consultation to be referring strictly to solving medical problems; they do not see the doctor willing to approach this kind of subjects or they consider these discussions as a violation of their privacy. The increased severity of oral affections and the difficult access to medical services, caused by either health issues or difficult financial condition which came along with retirement, turn the elderly patient into a vulnerable category. Hence, the psychological aspects become key elements in the medical act addressed to elderly patients.

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NEWS

TOPICAL XYLITOL ADMINISTRATION BY PARENTS FOR THE PROMOTION OF ORAL HEALTH IN INFANTS: A CARIES PREVENTION EXPERIMENT AT A FINNISH PUBLIC HEALTH CENTRE

Xylitol has been shown to be an effective tooth decay preventive agent. Clinical studies of xylitol have almost exclusively involved chewing gum or lozenges and evaluated school age children and tooth decay in permanent teeth. This demonstration programme tested topical use of xylitol as a possible oral health promoting regimen in infants at a Finnish Public Health Centre in 2002-2011. Parents (usually mothers) began once- or twice-daily administration of a 45% solution of xylitol (2.96 m) onto all available deciduous teeth of their children at the age of approximately 6-8 months. The treatment (xylitol swabbing), which continued till the age of approximately 36 months (total duration 26-28 months), was carried out using cotton swabs or a children's toothbrush; the approximate daily xylitol usage was 13.5 mg per each deciduous tooth. At the age of 7 years, caries data on the deciduous dentition of 80 children were compared with those obtained from similar, untreated children (n = 90). Xylitol swabbing resulted in a significant ($P < 0.001$) reduction in the incidence of enamel and dentine caries compared with the comparison subjects (relative risk 2.1 and 4.0, respectively; 95% confidence intervals 1.42-3.09 and 2.01-7.98, respectively). Similar findings were obtained when the children were 5 or 6 years old. The treatment reduced the need of tooth filling relative risk and 95% confidence intervals at 7 years: 11.86 and 6.36-22.10, respectively; $P < 0.001$). Compared with untreated subjects, the oral counts of mutans streptococci were reduced significantly ($P < 0.001$). Considerable improvement in dental health was accomplished in infants participating in a topical at-home xylitol administration experiment, which was offered to families in the area by the Public Health Centre as a supplement to standard oral health care. This study is the first to demonstrate that xylitol topical syrup at 8 g per day divided into two or three doses given during primary tooth eruption in children 15 to 25 months of age reduces tooth decay and could prevent up to 70 percent of decayed teeth. Caregiver assessment of the programme was mostly rated as high or satisfactory (Mäkinen KK, Järvinen KL, Anttila CH, Luntamo LM, Vahlberg T. Topical xylitol administration by parents for the promotion of oral health in infants: a caries prevention experiment at a Finnish Public Health Centre. *Int Dent J* 2013; 63(4): 210-224).

Gianina Iovan

PREDICTORS OF QUANTITATIVE MICROBIOLOGICAL ANALYSIS OF SPATTER AND AEROSOLIZATION DURING SCALING

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Predictors of quantitative microbiological analysis of spatter and aerosolization during scaling (Abstract): **The aim** of this study is to analyze the infection risk through spatter and aerosolization during scaling and to create a prediction model of the total number of hemolytic bacteria. **Material and methods:** Air samples were collected prior to patient's arrival and spatter and aerosol samples were collected during scaling procedure in 80 patients of 4 different dental clinics. The dentists calculated DI, CI, DMFT. Only patients with CI>=1 were included. The bacteriological results (CFU/m^3) were correlated with clinical indicators. Patients were divided into 2 groups: one that rinse with sterile water and the other with chlohexidinemouthrinse 0.1%. **Results and conclusions:** Medium size effect positive correlations were found between the number of decayed teeth and the total number of bacteria and the total number of hemolytic bacteria that grew on plate attached to the dentist's mask. The mean number of bacteria and the mean number of hemolytic bacteria that grew on plate attached to the dentist's mask were significantly lower in the group that rinse with chlorhexidine 0.1%, when compared to the group that rinsed with sterile water. When controlling for the total number of bacteria and the total number of hemolytic bacteria from air sampling, the total number of hemolytic bacteria that grew on the plate attached to the dentist's mask can be predicted by CI, group membership and DMFT score. **Keywords:** SPATER, AEROSOLS, HEMOLYTIC BACTERIA.

For dentists and oral health personnel there is a high risk of contamination with several communicable diseases, due to particularities of their work. Ultrasonic scalers are being used extensive in dental practice for the removing of dental plaque and calculus. Several authors have confirmed presence of bacteria in dental calculus (1, 2, 3, 4). Microbial aerosols are produced by ultrasonic scalers have a high risk of infection because they can contain microorganisms of oral cavity, including microbes from calculus, respiratory tract and blood, that can be carried through the aerosolisation and can contaminate skin, oral, nasal and eye membranes (5, 6). The microorganisms incriminated to be transmitted from patients through aerosolization could be Gram positive and Gram negative bacteria (7), viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), Herpes B virus, human immunodeficiency

virus (HIV), fungi and prions (8).

Regarding the exposure of dentists to aerosols, studies conducted in 1980-1995 had found higher concentrations of antigen and antibodies of HBV (9, 10) and HCV (11) than in general population. More recently other authors (12) had found significant increases in CFU/m^3 in air samples taken before the procedure and after the procedure in patient proximity. Rautemaa et al. (13) had found significant contamination at all distances sampled when high-speed instruments were used. They did not find higher densities in patient's close proximity. Gram-positive cocci, namely viridans streptococci and staphylococci, were the most common findings.

The aim of this study is to analyze the infection risk through spatter and aerosolization during scaling and to create a prediction model of the total number of hemolytic bacteria using patient's clinical features.

MATERIAL AND METHODS

The study included 4 dental practices and in the analysis were included 80 patients with a Calculus Index >=1. The patients were selected in a prior visit. These patients represented the first patient of the working day, after 12 hours lack of activity.

Air samples were taken before the patient arrived on different Petri plates that contained three different culture media: agar, blood agar and Sabouraud. For air sampling we used M.A.Q.S (Microbiological air quality sampler - Oxoid). The air is sucked by the turbine through a perforated filter. Air flow is directed to the Petri plate, situated under the grill. The air volume is 100 l/minute and for each sample is recommended the sampling of maximum 1.000 liters of air. Exciding this quantity will dehydrate the surface of Petri plate.

Another sample was taken during scaling on a blood agar culture plate attached to the dentist's mask.

Patients were divided into 2 groups and were randomized by order of arrival in the practice: one group that rinse with sterile water, and a group of patients that rinsed with an alcohol-free clorhexidine 0.1% mouth-rinse.

The DMFT, DI and CI were calculated by the dentist, respecting the WHO indication from 1997 (14, 15), after the patient sat on the dental chair. After this procedure, the dentist placed the mask that had a Petri plate attached and begun the intervention. When the intervention was over, the Petri plate was detached, and then covered. All plates were labeled and transported to the Microbiology laboratory. All plates were incubated 24 hours at 37°C. We used the following bacteriological indicators: the total number of bacteria (UFC/m^3), and the total number of hemolytic bacteria (UFC/m^3). The intervention consisted of scaling with an ultrasonic scaler.

Data were processed using IMB SPSS version 18 (2010). For the parametric correlation of data, Pearson's correlation was used. T-Tests were used in order to compare between study groups the number of CFU/m^3 (total and hemolytic) that grew on the plate placed on the operator's mask. For the prediction of the number of hemolytic CFU/m^3 that grew on the plate placed on the operator's mask we used the multiple hierarchal regression.

RESULTS AND DISCUSSION

Females represented 52.5% of total study population. Age range was between 20 and 65 years. Mean group age is 42.74 +/- 12.38 years, with median at 45 years. Mean DMFT score for the whole group was

21.3 +/- 4.94, with a minimum of 9 and a maximum of 28. We have found a significant positive correlation between DMFT score and the total number of bacteria ($r=0.37$, $p<0.01$), and between DMFT score and the number of hemolytic bacteria ($r=0.39$, $p<0.01$) that grew on the Petri plate that was attached to the operator's mask. Size effect of both correlation is small ($r^2=0.14$, respective $r^2=0.15$). Stronger correlations were found when using the decayed number of teeth with the total number of bacteria ($r=0.62$, $p<0.01$) and the total number of hemolytic bacteria ($r=0.57$, $p<0.01$) and the size effect of latter correlations is medium.

In accordance to our results, Korean researchers (16) found that the reduced glutathione level, a major endogenous antioxidant produced by the cells, showed a significant linear relationship with the salivary Lactobacilli level ($p=0.016$), DMFT score ($p=0.005$). In contrary to our results, Mannaa et al. (17) used a pooled supragingival plaque sample per participant, obtained from posterior approximal sites and analyzed it for 15 bacterial species using the checkerboard DNA-DNA hybridization technique, did not find significant relationships between the bacterial scores and DMFT/dmft nor D/d groups.

Mean DI score for the whole group was 0.28 +/- 0.27 with a minimum of 0 and a maximum of 1.0. Mean CI score for the whole group was 2.02 +/- 0.71 with a minimum of 1.0 and a maximum of 3.0.

The results of air sampling prior to intervention indicated that the mean number of bacteria that grew on the plate was 118.01 +/- 25.07 CFU/m^3 and the mean number of hemolytic bacteria that grew on the plate was 10.68 +/- 4.75 CFU/m^3.

Similar results were published by Azari et al. (18), that the total bacterial counts in the air of dental surgery rooms and in non-surgery rooms without direct involvements with dental operations were in the range of 120-280 CFU/m^3 and 49-128 CFU/m^3 respectively. Other Romanian researchers (19) had published similar results for the mean value for the total number of germs at the beginning of the day in the air was 129 CFU/m^3.

We have found that the mean total number of bacteria on the mask attached Petri plate were significantly lower in the group of patients that rinsed with chlorhexidine0.1% ($M=66.25$), when compared with the group that rinsed with sterile water ($M=121.35$ CFU/m^3) $t(78)=-5.37$, $p<0.001$ and size effect $\varepsilon=0.26$ is considered large according to Cohen. The mean number of hemolytic bacteria on the mask attached Petri plate were significantly lower in the group of patients that rinsed with chlorhexidine ($M=9.03$), when compared with the group that rinsed with sterile water ($M=15.78$ CFU/m^3) $t(78)=-6,75$, $p<0.001$ and size effect $\varepsilon=0.36$ is considered large according to Cohen.

In agreement to our study, Reddy et al. (20) when investigated the reduction of bacterial aerosol contamination during use of ultrasonic scaler comparing the efficacy of sterile water rinse with non-tempered chlorhexidine rinse and with a tempered chlorhexidine mouth rinse and found significant reductions in CFU in the group that used non-tempered chlorhexidine rinse when compared to the group that rise with sterile water. Similar results were obtained by Konig et al. (21) when they used cold (18°C) rinse versus wormed rinse (37°C), with better results for wormed solution of chlorhexidine. In a study by Rosin (22), that compared the effects in bacterial re-

duction of placebo *vs.* a chlorhexidine 0.12% rinse, the authors obtained significant reduction in bacterial count in the chlorhexidine 0.12% rinse group.

Multiple hierarchical regression was used to assess the ability of 4 measures (the group membership, the Debris Index and the Calculus Index, DMFT) to predict the number of hemolytic bacteria that grew on the plate attached to the dentist's mask, after controlling for the influence of total number of bacteria and the number of hemolytic bacteria cultivated from aerosols of the dental office, prior to intervention. Preliminary analyses were conducted in order to ensure no violation of the assumptions of normality, multicollinearity, homoscedasticity. In the first step, total number of bacteria and the number of hemolytic bacteria were included and the model containing these variables was not statistically significant. In the second step, besides total number of bacteria and the number of hemolytic bacteria we introduced the group membership, the Debris Index and the Calculus Index, DMFT. This model as a whole explains 69. 7% of the variance of total number of hemolytic bacteria $F(6.79)=27.9$, $p<0.001$. CI has the highest beta value (beta=0.71, $p<0.001$), followed by the membership to the group that rinsed with sterile water before the intervention with beta=0.19, $p<0.05$ and DMFT with beta=0.18, $p<0.05$. The unique variance explained by the significant predictors was 61.1% for CI, 15.8% for group membership and 15.7 for the DMFT score.

DI, total number of bacteria and the total number of hemolytic bacteria from air sampling did not contribute significantly to the model.

Prediction equation:Number of hemolytic bacteria that grew on the plate attached to the dentist's mask = -8.71 + 2.85 x sterile water group + 7.51 x CI + 0.28 x DMFT.

TABLE I

Regression coefficients for the prediction of predict the total number of hemolytic bacteria that grew on the plate attached to the dentist's mask

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	17.847	4.268		4.181	.000
	Total number of hemolytic bacteria from air initial	-.065	.181	-.041	-.360	.720
	Total number of bacteria initial from air	-.040	.034	-.134	-1.169	.246
2	(Constant)	-8.711	3.663		-2.378	.020
	Total number of hemolytic bacteria initial	-.082	.104	-.052	-.788	.433
	Total number of bacteria initial	-.025	.020	-.082	-1.247	.216
	Sterile water group	2.851	1.162	.190	2.452	.017
	DI	-2.155	2.444	-.079	-.882	.381
	CI	7.517	.793	.709	9.473	.000
	DMFT	.281	.115	.185	2.435	.017

CONCLUSIONS

Small size effect positive correlations were found between DMFT score and the total number of bacteria and the number of hemolytic bacteria that grew on plate attached to the dentist's mask. Medium size effect positive correlations were found between the number of decayed teeth and the total number of bacteria and the total number of hemolytic bacteria that grew on plate attached to the dentist's mask.

The mean number of bacteria and of hemolytic bacteria that grew on plate attached to the dentist's mask were significantly lower in the group that rinse with chlorhexidine 0.1%, when compared to the group that rinsed with sterile water.

When controlling for the total number of bacteria and the total number of hemolytic bacteria from air sampling, the total number of hemolytic bacteria that grew on the plate attached to the dentist's mask can be predicted by CI, group membership and DMFT score.

Simple measures like the prior rinse with a chlorhexidine 0.1% mouth rinse reduces bacterial counts in the mouth and implicit the number of aerosolized microbes. The level of calculus is also a predictor of the quantity of aerosolized bacteria. Decayed teeth maintain a higher level of microbial flora that translates to a higher quantity of aerosolized microbes.

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NEWS

ANTICANCER ACTIVITY OF *SCLEROCARYA BIRREA*

Sclerocarya birrea is used in traditional medicine for the treatment of many diseases. A study performed by Tanih *et al.* aimed to scientifically prove its therapeutic properties. For this reason, water and acetone extracts of *S. birrea* were evaluated in terms of their anti-cancer activity on HT-29, HeLa and MCF-7 cell lines. The methods used included: cell titre blue viability assay in 96-well plates was used, acridine orange and propidium iodide staining for evaluation of apoptosis, and scanning electron microscopy for morphological examination of the treated cells. The study showed remarkable antiproliferative activities of the acetone extract of *S. birrea.* Also, the extract exerted apoptotic potential against MCF-7-treated cells. The study concludes that *S. birrea* could be very useful in anticancer therapy (Tanih NF, Ndip RN.The Acetone Extract of *Sclerocarya birrea* (Anacardiaceae) Possesses Antiproliferative and Apoptotic Potential against Human Breast Cancer Cell Lines (MCF-7). *ScientificWorldJournal* 2013; 2013: 956206 doi: 10.1155/2013/956206).

Teodora Vremera

INCIDENCE AND PREVALENCE OF DENTAL - PERIODONTAL CONDITIONS AND EDENTATION IN MOLDAVIA

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INCIDENCE AND PREVALENCE OF DENTAL-PERIODONTAL CONDITIONS AND EDENTATION IN MOLDAVIA (Abstract): The proper rehabilitation of the partially extended edentulous patient should lead to a state of balance between the components of the stomatognathic system and the therapeutic option selected for an as long as possible. **Aim:** To outline the importance of a holistic biological perspective on the body-stomatognathic system relationships and the need for complex rehabilitation in view of obtaining a long lasting balance. **Material and methods:** 3340 individuals from urban and rural areas of Iasi County were evaluated. The evaluation was based on the correlation of physical exam with data from a specially drafted questionnaire aimed at determining oral health behavior pattern, a sine-qua-non condition for a clear identification of oral conditions specific to the study area. **Results:** General health status was poor in 72.1% of the subjects from rural areas as compared to 27.9% in those from rural areas, with cardiovascular diseases ranking first but with higher rates in urban than in rural areas. In urban areas, odontal lesions were the most common and had a higher prevalence in 2012 than in the previous years, followed by the periodontal lesions with the same ascending trend in 2012. **Conclusions: C**ontinuity with regard to the prophylaxis of general and oral conditions in the Iasi County in both rural and urban areas is a must, health education being essential for improving population health.
Keywords: POPULATIONAL SCREENING, EDENTULOUS RATE, GENERAL STATUS, ORAL PATOLOGY.

The treatment of edentulous patient involves the application in his/her oral cavity of certain artificial substitutes for restoring the morphological continuity of the dento-alveolar ridges and the functions of the stomatognathic system affected by edentation (1).

The tolerance of the components of the stomatognathic system to the contact with artificial substitutes – prostheses is a complex challenge (2).

The proper rehabilitation of the partially extended edentulous patient should lead to a state of balance between the elements of the stomatognathic system and the therapeutic option selected for as long as possible (3).

This study aimed at outlining the importance of a holistic biological perspective on relationships between the body and the stomatognathic system and the necessity of a complex rehabilitation in view of reaching a long lasting balance.

This study was focused on determining

the incidence and prevalence of odonto-periodontal conditions and edentation accompanied by various complications in the Moldavian territory.

MATERIAL AND METHODS

The 4th Edition of the Oral and General Diseases Prophylaxis Caravan, an already traditional event that started in 2009, took place in Iasi and other towns on May 22, 2012.

On this occasion, 3,340 individuals from urban and rural areas of Iasi County were evaluated. The female gender was prevalent (62% from urban and 55.4% from rural areas). Most subjects from urban areas (54.3%) were retired, while school-going population (43.9%) was prevalent in rural areas.

The evaluation consisted in the correlation of physical exam findings with the data from a specially drafted questionnaire aimed at determining the oral health behavior pattern, a sine-qua-non condition for a clear reflection of oral conditions specific to the study areas (4, 5).

RESULTS

General health status was poor in 72.1% of the subjects from urban areas as compared to 27.9% in the rural areas (fig. 1).

Cardiovascular diseases ranked first but with higher rates in urban than in rural areas.

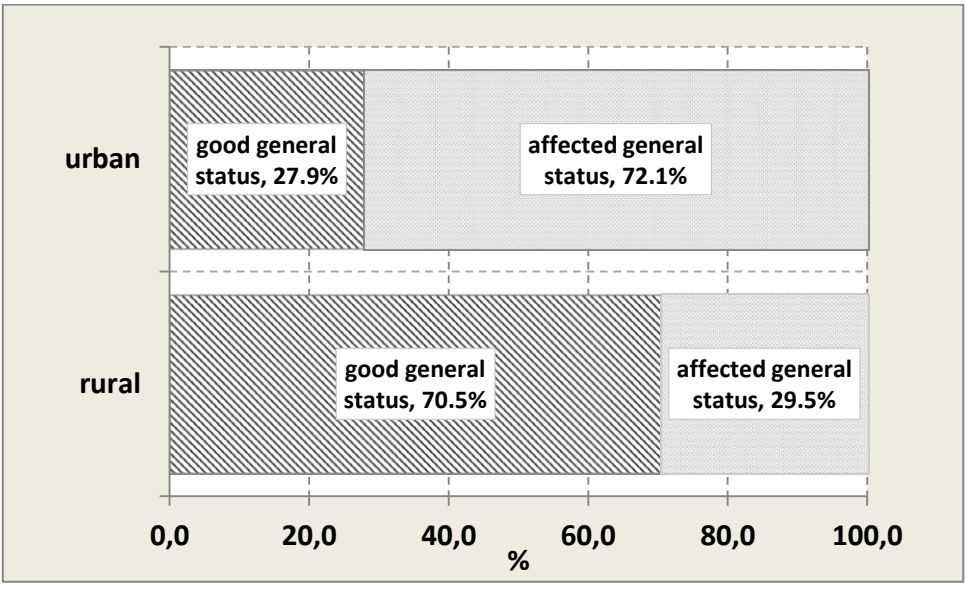

Fig. 1. General health status of the study population

Following the dynamic evolution during the whole time span of the caravan, an increase in the incidence of cardiovascular diseases starting from 19.1% in 2009, to 21.3% in 2010, 37.4% in 2011 and 42.2% in 2012 was found (fig. 2).

In the rural areas (fig. 2), further deterioration of the general health status from 2010 to 2012 was also noticed, cardiovascular diseases being the most common, followed by liver and neurologic diseases and diabetes.

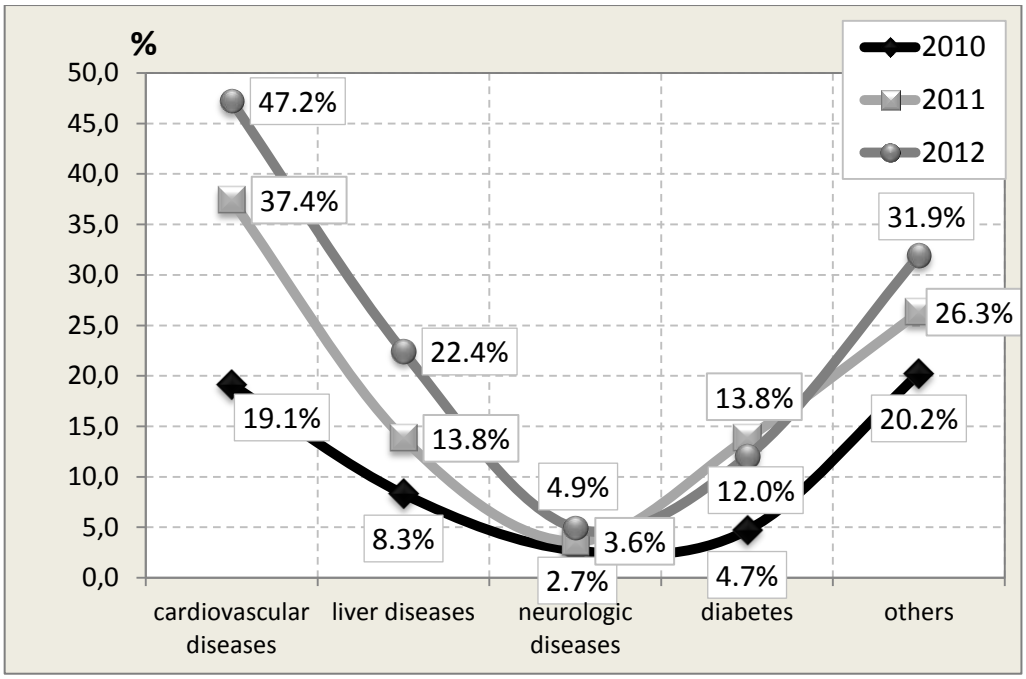

Fig. 2. City of IASI - General Pathology 2010 - 2012

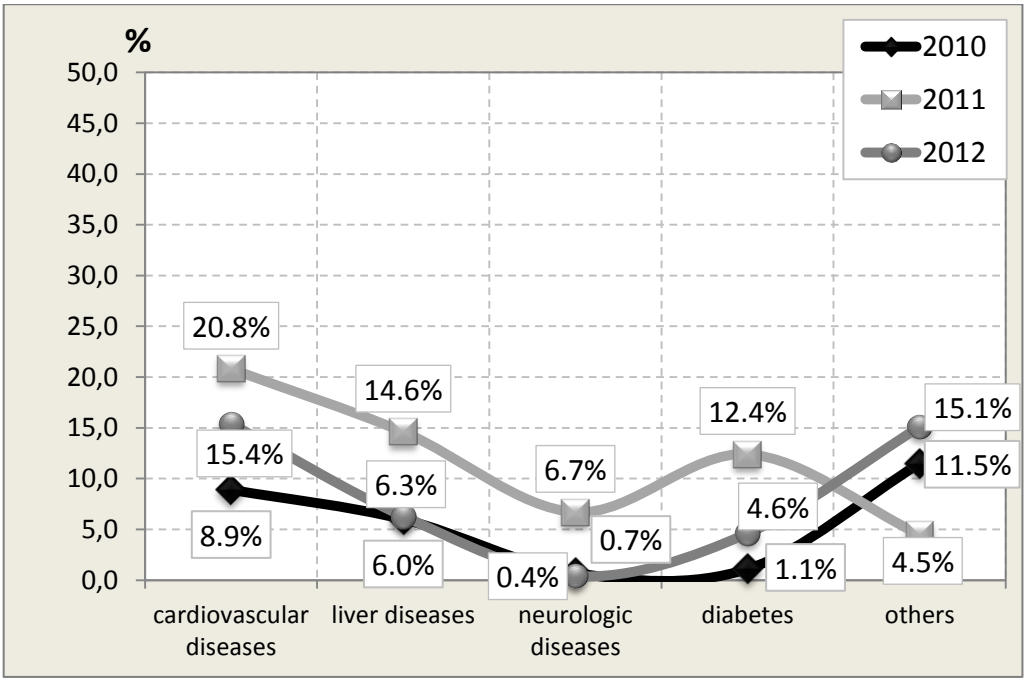

Fig. 3. Rural Area - General Pathology 2010 - 2012

The year 2012 gives a clear image on the prevalence of local oral pathology in the Iasi county (urban and rural areas), the odontal and periodontal lesions reaching significant prevalence rates of 74.7% in rural areas and 67,2% in the urban ones (fig. 4, 5).

In 2012, the prevalence of oral conditions in the urban areas of Iasi County was high, odontal lesions followed by periodontal lesions being the most common and sharing the same ascending trend.

In rural areas, the prevalence of oral conditions fluctuated, with a highest of 74.7% in 2012 for odontal lesions, while fistulas were found in small percentages, relatively equal for the 3 study years (fig. 5).

As to the type of edentation, the partially reduced edentation was prevalent, both in the urban and rural areas, 43.7%, and 22.1%, respectively. Following the dynamic of edentation during the 3 study years, the incidence of partially reduced edentation is increasing, calling attention to the need of treatment for this clinical entity.

In rural areas an obvious increase in the incidence of partially reduced and extended edentation was found in 2012 as compared to the previous years, so the need for both fixed and removable dentures will increase.

As to the therapy of edentation, in 2012 there was an increased prevalence of fixed restorations, followed by removable dentures, reaching 40.8% in urban areas as compared to 18.9% in rural areas. We also need to underline that 74.7% of the rural population does not need prosthetic therapy, while the same applies to only 39.9% of the urban population.

Regarding the use of oral hygiene products, in rural areas toothpaste is used by 94.0%, mouthwash by 18.9% and dental floss by 3.9% as compared to 97.2%, 41.4% and 11%, respectively in urban areas (fig. 6).

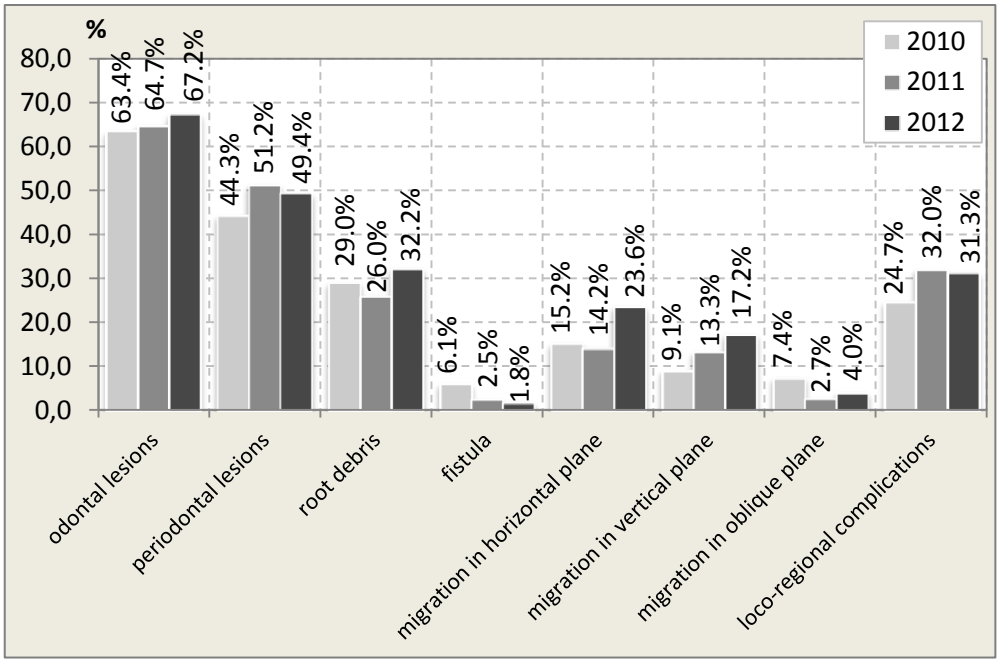

Fig. 4. Local pathology - City of Iasi 2010-2012

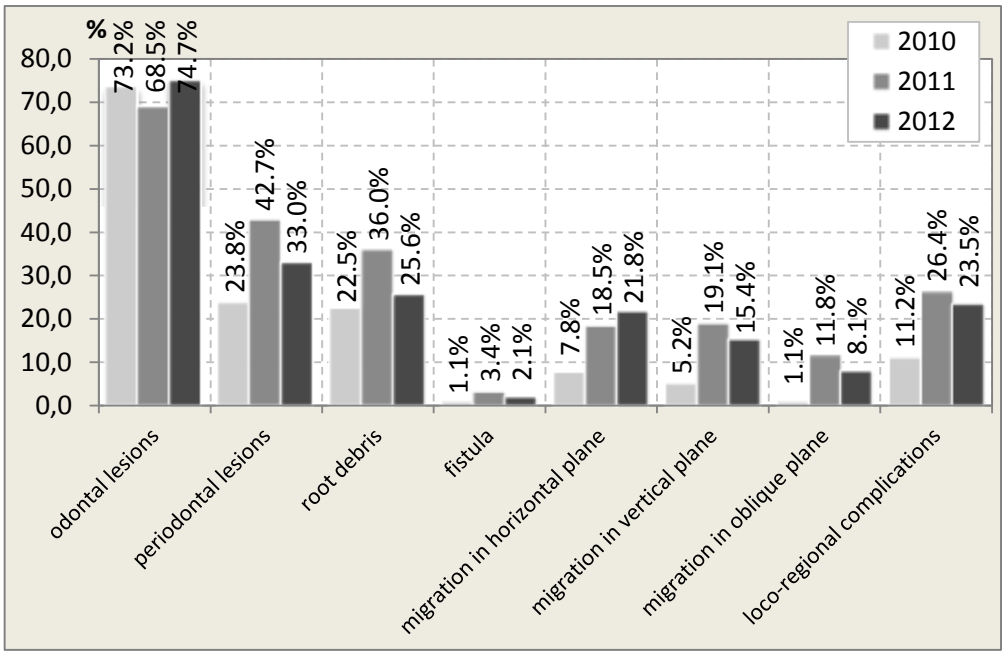

Fig. 5. Local pathology - Rural Area 2010-2012

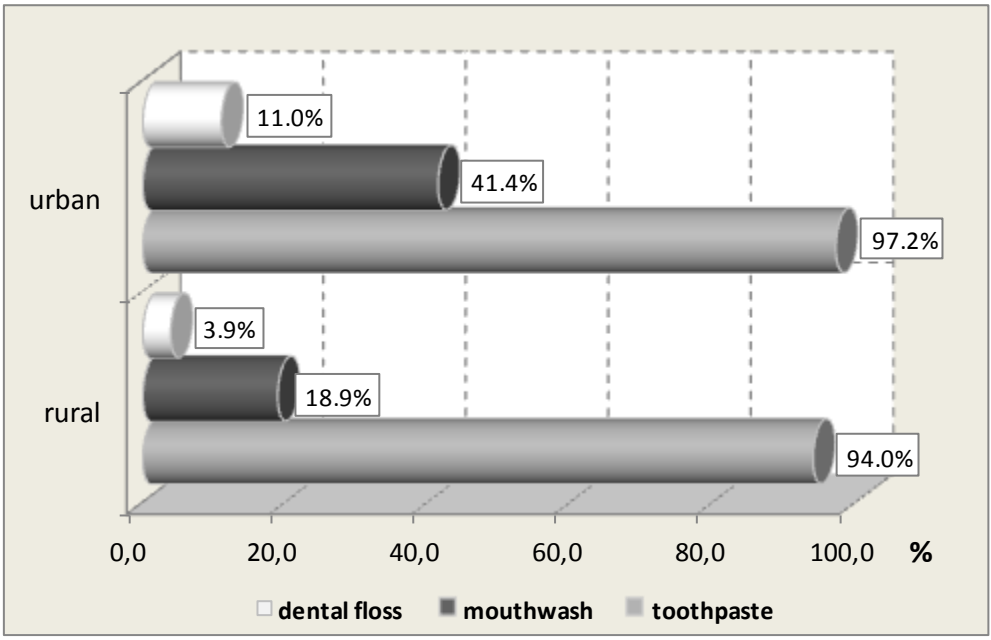

Fig. 6. The use of oral hygiene products in urban and rural areas

As to oral hygiene, 50.9% of the urban population and 42.1% of the rural one clean their teeth twice a day (fig. 7). Toothbrush is changed by 22.8% of the rural population of Iasi County once a year, while 31.6% of the urban population does so 4 times a year (fig. 8).

Following the dynamics of oral hygiene in the study population, the improvement in health education resulted in that more and more people brush their teeth more than once a day and use auxiliary oral products, but toothbrushes are replaced less often in both rural and urban areas.

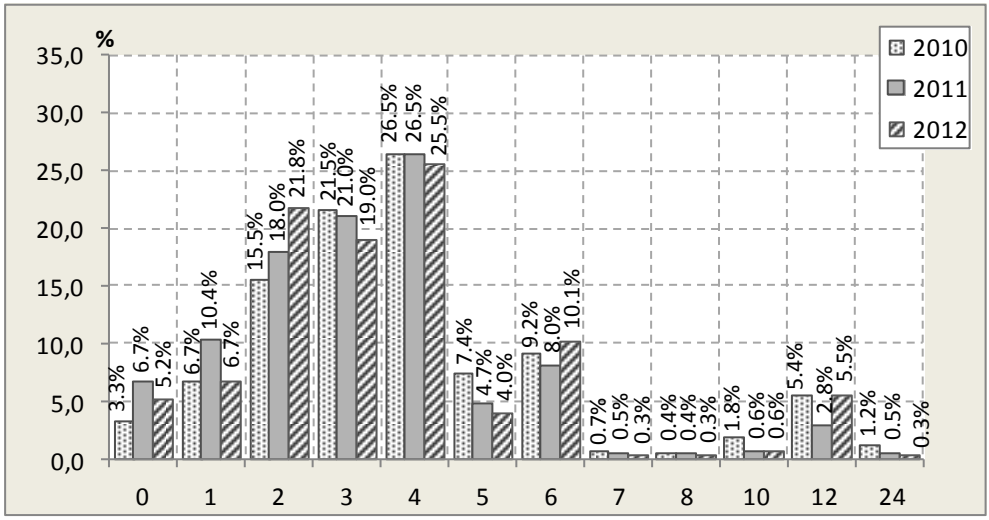

Fig. 7. City of Iasi – Number of toothbrush replacements per year (2010 – 2012)

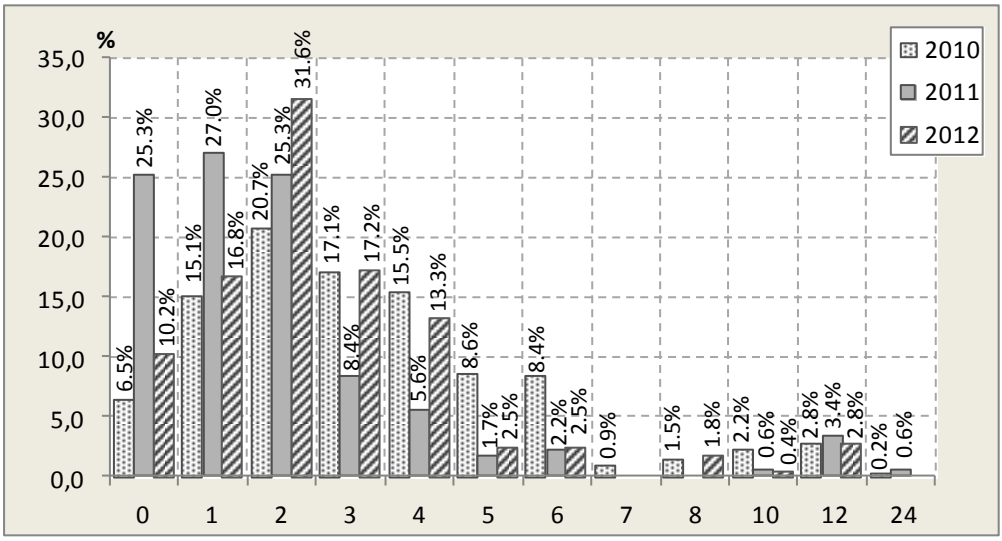

Fig. 8. Rural area – Number of toothbrush replacements per year 2010 - 2012

With regard to the number of dental visits, starting with 2009 there was a steady increase in the number of "when in need" visits", a worrying trend of possible local and loco-regional complications.

CONCLUSIONS

As a result of attending the activities of the caravan, more and more people gained better access to health education and care, oral hygiene products, learned correct tooth brushing techniques, and understood that neglect of health can have long-term effects on health and well-being.

Continuity of the prophylaxis of general and oral conditions in the Iasi county, both in the rural and the urban areas is a must, health education playing an essential role in health promotion and improvement process.

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NEWS

DIABETES IN THE COMPETITIVE ATHLETE

Athletes with diabetes range from the athlete participating in various youth sports to competitive olympic athlete and present a significant challenge to themselves and the medical staff who care for them on a daily basis. Each sport and the type of exercise have their own effects on diabetes management with numerous factors that significantly affect glucose levels, including stress, level of hydration, the rate of glycogenolysis and gloconeogenesis and the secretion of counter-regulatory hormones. An american research team from University of Missouri – Kansas City School of Medecine provides in this article a general overview of diabetes mellitus, the effects of exercise on glucose levels and a detailed review of the potential complications encountered in the management of diabetes in the athlete (Harris GD, White RD, Diabetes in the Competitive Athlete, *J Voice*, 2012, 26, 1: 44-50)

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SPECIFIC FEATURES OF THE STROMAL OVARIAN TUMORS

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CHARACTERISTICS OF THE STROMAL OVARIAN TUMORS (Abstract): **Aim of the study:** establishing a characteristic pattern for the stromal ovarian tumors. **Material and methods:** we realized the clinical-statistics analyze of 27 stromal tumors (lot B) from a total of 498 patients with ovarian tumors (lot A), diagnosed and treated between 1999 – 2008 in Elena Doamna Hospital of Obstetrics and Gynecology, Iasi. We used the clinical files of these patients, operatory protocols and anatomopathological results. **Results:** we analyzed a large number of parameters: age, location, first diagnoses, symptoms, anatomopathological diagnose, imunohistochemical diagnose, etc. When we compared the two lots of patients we pointed out significant differences for the following parameters: most affected group of age, first diagnose, and associated diseases, anatomopathological and imunohistochemical differences, and prognostic factors. **Conclusions:** stromal ovarian tumors are a less studied entity, with particular anatomopathological and imunohistochemical features which differentiates them from the rest of the ovarian tumors. **Keywords:** OVARIAN CARCINOMA, TUMOR, BIOMARKER

The malignity of stromal ovarian tumors is somehow between the same characteristics as in the case of the epithelial tumors. Based on histological grading it will be appreciated the tumoral invasion, short term prognostic, type of surgical procedure and its necessity, future oncologic protocol (1, 3, 4, 6, 7).

The interaction between tumor and ovarian stroma is an insufficient aspect of ovarian tumorogenesis. Tumoral aria, including stromal fibroblasts, infiltrative stromal cells, vascular and lymphatic network and extracellular matrix is an integrated part of carcinogenetic process, promoting cellular growth and metastatic disorders (1, 2, 5).

Stromal alterations which accompany the tumoral progression include breaches in basal membrane which surrounds the tumor, severe immune response and angiogenesis (new vascular network). Additional breakdowns of the mesenchymal connective tissue surrounding the tumor can also be noted (6, 7, 9, 10, 11). These changes resemble a lot with tissular response observed during the healing of wounds through fibrosis (8, 10, 11). Fibroblasts and their role in tumorogenesis are considered to be the new target in the development of

new therapies for the tumoral stroma (10, 11), tumor associated fibroblasts or their disturbed environment (8, 10).

Unfortunately today we have less information regarding the stromal ovarian fibroblastic activation because of the lack of a performant experimental research system.

Our study recognizes the implication of a double interaction between neoplasic cells and ovarian peritumoral stroma, demonstrating their relationship in sustaining and promoting the tumoral invasion. After the analyze of the two lots of patients and the correlation of data with the medical literature we realized a protocol based on the conclusions of this study.

MATERIAL AND METHODS

This personal study was realized in multiple directions (anatomical, clinical, statistical and anatomopathological) using the following components: *1.* we realized a retrospective study on 498 patients hospitalized in Clinical Hospital of Obstetrics and Gynecology **"**Elena Doamna**"** Iasi, between 1.01.1999 – 31.12.2008. The lot of 498 patients with ovarian pathology was selected from the total of 8898 general gynecological pathology; *2.* lot A represents all patients diagnosed with ovarian tumors, meaning all 498 cases; *3.* lot B represents patients with stromal ovarian pathology, selected from all cases; *4.* microscopic study was realized on paraffin included specimens selected from the lots of study (we selected patients from all groups of age with/without other associated pathology); *5.* macroscopic histopathological study followed the macroscopic aspect of all tumors, local extension, stages; *6.* microscopic histopathological study evaluated the specimens colored with HE for establishing the diagnose of organ and after that anatomopathological diagnose; *7.* imunohistochemical study used an indirect tristadial method and a panel of 5 antibodies: ER (estrogen receptor), PR (progesterone receptor), S100 (protein S100), SMA (smooth muscle actin) and Caldesmon. We realized dosage for 18 ovaries (for ovarian tumors in generally) and for 3 stromal ovarian tumors; *8.* Statistic analyze used many methods: *t-Student* test, test χ^2**,** relative risk, predictive value and tendency. Data was followed up and charged with statistic functions from *Excel* and *EpiInfo*.

RESULTS AND DISCUSSION

Lot A contains all 498 cases of ovarian pathology, representing 5.6% from all 8898 patients with gynecological pathology hospitalized between 1999-2008. Lot B contains 27 stromal and sex cords ovarian tumors: 7 granulosa tumors (one bilateral and one cystic) (26%), 11 ovarian fibromas (41%), one ovarian thecoma (4%), 5 ovarian fibrothecomas (18%), 3 ovarian mixomas (11%).

The distribution on group of age for lot A reveals: highest frequency for group 31-40 years (26.51%), closely followed by group 41-50 years (21.08%). For lot B the age average is 45.5 years, between the intervals 22-69 years. The result is congruent with the result from group A, 45.94 ± 15.68 years (t-Student=0,15; dF=496; p>0.05). The distribution on group of age for lot B reveals following aspects: highest frequency: 41-50 years (33.33%), followed by group 51-60 years (22.23%); the rest of the groups do not have frequency variations, each of the containing four cases (14.8%).

Comparing the associated co morbidities we can highlight a difference between the two lots: for lot A most associated were

cardiovascular diseases (30.8%), and for lot B genital pathology (47%).

Comparing clinical diagnose for the two lots we pointed out the following elements: patients from lot A had associated pain (100%), while lot B 98%; bleeding for lot A: 0.6% and for lot B: 28%.

For stromal ovarian tumors we followed many steps for ultrasound exam also: we wanted to correlate the ultrasound aspect with the histological subtype of the tumor; increased echogenity with posterior shadow suggested ovarian fibroma while diffuse echogenity without posterior shadow was correlated with pure thecoma; echodense tumors, without posterior shadow suggested fibrothecoma.

The analyze of CA 125: lot A revealed following aspects: malignant tumors had average values (163.63 ± 143.08 UI/ml) for stage II, borderline tumors had average values (39.59 ± 10.0 UI/ml) for stage I, benign tumors had average values (27.21 ± 18.22 UI/ml) for normal interval. Only one teratoma was evaluated (66.5 UI/ml). Stromal tumors were most of the cases in stage I (96± 106 UI/ml). For lot B we pointed out the following aspects: granulosa tumors had average values (111.6 ± 75.4 UI/ml) for stage I, for the rest of the tumor types the values were under the limit of 35 UI/ml (tab. I).

TABLE I

Correlation matrix of average values for CA 125 for different stromal ovarian tumors (test t-Student)

Tumors	granuloasa (n=7)	ovarian fibroma (n=11)	ovarian thecoma (n=1)	fibrothecom (n=5)
ovarian fibroma (n=11)	2.56 ($p<0.05$)	-		
ovarian thecoma (n=1)	2.95 ($p<0.05$)	2.54 ($p<0.05$)	-	
fibrothecom (n=5)	2.85 ($p<0.05$)	1.39 ($p>0.05$)	-	-
mixom (n=3)	3.10 ($p<0.05$)	3.57 ($p<0,01$)	0.43 ($p>0.05$)	1.36 ($p>0.05$)

We dosed CA125 for the patients with clinical and ultrasound suspicion of neoplasic tumor. We had values inside the normal interval but also very increased values, over 400 UI/ml. The evolutive stages of malignant tumors for which CA125 was positive were in most of the cases terminal stages, with distance and epiplonic dissemination.

Macroscopic anatomopathological exam offered important details regarding the aspect of the tumor, shape, consistence, colour. The lab dissection of the specimenes revealed the inside aspect, described the content (color, aspect, consistence, volume), septum and intratumoral vegetations.

Microscopic anatomopathological exam pointed out the invasion of ovarian stroma in benign and malign tumoral processes. The histological fragments were prelevated from different ovarian sites.

Imunohistochemical evaluation of ovarian tumors was used to point out the response of ovarian stromal cells to a specific marker. We followed both the ovarian tu-

mors in general and stromal ovarian tumors in particular and also the stromal reaction from the peritumoral area.

The main stromal reaction during a tumoral process is the stromal hyperplasia. Physiologically this is also increased with age. We choose to identify the stromal reaction in other types of ovarian tumors also, because we wanted to frame these changes in the global context of ovarian pathology. For *struma ovarii* ER was intense positive in stromal cell's nucleus from the peritumoral ovarian tissue and was negative in thyroid tissue (epithelial cells and stroma also). For serous cystic adenomas ER was positive in epithelial cells, weakly positive and moderate positive in nuclei from adjacent ovarian stroma. There were also cases when ER was not positive at all inside the stroma (fig. 1, 2).

For mucinous adenocarcinoma ER was negative in tumoral stroma and nuclei of epithelial tumoral cells but positive in peritumoral ovarian stroma (fig. 3, 4).

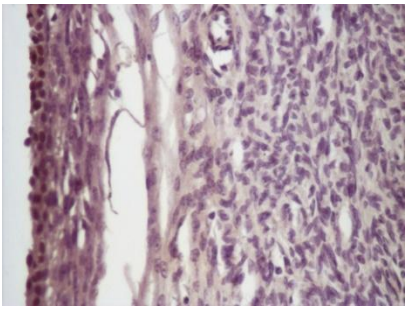

Fig. 1. ER (+) in epithelial cells, negative stroma

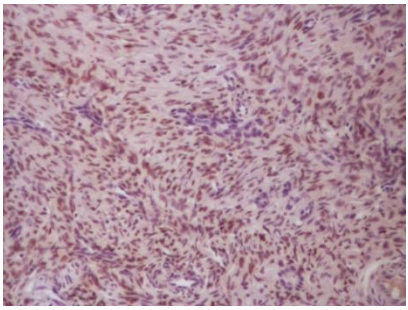

Fig. 2. Weak focal and moderate ER (+) stroma

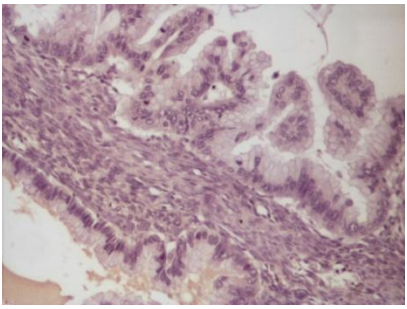

Fig. 3. ER (-) in tumoral tissue

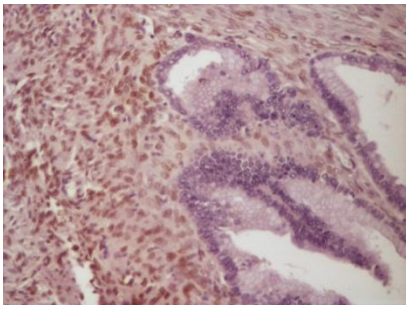

Fig. 4. ER (+) in tumoral stroma

For ovarian fibrothecoma ER was focal positive in tumoral stroma and negative in adjacent ovarian stroma (fig. 5, 6).

PR imunohistochemical detections were done especially in the nuclei. PR concentration for all groups of study was similar. It was detected inside nuclei, cystic invaginations developed from the isolation of invaginated epithelium, metaplastic epithelium and ovarian stroma. PR concentration was not dependent to menopause instalation.

For mucinous ovarian adenocarcinoma, PR was focal positive in tumoral stroma and negative in tumoral epithelial cells; and also positive in nontumoral ovarian stroma.

For cystic adenocarcinomas PR was negative in tumoral stroma and positive in nuclei of tumoral epithelial cells for one case and negative in tumor and focal weakly positive in stroma in the other case (fig. 7, 8, 9, 10).

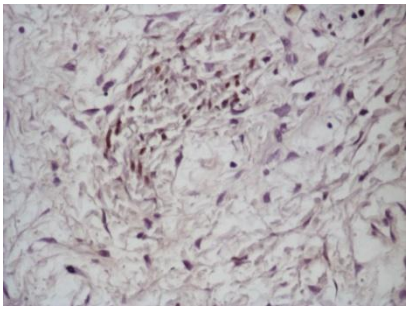

Fig. 5. ER(+) focal

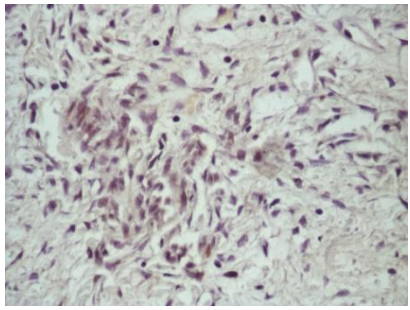

Fig. 6. ER increased (+) focal

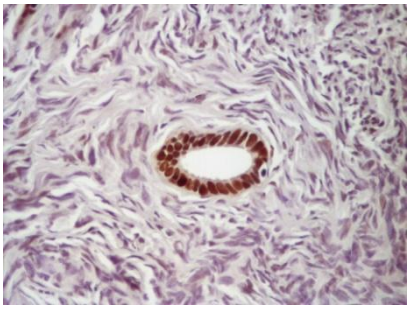

Fig. 7. Hiperplasic stroma with PR (-)

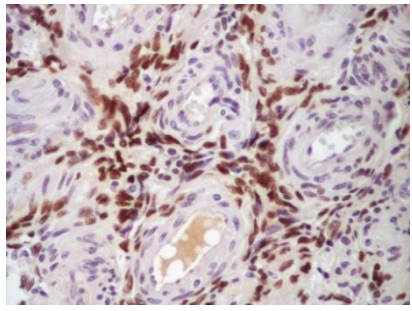

Fig. 8. Intense PR(+) medular stroma

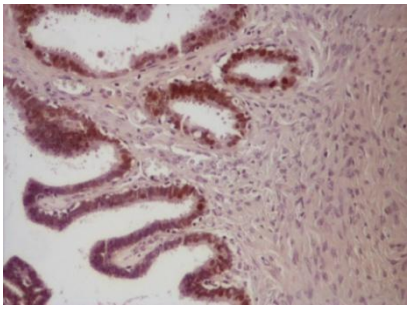

Fig. 9. PR intense (+) in the tumoral epithelium

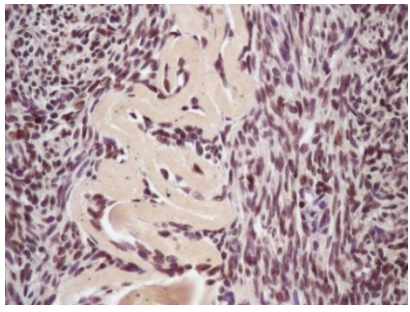

Fig. 10. Ovarian stroma weakly PR (+)

For ovarian fibrothecoma PR was positive in different degrees inside tumoral stroma and also inside adjacent stromal tissue.

For the dosage of SMA inside *struma ovarii* was negative inside epithelial tumoral cells and positive in tumoral stroma. In the extratumoral stroma SMA was also positive, same as vascular walls and other smooth muscular fibers. For the mucinous ovarian adenocarcinoma SMA was positive

in tumoral stroma and negative in epithelial tumoral cells. Inside normal stroma SMA is lightly positive, positive in vascular walls and other smooth muscular fibers. Fibrothecal tumoral cells were also intense positive for SMA (fig. 11, 12, 13, 14).

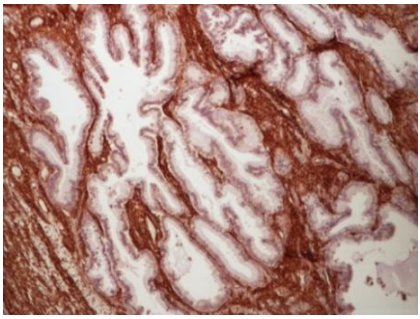

Fig. 11. SMA intense (+) inside tumoral stroma

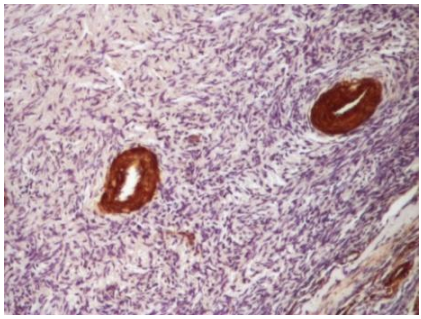

Fig. 12. SMA(-) inside ovarian stroma

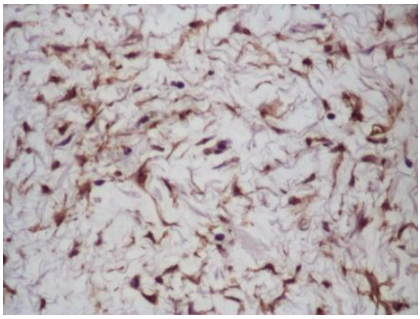

Fig. 13. SMA intense (+) tumoral cells

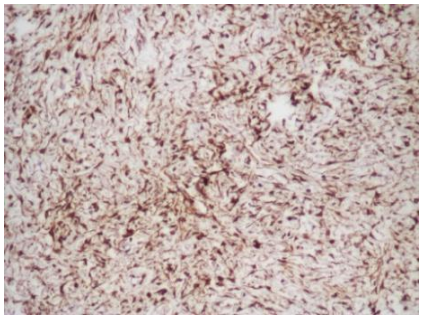

Fig. 14. SMA intense (+) tumoral cells

Nuclear expression of S100 in different stages is correlated with the aggressiveness of an ovarian cancer, autocrine and paracrine conditions playing a main role in the determination of the aggressiveness of ovarian neoplasic cells. For the studied ovarian stroma S100 was negative in the selected cases, we had only cases with positive intern marker such as small amielinic nervous fibers (fig. 15, 16).

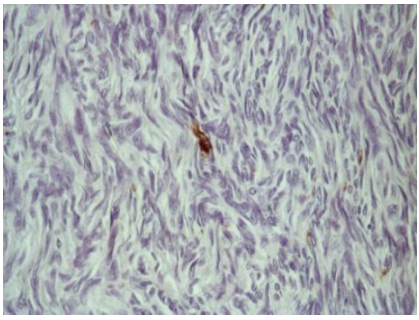

Fig. 15. S100 (-) in ovarian stroma

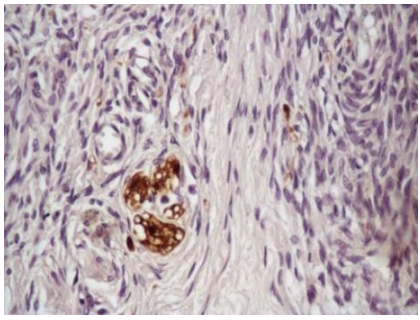

Fig. 16. S100 (-) in ovarian stroma

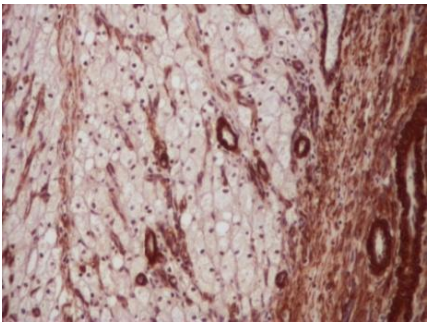

Fig. 17. Caldesmon (+) stroma

Fig. 18. Caldesmon intense (+) tumoral cells

Caldesmon dosage revealed variable results and was positive mainly inside stromal ovarian tumors but also inside tumoral stroma and adjacent peritumoral stroma from other types of tumors (fig. 17, 18).

CONCLUSIONS

The increasement of stromal network installed with age determined the reduction of vascular support. The tumoral progression also induces the multiplication of the stromal network. Our study developed on two lots of patients pointed general epidemiologic parameters, statistic differences for clinical, paraclinical and anatomopathological diagnose for both categories.

We pointed a frequent positivity for ER inside the ovarian stroma, in all types of tumors not only for those with main stromal component. The figures we presented demonstrated the behavior of ovarian stroma for ER in general, for all groups of age.

We noticed the overexpression of estrogen and progesteron receptors in ovarian stroma from the ovaries with fields of stromal thecal hyperplasia both nodular and diffuse.

For the current medical practice we can suggest the utility of SMA dosage for certitude histological diagnosis of ovarian tumors. We did not remark the necessity of usage of S100 for the differentiation of ovarian tumors for both lots. The positivity of ovarian stroma for Caldesmon is obvious especially for stromal tumors. In this case we also noted the positivity of the adjacent stroma and vascular stromal elements. We also noted the negativity of this marker for epithelial components and epithelial originated tumors. To conclude, Caldesmon can be used for the diagnosis of ovarian stromal tumors.

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NEWS

ELAFIN-EXPRESSING BACTERIA, BENEFICIAL FOR INFLAMMATORY BOWEL DISEASE

In a study by Motta *et al*, the intestinal mucosal expression of Elafin, a natural protease inhibitor, was determined in patients with inflammatory bowel disease. It was found that Elafin expression is diminished in those patients. Elafin has anti-inflammatory properties proved both *in vitro* and in animal models. Lactic acid bacteria were engineered to express and deliver Elafin to the inflammation site in colon. In mouse models of acute and chronic colitis, oral administration of lactic acid bacteria secreting Elafin decreased intestinal inflammation. In cultures of human intestinal epithelial cells treated with lactic acid bacteria expressing Elafin, the epithelium was protected from increased intestinal permeability and the release of cytokines or chemokines, which suggests that oral delivery of such bacteria could be useful in treatement of inflammatory bowel disease (Motta JP, Bermudez-Humaran LG, Deraison C *et al*. Food-Grade Bacteria Expressing Elafin Protect Against Inflammation and Restore Colon Homeostasis. Science Translational Medicine 2012; 4 (158): 158ra144 DOI: 10.1126/scitranslmed.3004212).

Teodora Vremeră

LISFRANC MIDFOOT DISLOCATIONS: CORRELATIONS BETWEEN SURGICAL TREATMENTAND FUNCTIONAL OUTCOMES

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LISFRANC MIDFOOT DISLOCATIONS: CORRELATIONS BETWEEN SURGICAL TREATMENT AND FUNCTIONAL OUTCOMES (Abstract): Lisfranc dislocations and fracture dislocations are the most common severe injuries of the foot. **Aim:** To assess the functional outcome of patients with Lisfranc dislocations of the midfoot by applying the latest methods of diagnosis and treatment. **Material and methods:** The study reviewed 31 patients with dislocations and fracture- dislocations of the Lisfranc joint over a 10 years period. The average follow-up period was 44 months (range 12 - 108). Injuries were classified according to Myerson scale. **Results:** The outcomes were evaluated using the Baltimore Painful Foot score (PFS) and American Orthopaedic Foot and Ankle Society (AOFAS) mid-foot scoring scale. 10 patients had an excellent outcome on the PFS scale, 8 were classified as good, 13 fair and poor. The average AOFAS score for the midfoot, used for results interpretation was 72 (range 52-92). **Conclusions:** Of all methods of surgical treatment used, the highest scores were obtained by internal fixation with screws. Eight patients (25.8 %) developed posttraumatic arthritis of the tarsometatarsal joints. **Key words:** TARSOMETATARSAL DISLOCATION, LISFRANC JOINT, INTERNAL FIXATION, FUNCTIONAL OUTCOME

Lisfranc injury is relatively rare, with an incidence variously reported between one per 5,500 fractures per year (1), one per 60,000 populations per year (2)_or accounting for 0.2% of all fractures (3). This term is used in present to describe a large spectrum of posttraumatic injuries of the midtarsal region of the foot (1). Diagnosis is aided by high-quality radiographs (AP, 30° oblique and lateral views). Diagnosis is often overlooked, especially in the multiply injured patient with other distracting injuries (3, 4), which can significantly reduce outcome (2) with resultant long-term disability (5, 6). A spontaneous partial or complete relocation is not unusual (4, 7) and may complicate accurate assessment. A high index of suspicion must be maintained for these injuries, and additional imaging, stress radiographs (2), weight-bearing radiographs (6), CT (8, 11) or MRI (4) performed as indicated.

Although some controversy exists about the optimal method of treatment (6, 8, 10, 12) most authors now advocate anatomic reduction with internal fixation as the

treatment method of choice (2, 13, 14). The internal fixation methods of Lisfranc midfoot injuries were exposed in The Manual of Internal Fixation (7), recommended by Association of Osteosynthesis (AO). Although pin fixation has their own supporters (3), the postoperative and functional outcomes have a relatively high failures rate (7). This study reviews the outcome of Lisfranc midfoot injuries treated by different methods. In addition, it attempts to determine whether there is a correlation between traditional physician-based outcome and patient based outcomes in order to establish a benchmark for future assessments (3, 11).

MATERIAL AND METHODS

We conducted a preliminary study on a series of 31 patients with Lisfranc tarsometatarsal fracture-dislocations, investigated and operated between January 1, 2001 and 31 of December 2010 in the Orthopedic Unit of the Suceava Emergency County Hospital, Romania. A historical retrospective study was performed and all case notes and radiographs were examined. Data was collected from patient charts under the following headings: age, gender, date of injury, side of injury, delay in diagnosis, mechanism of injury, classification of injury, associated injuries, treatment method, method of wound closure and any resultant complications. Injuries were classified on reviewing the initial radiographs according to the scale proposed by Myerson (14). The patients were evaluated in terms of postoperative course, incidence of early and late complications, functional outcome of surgery, depending on the severity of injuries and treatment methods applied. A descriptive statistical analysis and a correlation study were conducted in order to evaluate the relation between different internal fixation methods and functional outcome scores at 3 and 6 months following surgery. Outcome instruments used were the Baltimore Painful Foot score (PFS) (14) and the American Orthopaedic Foot and Ankle Society (AOFAS) mid-foot scoring scale (9).

RESULTS

The annual incidence of Lisfranc injuries kept increasing especially in the last 4 study years, culminating in 2010 with 5 cases out of 2100 admissions to our unit. Age distribution of the 31 patients available for follow-up was 18 to 67 years (mean age 39 years). There were 19 male (61.3 %) and 12 female patients (38.7 %). Left foot was involved in 18 patients and the right in 13. In one patient with multiple injures a diagnosis of |Lisfranc injury was initially missed but treated within 2 weeks of the original injury. Ten patients (24%) with multiple associated injures, but only two of them in traumatic or hemorrhagic shock, were admitted to ICU for monitoring of the general condition and hemodynamic rebalancing (6, 11). Isolated foot injuries occurred in 21 patients.

The mechanism of injury was: crush injury at workplace in 4 patients; motor vehicle accident in 12 cases, motorbike accident in 7 cases („high velocity trauma": 74.2%), and in 8 cases the injury occurred during sports activities or falling from height („low velocity trauma": 25.8%). Associated injuries in polytrauma patients were: neurological trauma in 2 patients, rib fractures in 3, long bone fractures in 5, intraabdominal injuries in 1 patient. Twenty patients had associated tarsal fractures (4 cases, 12,9%), metatarsal fractures (5 cases, 16.1%), metatarso-phalangeal dislocation (1 case), with only 11 patients having

neither (35.4%). The loco-regional complications were quite common, involving the dorsalis pedis artery (n=4), the peroneal nerve (n=2), open fracture-dislocations (n=4), crushing syndrome (n=2), compartment syndrome (n=5). Twenty-nine patients had primary wound closure, and 2 secondary closures, split skin grafting being performed in 2 cases. Average follow-up was 44 months (range 12–108).

Injuries were classified according to Myerson scale. A type A1 injury was found in 9.7% (*n*=3) of the cases, type A2 in 19.3% (n=6), B1 in 6.4% (*n*=2), B2 in 12.9% (*n*=4), C1 in 32.2% (n=10), and C2 in 19.3% (*n* =6) (fig.1).

Fig. 1. Lisfranc divergent fracture dislocation type C2 Myerson

TREATMENT PRINCIPLES

The patients with closed Lisfranc mediotarsal traumas were subjected to surgery in a variable period of time that did not exceed 24 hours since their admission (12). Generally, the surgical approach was by the two longitudinal, standard incisions, recommended by the specialty literature (6). This approach was practiced in 28 patients with dislocations or midtarsal fracture–dislocations, including those who developed a compartment syndrome (5 patients), this way both syndrome diffusion (11) and open reduction and internal fixation being obtained. In 3 patients we practiced a single medial incision due to the presence of the isolated tarsal-metatarsal lesions, strictly located at the level of the internal spine (4). By this approach we have performed the open reduction and the fixation of the first three tarsal-metatarsal joints.

The surgical technique used in all the patients was that of anatomical open reduction followed by the internal fixation under direct visual control (12) by different fixation techniques. We have chosen this technique, being the most modern and accepted technique by most of the nowadays specialists (7, 13, 15).

In twenty patients (64.4%) surgery was performed between 2001 and 2008. The technique and algorithm for introducing the pins were not generally standardized. 1, 8 and 2 mm Kirschner wires were used. Usually the lesions were approached by the two previously mentioned longitudinal incisions

Between 2008 and 2010, we have tried to standardize the internal fixation according to AO-ASIF principles in 11 patients (35,6%). Therefore, we introduced internal fixation by screws, observing the internal fixation principles and algorithm in order to minimize the error rate and to ensure a generally rigid stabilization of the mediotarsal and especially of the internal spine. This algorithm strictly follows the steps recommended by the AO school (6,7), the screws being introduced after the anatomic tarsometatarsal reduction. The initial temporary fixation was done by K-wires, after the entire exposure of the lesions and their reduction through the two parallel longitudinal incisions. Fixation of the medial column was done after having tested the intercuneiform instability neutralized by screw fixation. Fixation of the lateral column

was not always necessary after the fixation of the medial column due to post-reduction secondary stability (6, 7) (in only 5 of 11 patients treated by screwing the medial column); other 4 patients (12.9%) presented only isolated lesions to the medial column, without surgically approaching the lateral column (fig.2). The associated metatarsal fractures were fixed by K-wiring in 3 patients (9.6%) or by fixation with miniplates to the comminute fractures in 2 cases (6.4%).

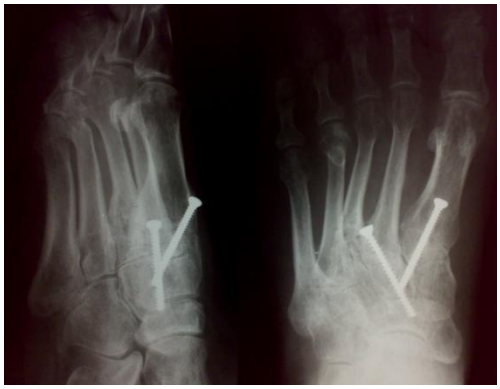

Fig. 2. Isolated injury of the medial column (type A2 Myerson) with screw fixation: radiograph images 6 month after surgery

Post-surgical care included the immobilization with well cushioned below-knee cast for a period of 6 weeks. Sutures were removed 14 to 21 days after surgery, and anticoagulation Low Molecular Weight Heparin (LMWH) treatment was extended up to 6 weeks after surgery (12).

In the patients in whom the pinning method was used, we usually chose to extend the immobilization period up to 8-10 weeks for the prevention of secondary displacements. A foot support was usually done late in the patients with pin fixation, 10-12 weeks after surgery, respectively. The osteosynthesis materials (K-wires) were usually removed 6-8 weeks (6, 14) after the immobilization period ended in 29 patients (with only pins or pins associated to screws). We have also chosen to initiate an early recovery treatment plan (at 6 weeks), with a progressive relapse of the partial foot support, followed by total foot support after 8-10 weeks (16). Algoneurodystrophic Leriche syndrome had a higher incidence (2, 4), especially in the patients with mediotarsal dislocation when the exclusive pin-fixation technique was applied. Fourteen of a total of 20 patients operated by this technique (70%) had pain, paresthesia, swelling, cyanosis of the foot, and loco-regional osteoporosis on radiographic images in the late postoperative period after plaster immobilization suppression (8 to 12 weeks) (14, 15).

The patients operated during the last three study years by stable internal fixation (screwing techniques) had a much lower incidence of Leriche syndrome as a result of lower assets (6 weeks) associated with active rehabilitation treatment (15) (2 of 11 patients developed this complication, 18.2%, respectively).

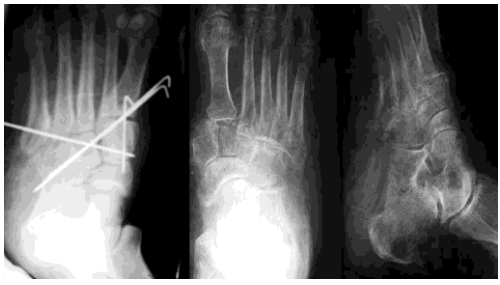

Fig. 3. Severe midfoot arthritis after Lisfranc fracture dislocation with K-wire fixing, secondary displaced (postoperative and after 6 month radiographs images).

Midtarsal osteoarthritis had a fairly high incidence (16, 17), especially in patients operated during the first study years by K-

wire fixation (fig. 3). Of these 20 patients, 6 patients developed mediotarsal osteoarthritis (30%). In the group operated with screw techniques of the medial column, only 2 of 11 patients developed midtarsal arthritis, respectively 18.2%. Overall, the incidence of posttraumatic midtarsal arthritis was 25.8% (8 patients).

DISCUSSION

The aim of the study was to review: the functional results after these injuries; which factors influence the outcomes; which surgical treatment yielded the best functional outcomes; the incidence of posttraumatic midtarsal arthritis following these injuries. Functional outcome was assessed in all patients included in the study, using the AOFAS scale for midtarsal region of the foot, developed by the American Orthopedic Foot and Ankle Society (3). This scale has three major items, pain, function and alignment, the total score being set at 100 points. Each item is scored depending on the severity of organ or functional damage). Thus, for a healthy foot, the highest score 100; the score gradually decreasing according to the functional impairment of the midtarsal region found.

We intentionally joined the group of 20 patients operated in the interval 2001-2008, who exclusively underwent internal pin fixation, with the group of 11 patients operated in 2008-2010, in which we applied stable internal fixation, according to the AO screw fixation techniques of the medial column. The functional assessment scale was administered individually to all patients. The incidence of late postoperative complications was high, especially in patients with internal pin fixation: midtarsal osteoarthritis, foot deformities, and painful neurodystrophic syndrome, as compared to the group of patients operated by means of modern internal fixation. The functional prognosis of patients with midtarsal dislocations, measured by AOFAS scale (3) is quite poor in the group of patients treated by pin fixation methods.

The end results showed an average AOFAS score of only 62 in the patients operated exclusively by pin fixation techniques, and an average AOFAS score of 90 in the group of patients operated by AO standard fixation methods. Overall, the study patients had an average AOFAS score of 72 points.

In our study, the Baltimore Painful Foot Score (12, 14), that quantifies the outcomes of the treatment measuring pain, function, gait, cosmesis and motion, the outcome was excellent (90 to 100) in 10 patients, good (75 to 90) in 8, fair (60 to75)in 8, and poor (less than 60) in 5. The average PF score was 75. There were 18 patients (58%) with good and excellent results, and 13 with fair or poor results (42%). Twenty patients with open reduction and pinning fixation performed between 2001 and 2008 had an average PF score of only 68. The reminder of 11 patients with open reduction and internal screw fixation performed between 2008 and 2010 had an average PF score of 92.

CONCLUSIONS

The treatment of these lesions should be standardized for all tarsometatarsal lesions, but with the therapeutic characteristics of open traumas, crush syndrome, or associated vasculoneural lesions (11). As a rule we applied first-intention surgical treatment with the first 24 hours, regardless of the anatomoclinical variety, comprising open anatomic reduction and screw fixation, using the screw insertion algorithm for fixing the medial column (7, 9, 11). Pin fixation of

the lateral column, of the metatarsus, or fixation with screwed-in miniplates can also be included (13). Internal fixation of the medial column using open or percutaneous pin fixation has a poor prognosis because of the high incidence of secondary displacements and posttraumatic arthritis (17), so we abandoned this technique.

Starting physical therapy immediately after the 6-8 weeks period is compulsory, progressively resuming the support function of the foot until full support at 10-12 weeks, and wearing foot ware arch cushions up to 12 months after surgery to support the arch of the foot. The goal is to lower the incidence of the complex regional pain syndrome, foot stiffness, and walking disorders (14, 15).

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BEHAVIORAL INVOLVEMENT OF IMIDAZOLINE SYSTEM

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BEHAVIORAL INVOLVEMENT OF IMIDAZOLINE SYSTEM (Abstract): The imidazoline system consists in imidazoline receptors I_1, I_2 and I_3 and the active as well as that there are some endogenous ligands. We consider that one of the most important functions of this system is modulation of different types of behavior. Existent data show that I_1 and I_2 receptors have different effects on some behavior components, such as suicidal behavior, stress, anxiety, food intake etc. We consider that selective I_1 and I_2 agonists could become drugs to be used in the therapy of some behavioral disorders. The fact that some agonists or antagonists of imidazoline receptors have also effect on alpha-adrenergic receptors or MAO (monoamine oxidase) enlarges the use area of the compounds in behavioral disorders. **Key words**: IMIDAZOLINE RECEPTORS I_1 AND I_2, BEHAVIOR, DEPRESSION, FOOD INGESTION

The imidazoline system consists in imidazoline receptors I_1, I_2 and I_3 and the active endogenous substances acting on them and it represents an important system for regulating the cell functions. Existing evidence show the involvement of the importance of imidazoline in modulating central nervous system (1). One of the most important natural agonists is agmatine. The imidazoline system has at least two important characteristics: some agonist and antagonists also act on the alpha-adrenergic system and agmatine is synthesized out of arginine, which is also a source of nitric oxide. One of the important endogenous systems in our body is the imidazoline system. It is known that there are two types of well-described receptors for this system (I_1 and I_2 receptors), as well as that there are some endogenous ligands. Also, there are known certain types of different effects mediated through this biological system. One of the most important fields where the imidazoline system is known to be involved in is represented by human and animal behavior. Imidazoline system is important for both normal and pathological behavior. In case of certain neurological and especially psychiatric diseases, changes in the imidazoline system balance have been detected:

a. Involvement in depression and suicidal behavior;
b. Involvement in mood disorder;
c. Involvement in stress;
d. Involvement in the locomotor activity.

DEPRESSION AND SUICIDAL BEHAVIOR

One of the fields of real scientifical interest is represented by the involvement of

imidazoline system in major depression and in suicidal behavior. Piletz et al., 2000 (2) reported a down regulation of I_2 receptors in frontal cortex of suicidal victims with major depression. In case of deceased patients with major depression the imidazoline receptor binding protein band (40 - 50kDa) from the hippocampus of the subjects with major depression was significantly lower compared to controls. An extremely interesting fact is that the amount of protein-receptors decreases not only in the brain but also in the platelets (2).

Differential analysis of the amount of receptor protein for I_1 and I_2 receptors in the brain of major depression deceased suicidal patients showed that there is also an imbalance in the expression of the two receptors subtypes in the brain and also in the platelets. In the brain, the amount of 45kDa protein corresponding to I_1 receptors increases with 51% in the brain of suicidal victims, whereas in major depression patients with no suicidal attempts it decreases with 40% (3). The I_2 receptor subtype has been especially involved in the pathogenic mechanisms of psychiatric diseases (4).

One of the extremely interesting characteristics of these binding situs (I_2) is that some of the situs are localized to a specific domain of monoamineoxidase. Thus, it could be influenced the metabolism of biogenic amines and especially of brain catecholamine. Morever, it has been raise the problem that agmatine (natural I_1 and I_2 receptor agonist) should be considered a new neurotransmitter (5). Imidazoline I_2 receptors have a role in antidepression activity and probably in preventing the apparition of depression. An I_2 receptors agonists, 2-BFI, a compound which selectively inhibits (MAO-A) has a pronounced antidepressive effect in case of experimental animal depression. 2-BFI (100-300 micromol/kg) significantly reduced the immortality time in the tail suspension test on mice. The I_2 receptors agonists, 2-BFI, as well as agmatine (agonist of both I_1 and I_2 receptors) (5-10 μmol/kg i.p.) potentates the antidepressant effect of agmatine. We consider that brain agmatine has a physiological role as an endogenous antidepressant as in mice exposed to FST the brain level of agmatine increases SSRIs (but not imipramine) determined an increase in agmatine brain level (6). Unlike I_2 receptors, which stimulatins determines antidepression effects, The I_1 receptors are increased in platlets but also on peripheral. Agmatinase, an inactivator of the putative endogenous antidepressant agmatine, is strongly upregulated in hippocampal interneurons of subjects with mood disorders (7).

The antidepressive effect of the selective serotonin re-uptake inhibirotors involves the imidazoline receptors activation. Regarding the mechanism of antidepression action of agmatine, it is considered as been produced on the following routes (which are activated upon imidazoline receptors activation):

a. agmatine reverses the imidazoline induced calcium concentration increase (8);
b. agmatine reverses the norepinephrine and dopaminic cell content;
c. agmatine inhibits the nitric oxide synthase and down regulates inducible nitric oxide synthase (4).

Also, it is possible that alpha-2 receptors are also involved in agmatine antidepressant activity, as this kind of action is reduced by agmatine (9).

ANXIETY

Anxiety is also influenced by the imid-

azoline system. Agmatine is considered as an endogenous neuromodulator (4). Intracerebroventricular, as well as intraperitoneal agmatine administration elicits anxiolitic-like effects (and also anticonvulsivant and neurotoxic action). This compound has also an anxiolitic effect at 300 μmol/kg s.c. (10). The antidepressant-like effect of tramadol in the mouse forced swimming test (FST) is partially mediated by imidazoline system stimulation in the brain (11). The imidazoline system (including I_1 and I_2 receptors) is also involved in modulating stress response (1). Both hypothalamic-pituitary-adrenal (HPA) axis and the activity of other cerebral structures are influenced (12). Agmatine is considered as an endogenous neurotransmitter, which has the ability to influence the response to stress factors on at least two ways: by stimulating I_1 and I_2 receptors and by acting on adrenergic receptors, such as α_2. In brain and also in spinal cord, has been identified agmatine decarboxylases (enzymes involved in agmatine decarboxylation and agmatine synthesis). Agmatine is packaged in presynaptic vesicles and released after neuronal depolarization (1). Intraperitoneal administration of agmatine elicit in rats anxyolitic effect on experimental acute stress. Agmatine reversed the increase of intracellular Ca^{2+} concentration in neurons after NMDA receptors activation (8). The activity of stress agents increase the activity of brain glutamatergic system and brain agmatine level and may determine a modulatory effect on these systems. Agmatine or agmatine-analogues administration may determine beneficial effects in stress or stress-related disorders (1). It has been proved that the anxiolytic-like effect of ethanol in rats is potentiated by pre-treatment with agmatine (10 – 20 mg/kg, i.p.) as well as other substances which increase brain agmatine concentration, such as l-arginine and DFMO (a ornitine-decarboxylase inhibitor) (13). Also, agmatine reduced withdrawal anxiety. In case of this effect of agmatine, stimulation of both I_1 and I_2 receptors is involved, as it is decreased by both efaroxan (1mg/kg, i.p.) and idazoxan (0.25 mg/kg, i.p.). There are some differences regarding the involvement of imidazoline system in depression and anxiety. In depressed patients a decreased I_2 receptors density in platelets and other tissues was found, whereas this is not present in patients with generalized anxiety disorder (14).

NEUROPROTECTION

Imidazolines are also involved in neuroprotection, which we consider important for protection in case of stress, including post-traumatic stress.

Agmatine reduced the glutamate induced neurotoxicity in cultured neurons by blocking the NMDA glutamate receptors activity. The other imidazoline drugs as antazoline and cirazoline fully prevented fully prevented the glutamate neurotoxicity. Besides this, agmatine attenuated the brain posttraumatic edema by decreasing the aquaporin 1 expression in various types of central ischemia (15). Another behavioral effect is the ultrasonic vocalization produced by rat pups in isolation. Neonatal ethanol exposure produced this behavior and agmatine reduced this effect (16). Moxonidin, another I_1 agonist, determined a protective effect on glutamate induced neurotoxicity (17).

STRESS

Regarding the complex involvement of imidazoline receptors in stress, it is worth

mentioning that a synthetic I_2-receptors agonist named BU224 increased the immuno-like reactivity in a lot of brain area (hypothalamic paraventricular nucleus, amygdala, locus coeruleus) in both naïve rats and restrained-stressed rats (18).

FOOD INTAKE

The central imidazoline system also seems to be involved in eating behavior. Clonidine (alpha$_2$ and I_1 agonist) injected into the median raphe nucleus in rats changed the food intake in the anxiety-like in rats (19). On the other hand, water intake doesn't seem to be influenced by imidazoline system (20).

PAIN BEHAVIOR

The I_1 receptors stimulation by moxonidine combined with some opioid agonists of μ receptors produced a spinal antinociceptive synergy and inhibits substance P-elicited behavior (21). We think that administration of moxonidine opioids association may be effective not only to manage the pain, but also to reduce pain-related anxiety.

CONCLUSIONS

Regulation of human behavior is dependent on lots neuromodulators or neurotransmitters, molecules with specific roles in central nervous system. Most of these molecules act on receptors present in neurons or nevroglia. Imidazoline system has permanent interactions with other brain neurotransmitters systems, and mainly with catecholamine system. Existent data suggest that interactions between imidazoline system and other neuromediators systems are essential in behavior regulation. Drugs acting as selective agonists and antagonists of imidazoline receptors will have clinical uses as behavior modulators in the future.

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ANATOMIA CLINICĂ A JONCȚIUNII ESO-GASTRICE

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CLINICAL ANATOMY OF THE ESOPHAGOGASTRIC JUNCTION (Abstract): The esophagogastric junction is a controversial anatomical area, due to its sphincteric mechanism which does not show an obvious anatomical basis. The **aim** of this study is to investigate the anatomical components that endoscopically indicate the mucosal esophagogastric junction in hiatal hernia patients. **Material and methods**: The esophagogastric junction was investigated in 27 hiatal hernia patients undergoing surgery. Hiatal hernia is an extension of the stomach situated between the esophagogastric junction and the diaphragmatic indentation. The following types of hiatal hernia were found: sliding hiatal hernia (type I) in 4 patients (14,81%), rolling hiatal hernia (type II) in 2 (7,4%), mixed hiatal hernia (type III) in 12 (44,44%), type IV hiatal hernia in 4 (14,81%) and recurrent hiatal hernia in 5 (18,51%). Of the 27 hiatal hernia patients, 8 (29,6%) were operated using classical procedures: laparotomy – 6 (75%) and laparoscopic surgery – 2 (25%). **Results**: The angle of His cannot be used for marking the mucosal esophagogastric junction due to the severe damage of the lower esophageal sphincter in hiatal hernia patients. The squamocolumnar junction is displaced in hiatal hernia patients and was not an option for the study group. The distal end of the esophageal longitudinal palisading vessels needs medication (proton pump inhibitors that reduce the gastric acid production), in order to enhance the visibility of these vessels. The proximal end of gastric longitudinal mucosal folds proved to be the most reliable site to identify endoscopically the mucosal esophagogastric junction. **Conclusions**: The anatomical structure of the esophagogastric junction differs in hiatal hernia patients and these peculiarities are very important in surgery. **Key words**: ESOPHAGOGASTRIC JUNCTION, CLINICAL ANATOMY, HIATAL HERNIA

Joncțiunea eso-gastrică reprezintă o zonă anatomică extrem de controversată, din cauză că, deşi în regiunea aceasta care leagă esofagul de stomac nu s-a descoperit niciun sfincter muscular neted, există totuşi un mecanism funcțional care indică relaxarea şi contracția unui sfincter. Presiunea exercitată de joncțiunea eso-gastrică normală este extrem de importantă pentru digestie, prin controlul intrării alimentelor în stomac, dar şi prin blocarea revenirii acestora. Din cauza fiziologiei sale, care nu pare să aibă o bază anatomică, joncțiunea eso-gastrică rămâne o regiune a cărei microstructură nu este încă bine înțeleasă. Chiar dacă o serie de studii anatomice au demonstrat o uşoară creştere a stratului muscular circular din jurul joncțiunii eso-gastrice, anatomiştii resping totuşi utilizarea termenului „sfincter", dată fiind absența unei îngroşări a stratului muscular (1). Muşchiul diafragmatic din hiatusul esofa-

gian funcționează ca un sfincter (2). De fapt însăşi natura acestei zone de graniță între esofag şi stomac nu este clară, ea fiind considerată fie musculară, fie mucozală.

Dat fiind că joncțiunea eso-gastrică este investigată nu doar în anatomie, fiziologie, gastroenterologie şi chirurgie, ci şi în imagistică, imaginile obținute prin rezonanță magnetică nucleară au permis deja crearea unor modele computerizate tridimensionale (3) ale acestei zone care continuă să fie investigată şi în prezent.

În stările patologice, structura musculară a joncțiunii eso-gastrice, care formează sfincterul esofagian inferior, degenerează şi devine relaxată, provocând dilatarea esofagului distal şi a joncțiunii eso-gastrice. Acest proces duce ulterior la apariția herniei hiatale.

MATERIAL ŞI METODE

Studiul de față abordează anatomia joncțiunii eso-gastrice din perspectiva chirurgului practician şi cu scopul de a verifica metodele de identificare a joncțiunii eso-gastrice la pacienții cu hernie hiatală. Cercetarea s-a efectuat în perioada ianuarie 2007 – martie 2011, pe un lot de 27 de pacienți cu hernie hiatală voluminoasă operați la Secțiile Chirurgie I şi Chirurgie II ale Spitalului Universitar de Urgență Militar Central „Carol Davila", Bucureşti. Din cei 27 de pacienți cu hernii hiatale voluminoase, 8 (29,6%) au fost operați prin procedeele clasice. Dintre aceştia, în 6 cazuri (75%) s-a intervenit prin laparotomie de la început, iar în 2 cazuri (25%) s-a procedat la conversie prin chirurgie laparoscopică.

REZULTATE ŞI DISCUȚII

În lotul de pacienți luați în studiu, s-au identificat următoarele tipuri de hernii hiatale: hernie hiatală de alunecare (de tip I) la 4 pacienți (14,81%), hernii hiatale de rostogolire (de tip II), la 2 (7,4%), hernii hiatale mixte (de tip III), la 12 (44,44%), hernii hiatale de tip IV, la 4 (14,81%) şi hernii hiatale recidivate, la 5 pacienți (18,51%) (tab. I).

TABELUL I

Tipuri de hernii hiatale voluminoase incluse în lotul de studiu

Tipul de hernie hiatală	Nr. de cazuri	%*
I (de alunecare)	4	14,81
II (de rostogolire)	2	7,4
III (mixte)	12	44,44
IV	4	14,81
Recidivate	5	18,51

*Pentru valorile mai mici de 10, proporțiile sunt orientative.

Diagnosticarea pacienților a fost complicată de problema definirii joncțiunii eso-gastrice din punct de vedere endoscopic şi histologic. Manometria nu a reprezentat o opțiune pentru diagnosticarea herniilor hiatale, ci doar o metodă de evaluare a intervenției chirurgicale în varianta manometriei intraoperatorii sau postoperatorii, când presiunea sfincterului esofagian inferior (structura musculară a joncțiunii esofagiene) a fost apreciată la peste 20 mmHg.

Pentru identificarea liniei Z, numite astfel din cauza formei sale în zig-zag (4) – joncțiunea dintre mucoasa esofagiană şi cea gastrică – s-au observat principalele elemente anatomice ale zonei esofagogastrice pe lotul de 27 de pacienți cu hernie hiatală voluminoasă operați la Spitalul Universitar de Urgență Militar Central „Carol Davila", Bucureşti. Joncțiunea eso-gastrică este poziționată în stânga vertebrei T11, pe planul orizontal care trece prin vârful procesului

xifoid. Joncțiunea eso-gastrică joacă un rol extrem de important în prevenirea refluxului gastrointestinal, prin funcționarea corectă a sfincterului esofagian inferior (5).

La pacienții cu hernie hiatală s-a observat că joncțiunea scuamocolumnară este dislocată proximal, în diverse grade, față de joncțiunea eso-gastrică. Se ştie că la persoanele sănătoase, joncțiunea eso-gastrică mucosală se suprapune pe joncțiunea scuamocolumnară.

Unghiul lui His nu s-a putut folosi pentru identificarea joncțiunii eso-gastrice la pacienții cu hernie hiatală voluminoasă, deoarece în cazul lor întregul sfîncter esofagian inferior este distrus în diferite grade, iar acest reper este lezat.

Capătul proximal al pliurilor gastrice longitudinale, un reper endoscopic al joncțiunii eso-gastrice mucozale (6), este strâns legat de joncțiunea eso-gastrică musculară. La persoanele sănătoase, joncțiunea scuamocolumnară este localizată la evaluarea endoscopică la 2 cm de capătul proximal al pliurilor longitudinale ale mucoasei gastrice. Pentru pacienții cu hernii hiatale, capătul proximal al pliurilor gastrice longitudinale este considerat în cadrul endoscopiei un reper anatomic fix al joncțiunii eso-gastrice mucozale (7). Obiecțiile care se pot aduce acestui reper se referă la faptul că pliurile mucoasei gastrice pot fi aplatizate în mod artificial prin dilatarea indusă endoscopic sau modificate în timpul respirației. Observațiile obținute la pacienții noştri sugerează că acest reper poate fi totuşi folosit la pacienții cu hernie hiatală voluminoasă.

Capătul distal al vaselor esofagiene longitudinale dispuse în palisadă reprezintă o altă modalitate de identificare endoscopică a joncțiunii eso-gastrice, care a fost folosită până în prezent în Japonia (8). Astfel, în esofagul distal, există multe vene longitudinale paralele, care străbat *lamina propria* şi se termină în joncțiunea mucozală a stomacului. Aceste vene sunt vizibile în timpul endoscopiei ca vase dispuse în palisadă, vizualizarea este însă posibilă numai în timpul unei inspirații profunde. Întrucât asemenea vase nu apar şi la nivelul stomacului, ele pot fi utilizate ca reper al joncțiunii eso-gastrice. Nu am aplicat această metodă la pacienții luați în studiu, din cauză că ar fi trebuit să le administrăm pacienților inhibitori de pompă de protoni, pentru a reduce nivelul de acid gastric şi pentru a îmbunătăți astfel vizibilitatea acestor vase, mai ales la cei care prezintă esofagită indusă de hernia hiatală voluminoasă.

Joncțiunea scuamocolumnară, pe baza căreia la subiecții normali se poate identifica joncțiunea eso-gastrică, nu a putut fi luată drept reper la pacienții cu hernie hiatală voluminoasă, deoarece în cazul acestora joncțiunea scuamocolumnară este dislocată şi se poziționează deasupra joncțiunii eso-gastrice mucozale.

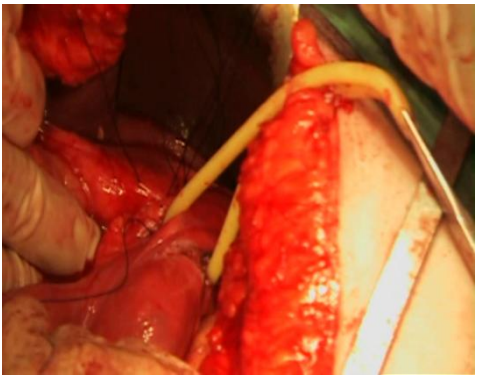

Fig. 1. Joncțiunea eso-gastrică la un pacient cu hernie hiatală voluminoasă

CONCLUZII

Anatomia joncțiunii eso-gastrice poate suferi modificări ample atât în procesele patologice maligne, cât şi în cazul patolo-

giei benigne. Dacă achalazia cardiei implică mai ales o îngroşare a peretelui esofagian inferior datorată hipertrofiei musculare circulare de la nivelul sfincterului esofagian inferior, în cazul herniilor hiatale modificările anatomice sunt diverse şi se datorează migrării joncțiunii eso-gastrice sau/şi a unei porțiuni de stomac din abdomen în mediastin. Herniile hiatale voluminoase, in care cel putin 30% din stomac, singur sau însoțit de alte organe, se află în cavitatea toracică, pot determina alterări profunde ale anatomiei locale, uneori foarte greu de restabilit. Astfel, identificarea esofagului şi a joncțiunii eso-gastrice se pot face numai după disecția minuțioasă a sacului de hernie şi abdominalizarea viscerelor.

Studiul de față a demonstrat că la pacienții cu hernie hiatală voluminoasă capătul distal al vaselor esofagiene longitudinale dispuse în palisadă s-a dovedit un element anatomic util în identificarea endoscopică a joncțiunii eso-gastrice.

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RELAŢIA DINTRE ADIPOKINE, MARKERI INFLAMATORI, SCĂDEREA SENSIBILITĂŢII LA INSULINĂ ŞI ELIMINAREA URINARĂ DE ALBUMINĂ LA SUBIECŢI NEDIABETICI

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ASSOCIATION OF ALBUMINURIA WITH ADIPOKINES, INFLAMMATION AND INSULIN RESISTANCE IN NON-DIABETIC SUBJECTS (Abstract): The objective of this study was to evaluate the association of traditional cardiovascular risk factors, adipokines, inflammation and insulin resistance with low-grade urinary albumin-to-creatinine ratio (ACR) in nondiabetic, healthy subjects. **Material and methods**: The study included 117 healthy subjects (64 men and 53 women), who were classified according to their body mass index (BMI) into three groups: lean (BMI<25 kg/m^2), overweight (25≤BMI<30 kg/m^2) and obese (BMI≥30 kg/m^2). **Results**: ACR was higher in obese men and women compared to overweight and lean men and women (men – 3 mg/g vs. 6.1 mg/g vs. 8.9 mg/g, p=0.018; women – 2.1 mg/g vs. 6.7 mg/g vs. 9.1 mg/g, p=0.03). In univariate analyses ACR was associated in obese men with adiponectin (r=-0.43, p=0.021) and systolic blood pressure (r=0.51, p=0.006) and in non-obese men with systolic blood pressure (r=0.39, p=0.041). In women systolic blood pressure was the only determinant both in obese (r=0.49, p=0.022) and non-obese subjects (r=0.42, p=0.04). **Conclusions:** Adiponectin is an important mediator of low-grade albuminuria in obese, non-diabetic men. **Key words**: LOW-GRADE ALBUMINURIA, ADIPONECTIN, OBESE, INFLAMMATION

Microalbuminuria este definită ca prezenţa unei eliminări urinare cuprinsă între 30-300 mg în urina colectată în 24 ore, sau ca raportul urinar albumină/creatinină, dintr-o emisie spontană de urină, cuprins între 30-300 microg/mg (1).

Datele epidemiologice arată că prevalenţa microalbuminuriei la persoanele adulte este de 30% la subiecţii cu diabet zaharat şi de 10-15% la subiecţii nediabetici (2). Eliminarea urinară de albumină, rezultat al creşterii permeabilităţii la nivelul endoteliului renal, este un marker al disfuncţiei endoteliale sistemice (2). Cossonert al (3) au demonstrat la un număr de 87 pacienţi cu diabet zaharat tip 2, faptul că subiecţii cu microalbuminurie, comparativ cu cei normoalbuminurici, asociau o afectare severă a răspunsului vasodilatator endoteliu-dependent la nivel coronarian. Creşterea eliminării urinare de albumină se asociază cu majoritatea factorilor de risc cardiovasculari: hiperglicemie, hipertensiune arterială, disfuncţie renală, dislipidemie, hiperhomocisteinemie, creşterea aportului proteic, fumat, prezenţa markerilor de fază acută. Dintre aceştia, diabetul zaharat şi hipertensiunea arterială sunt principalii factori

de risc pentru apariția microalbuminuriei (1). Microalbuminuria se asociază cu creşterea riscului cardiovascular atât la subiecții cu diabet zaharat, cât şi la subiecții nediabetici (4,5,6,7,8).

Studiile efectuate la subiecții nediabetici au arătat faptul că există o corelație pozitivă între eliminarea urinară de albumină, la valori mai mici decât valoarea prag pentru stabilirea diagnosticului de microalbuminurie şi riscul de apariție al afecțiunilor cardiovasculare (9,10,11,12).

O lungă perioadă de timp, s-a considerat că țesutul adipos are un rol pasiv, de depozitare, sub formă de trigliceride, a excesului de energie. Odată cu identificarea produşilor de secreție ai adipocitului, denumiți adipokine, cu acțiuni atât locale, cât şi la distanță, a devenit clară legătura dintre țesutul adipos visceral şi factorii de risc metabolici. Adipokinele (13,14) sunt principalii mediatori ai legăturii dintre obezitate şi modificările metabolice. Aceşti produşi de sinteză ai țesutului adipos au atât efecte directe cât şi indirecte în reglarea apetitului, a metabolismelor glucidic, lipidic şi proteic şi a procesului inflamator. La subiecții obezi, majoritatea adipokinelor sunt sintetizate în exces, conducând la accentuarea disfuncției metabolice. Adiponectina este un hormon unic al țesutului adipos cu efect antidiabetic, antiinflamator şi antiaterogen (15,16). Concentrația plasmatică a adiponectinei este scăzută atât la subiecții obezi (17), cât şi la subiecții cu diabet zaharat tip 2 (18). Rolul antiaterogen al adiponectinei se manifestă la nivelul tuturor etapelor procesului aterosclerotic: disfuncție endotelială, formarea şi progresia plăcii de aterom, ruperea plăcii de aterom şi formarea trombusului (19,20). Date recente arată că adiponectina are un rol cheie în medierea relației dintre țesutul adipos şi microalbuminurie, asociere ce poate avea un rol important în creşterea riscului cardiovascular la subiecții obezi (21, 22, 23, 24).

În acest studiu am evaluat corelațiile dintre adipokine (adiponectină şi leptină), markeri inflamatori (PCR-reactivă cu sensibilitate crescută), markeri ai sensibilității la insulină (insulină şi HOMA-IR) şi eliminarea urinară de albumină la subiecți nediabetici, în funcție de indicele de masă corporală.

MATERIAL ŞI METODĂ

Subiecți

În studiu am incluşi un număr de 117 subiecți (64 bărbați şi 53 femei), nediabetici, fără afecțiuni cardiovasculare, selectați pe o perioada de 1 an (01.02.2009-30.01.2010) atât din rândul pacienților aflați în evidența Ambulatoriului Spitalului Clinic Colentina – Cabinetul de Diabet, Nutriție, Boli Metabolice, cât şi din rândul pacienților internați în Secția de Diabet, Nutriție, Boli Metabolice a Spitalului Clinic Colentina. Înainte de includerea în studiu, toți subiecții au semnat consimțământul informat. Participanții la studiu au fost împărțiți în funcție de indicele de masă corporală în următoarele trei grupe: obezi (IMC≥30 kg/m2) – 56 subiecți (31 bărbați şi 25 femei), supraponderali (25≤IMC<30 kg/m2) - 43 subiecți (24 bărbați şi 19 femei) şi normoponderali (IMC>25 kg/m^2) - 18 subiecți (9 bărbați şi 9 femei). Au fost excluşi subiecții fumători, aflați în tratament hipotensor, hipolipemiant, cu neoplasm, afecțiuni endocrinologice (acromegalie, hipotiroidie, hipertiroidie, sindrom Cushing), pancreatită acută, pancreatită cronică, hemocromatoză, pancreatectomie, hepatită cu virus B, hepatită cu virus C, boli cronice inflamatorii sistemice, cu va-

lori ale tensiunii arteriale ≥ 170/100 mmHg, trigliceridelor ≥ 400 mg/dL, colesterolului total ≥ 300 mg/dL şi creatininei serice ≥ 1,5 mg/dL.

Determinări de laborator

Probele de sânge au fost prelevate în cursul dimineții, între orele 8.00-10.00 (după repaus alimentar de minim 8 ore), pentru determinarea: hemoleucogramei, glicemiei, colesterolului total, HDL-colesterol, trigliceride, hemoglobina glicozilată (HbA1c), acid uric, fibrinogen, TGO, TGP, GGT. LDL-colesterol a fost calculat folosind formula Friedewald (LDL-colesterol = colesterol total – (HDL-colesterol + triglicerid/5). Adiponectina, leptina, proteina C-reactivă înalt sensibilă (hs-CRP) şi insulina au fost determinate folosind kituri produse de către DRG International, Inc., prin tehnica ELISA, utilizând un autoanalizator Dynex. Analizele au fost efectuate în cadrul Laboratorului Gral Medical Colentina. Sensibilitatea la insulină am evaluat-o folosind HOMA-IR (homeostasis model of insulin resistance) după următoarea formulă: glicemia „à jeun" (mg/dL) x insulinemia „à jeun" (µU/mL)/ 405. Folosind această formulă, subiecții cu valori scăzute ale HOMA-IR prezintă un grad mai mare de insulinosensibiliate. Masa de țesut adipos am calcalat-o prin metoda bioimpedanței, folosind un analizor Omron BF500.

Prelucrarea statistică

Analiza statistică am realizat-o cu ajutorul aplicațiilor GraphPad Instant 3, NCSS 2004, PASS 2002 şi Microsoft Excel 2003. Variabilele continui cu distribuție normală, le-am prezentat sub formă medie şi deviație standard (DS), datele cu distribuție non-normală (adiponectina, leptina, hs-PCR, insulina, HOMA-R, raportul albumină/creatinină urinară) le-am prezentat sub formă mediană şi diferența între quartila a treia şi prima quartilă. Variabilele continui au fost testate pentru distribuția normală cu testul Kolmogorov-Smirnov. Compararea mediilor între două grupuri, am realizat-o folosind testul *t-Student*, pentru variabilele continui cu distribuție normală şi folosind testul Mann Whytney U pentru variabilele continui cu distribuție non-normală. Pentru compararea mediilor între trei sau mai multe grupuri, am folosit testul de analiză a variației (ANOVA), pentru variabilele continue cu distribuție normală şi pentru variabilele cu distribuție non-normală testul Kruskal-Wallis, cu testul Dunn pentru comparațiile multiple dintre variabile. Analiza de corelație între variabilele continui, am efectuat-o prin determinarea coeficienților de corelație Pearson, variabilele continui cu distribuție non-normală fiind transformate logaritmic (log_{10}). Pentru toate testele, pragul de semnificație statistică a fost de p=0,05.

REZULTATE

Caracteristicile subiecților incluşi în studiu

Caracteristicile subiecților incluşi în studiu sunt prezentate în tabelul 1. Nu au existat diferențe între grupele analizate în ceea ce priveşte vârsta. Valorile medii ale următorilor parametri cresc semnificativ statistic odată cu indicele de masă corporală: circumferința abdominală, procentul de țesut adipos, glicemia bazală, tensiunea arterială sistolică, tensiunea arterială diastolică (diferența a fost semnificativă doar în cazul bărbaților), trigliceridele, TGO, TGP, GGT, acid uric. Procentul de masă adipoasă a fost semnificativ mai mare la femei comparativ cu bărbații la toate cele trei grupe analizate. Valoarea medie a HDL-colesterol a scăzut odată cu creşterea IMC. În ceea ce priveşte concentrația markerilor inflamatori, rezultatele obținute

arată, la ambele sexe, creşterea medianei hs-CRP şi a valorilor medii ale numărului de leucocite, odată cu creşterea indicelui de masă corporală. În cazul adipokinelor, la ambele sexe mediana adiponectinei scade odată cu creşterea indicelui de masă corporală, în timp ce mediana leptinei creşte odată cu creşterea indicelui de masă corporală. În ceea ce priveşte evaluarea insulinosecreției şi a sensibilității la insulină, atât mediana insulinemiei bazale, cât şi a HOMA-IR cresc odată cu creşterea IMC.

TABELUL I

Caracteristicile subiecților incluşi în studiu

	Normoponderali	**Supraponderali**	**Obezi**	**p**
Vârsta (ani)	54,6±7,1	54,5±9,4	52,9±8,7	0,67
IMC (kg/m^2)	24±0,5	27,2±2,9^a	34,1±3,3^{bc}	**<0,0001**
Masa de țesut adipos (%)	22,7±0,6	27,7±0,8^{a,}	34,9±0,8^{bc}	**<0,0001**
Glicemia bazală (mg/dL)	80,3±10,2	87,5±14,2	96,2±15,3^b	**0,044**
TAS (mmHg)	123,6±10,7	136,5±13,6^a	140,5±14,5^b	**0,02**
TAD (mmHg)	75,8±8,1	78,9±9,4	83,4±6^b	**0,038**
HDL-colesterol (mg/dL)	51,3±6,6	41,9±7,1^a	38,7±6,2^b	**0,034**
LDL-colesterol (mg/dL)	126,4±35	132,9±36,7	141,6±31,2	0,09
Trigliceride (mg/dL)	97,6±27,5	126,7±34,5	176,2±53,1^{bc}	**0,03**
TGO (U/L)	19,7±6,2	26,2±9,1^a	35,4±12,6^{bc}	**0,022**
TGP (U/l)	21,5±7,5	28,9±10,1^a	37,8±13,5^{bc}	**0,019**
GGT (U/l)	18,1±4,8	23,4±6,9^a	31,3±11,8^{bc}	**0,009**
Hs-CRP (mg/l)	1,4 (0,78-1,83)	2,35 (1,33-3,53)^a	2,95 (1,75-6,55)^b	**<0,0001**
Leucocite (10^3/mm^3)	5,84±1,6	6,41±1,8	7,36±2,1	**0,039**
Acid uric (mg/dL)	4,3±1,4	4,7±1,6	5,3±1,9^b	**0,021**
Insulinemie (µU/ml)	6,4 (4,97-8,79)	13,9 (9,75-19,53)^a	15,8 (10,04-27,35)^b	**0,0002**
HOMA-IR	1,77 (1,48-2,91)	3,16 (2,17-5,34)	3,86 (2,31-8,43)^b	**0,012**
Adiponectina (µg/dL)	6,26 (4,51-8,34)	4,31 (2,15-5,46)^a	3,48 (1,54-5,43)^b	**0,0004**
Leptina (ng/ml)	25,4 (10,8-37,1)	30 (19,18-48,18)	40,05 (20,07-73,65)	**0,042**

Datele sunt prezentate sub formă de medie±DS şI mediană (intervalul dintre prima quartilă şi a treia quartilă); a) p<0,05 ,SP vs. NP; b) p<0,05, OB vs. NP; c) p<0,05, OB vs. SP.

Eliminarea urinară de albumină în funcţie de indicele de masă corporală

Eliminarea urinară de albumină evaluată cu ajutorul raportului albumină/creatinină urinară a crescut odată cu creşterea indicelui de masă corporală atât la bărbaţi (normoponderali *vs*. supraponderali vs. obezi, 3 mg/g *vs*. 6,1 mg/g vs. 8,9 mg/g, p-0,018), cât şi la femei (normoponderali vs. supraponderali *vs*. obezi, 2,1 mg/g vs. 6,7 mg/g *vs*. 9,1 mg/g, p=0,03). Nu au existat diferenţe în ceea ce priveşte eliminarea urinară de albumină între cele două sexe (tab. II). Dintre subiecţii analizaţi, microalbuminuria a fost prezentă la 5 subiecţi (4,27%), dintre care 3 bărbaţi (4,69%) şi 2 femei (3,77%). Distribuţia subiecţilor cu microalbuminurie în funcţie de indicele de masă corporală, a fost următoarea: 2 subiecţi supraponderali (un bărbat şi o femeie) şi 3 subiecţi obezi (2 bărbaţi şi o femeie).

Analiza relaţiilor dintre parametrii analizaţi şi eliminarea urinară de albumină

La toţi bărbaţii nediabetici incluşi în studiu, în analiza univariată, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,46, p=0,0003) şi s-a corelat negativ cu adiponectina (r=-0,26, p=0,047) (tabel 3). La bărbaţii neobezi, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,39, p=0,041). La bărbaţii obezi, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,51, p=0,006) şi s-a corelat negativ cu adiponectina (r=-0,43, p=0,021) (fig. 1).

La subiecţii obezi, corelaţia dintre raportul albumină/creatinină urinară şi adiponectină a rămas semnificativă din punct de vedere statistic după ajustarea pentru tensiunea arterială sistolică (coeficient de regresie = - 0,348; 95% interval de încredere, 0,0075, 0,059; p=0,042).

La toate femeile nediabetice incluse în studiu, în analiza univariată, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,471, p=0,001) (tab. IV). La femeile obeze, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,485, p=0,022). La femeile neobeze, raportul albumină/creatinină urinară s-a corelat pozitiv cu tensiunea arterială sistolică (r=0,421, p=0,041) (tab. IV).

TABELUL II

Eliminarea urinară de albumină, în funcţie de indicele de masă corporală

	Raport albumină/creatinină urinară (mg/g)			**p**
	Normoponderali (IMC<25 kg/m^2)	**Supraponderali (IMC: 25-29,9 kg/m^2)**	**Obezi (IMC>30 kg/m^2)**	
Femei	2,1 (0,55-5.2)[a]	6,9 (3,3-11.1)	9,1 (4,35-15.6)	0,018
Bărbaţi	3 (0,95-5,75)[a]	6.1 (1.8-11.48)	8,9 (4,7-18,7)	0,03
p bărbaţi vs. femei	0,66	0,53	0,82	

a) p<0.05, OB vs. NP

TABELUL III

Corelația dintre raportul albumină/creatinină urinară şi factorii de risc, la subiecții de sex masculin

Variabila	Lot total		Obezi		Supraponderali / Normoponderali	
	r	P	r	p	r	P
Vârsta	0,03	0,69	0,08	0,392	0,02	0,79
IMC	0,16	0,12	0,19	0,094	0,13	0,17
Circumferința abdominală	0,13	0,16	0,21	0,058	0,11	0,21
Masa de țesut adipos	0,09	0,31	0,17	0,183	0,07	0,54
Colesterol total	-0,06	0,69	-0,02	0,812	0,03	0,76
LDL-colesterol	0,03	0,77	-0,05	0,677	0,06	0,58
HDL-colesterol	-0,1	0,28	-0,12	0,194	-0,05	0,43
Trigliceride	0,07	0,61	0,11	0,236	-0,02	0,83
Glicemie	0,14	0,13	0,2	0,073	0,09	0,55
HbA1c	0,08	0,6	0,1	0,471	0,03	0,72
Fibrinogen	-0,02	0,79	0,05	0,689	-0,06	0,61
Tensiunea arterială sistolică	**0,46**	**0,0003**	**0,51**	**0,006**	**0,39**	**0,041**
Tensiunea arterială diastolică	0,17	0,11	0,22	0,053	0,14	0,18
Leptina*	0,06	0,416	0,12	0,25	0,03	0,83
Adiponectina*	**-0,26**	**0,047**	**-0,43**	**0,011**	-0,13	0,16
Leptina/adiponectina*	0,09	0,362	0,14	0,19	0,06	0,52
Hs-CRP*	-0,1	0,266	-0,15	0,14	0,02	0,78
Insulina bazală*	0,07	0,672	0,161	0,089	0,03	0,71
Proinsulina*	0,05	0,775	0,11	0,46	-0,07	0,67
HOMA-IR*	0,12	0,267	0,17	0,11	0,08	0,41

** valori transformate logaritmic ; r-coeficient de corelație Pearson*

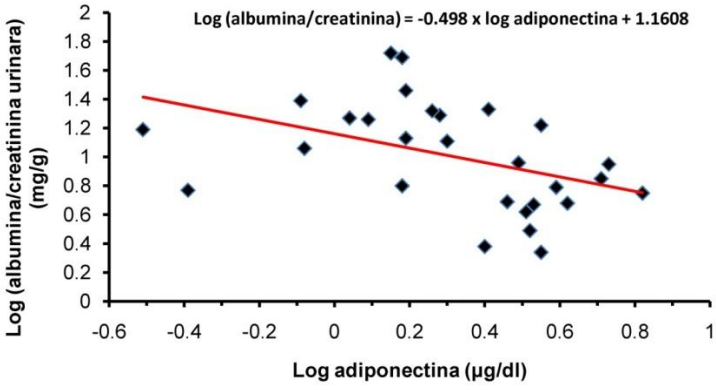

Fig. 1. Corelația dintre adiponectină şi raportul albumină/creatinină urinară la bărbați obezi nediabetici

TABELUL IV

Corelaţia dintre raportul albumină/creatinină urinară şi factorii de risc, la subiecţii de sex feminin

Variabila	Lot total		Obezi		Supraponderali / normoponderali	
	r	p	r	p	r	P
Vârsta	0,083	0,641	0,114	0,327	0,043	0,718
IMC	0,061	0,908	0,096	0,513	-0,025	0,813
Circumferinţa abdominală	0,114	0,304	0,152	0,171	0,067	0,602
Masa de ţesut adipos	0,074	0,792	0,126	0,478	0,036	0,767
Colesterol total	0,038	0,846	-0,091	0,577	0,074	0,594
LDL-colesterol	-0,057	0,711	-0,128	0,344	0,029	0,858
HDL-colesterol	-0,148	0,127	-0,192	0,083	-0,098	0,388
Trigliceride	0,132	0,176	0,167	0,128	0,062	0,464
Glicemie	0,094	0,604	0,131	0,412	0,051	0,627
HbA1c	0,122	0,247	0,172	0,138	0,104	0,352
Fibrinogen	0,086	0,846	0,092	0,496	0,054	0,541
Tensiunea arterială sistolică	**0,471**	**0,001**	**0,485**	**0,022**	**0,421**	**0,041**
Tensiunea arterială diastolică	0,146	0,13	0,184	0,122	0,092	0,396
Leptina*	0,085	0,765	0,103	0,492	0,051	0,533
Adiponectina*	0,105	0,276	0,137	0,397	0,043	0,582
Leptina/adiponectina*	0,127	0,226	0,149	0,193	0,076	0,476
Hs-CRP*	0,078	0,792	0,113	0,314	0,024	0,851
Insulina bazala*	0,14	0,142	0,173	0,113	0,1	0,284
Proinsulina*	0,09	0,685	0,142	0,206	0,077	0,586
HOMA-IR*	0,163	0,095	0,208	0,074	0,116	0,242

** valori transformate logaritmic ; r-coeficient de corelaţie Pearson*

DISCUŢII

În lucrarea de faţă, am arătat, la subiecţii nediabetici, fără afecţiuni cardiovasculare, existenţa unei corelaţii între creşterea masei de ţesut adipos şi creşterea eliminării de albumină (evaluată cu ajutorul raportului albumină/creatinină urinară), în concordanţă cu datele altor studii (21,22,23). Shaw (23) arată la populaţia din Asia de Sud, faptul că, obezitatea centrală (evaluată prin intermediul raportului talie/şold) se corelează independent cu albuminuria la subiecţi normoglicemici - rude de gradul I ale pacienţilor cu diabet zaharat tip 2.

De asemenea, am evidenţiat diferenţe atât în funcţie de sex, cât şi în funcţie de IMC, în ceea ce priveşte determinanţii eliminării urinare de albumină. La bărbaţii obezi, eliminarea urinară de albumină, la valori mai mici decât cele considerate necesare pentru stabilirea diagnosticului de microalbuminurie s-a corelat independent cu tensiunea arterială sistolică (pozitiv) şi cu adiponectina (negativ), în timp ce la

bărbații neobezi şi la femei, indiferent de IMC, s-a corelat numai cu tensiunea arterială sistolică. Tsioufis (24) arată că adiponectina este predictor negativ pentru albuminurie, independent de vârstă, IMC şi tensiunea arterială, la bărbați hipertensivi. Yano (21) arată diferențe între subiecții obezi şi neobezi în ceea ce privește determinanții microalbuminuriei. La subiecții obezi, nediabetici, microalbuminuria s-a corelat negativ cu adiponectina şi pozitiv cu tensiunea arterială şi glicemia, în timp ce, la subiecții neobezi, microalbuminuria s-a corelat pozitiv cu vârsta şi tensiunea arterială. Sharma (22) arată existența unei relații negative strânse între adiponectină şi albuminurie, adiponectina reglând albuminuria prin intermediul căii AMPK, care contribuie la inhibarea stresului oxidativ la nivelul podocitelor.

În această lucrare, nu am evidențiat existența unei corelații între markerii inflamatori şi eliminarea urinară de albumină. Yudkin et al (25) arată existența unei asocieri între markeri inflamatori (IL-6, TNF-α şi proteina C-reactivă) şi markeri ai disfuncției endoteliale (factor von Willebrand, trombomodulina, fibronectina şi albuminuria). Astfel, TNF-α s-a corelat pozitiv cu factorul von Willebrand, trombomodulina, fibronectina şi albuminuria, IL-6 s-a corelat pozitiv cu albuminuria, în timp ce proteina C-reactivă s-a corelat pozitiv cu factorul von Willebrand şi fibronectina.

CONCLUZII

Aceste rezultate arată că la bărbații obezi, nediabetici, fără afecțiuni cardiovasculare, adiponectina are un rol important în controlul eliminării urinare de albumină.

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STATUSUL LIMFOGANGLIONAR – FACTOR PROGNOSTIC ÎN CANCERUL MAMAR

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THE LYMPH NODES STATUS – PROGNOSTIC FACTOR IN BREAST CANCER (Abstract): **Aim**: The assessment of the survival rate according to axillary lymph nodes status in patients diagnosed with breast cancer. **Material and methods**: The study group included 206 women, treated in the 4th Obstetrics and Gynecology Clinic during 2002-2005. All patients were treated surgically with lymph nodes dissection. The analyzed variables included clinical and morphological features of both primary tumor and axillary lymph nodes. We have evaluated 5 years survival in relation to positivity and number of dissected lymph nodes. In order to determine the prognostic value of evaluated variables we have used Kaplan Meir method. **Results**: 77.7% of all patients were aged between 40 and 49 years old and 62.6% of them presented stage II tumors. Invasive ductal carcinoma was the most frequent histologic type (79.12%) and 71.2% of tumors were moderated of poorly differentiated, lymph nodes invasion being presented in 93 cases. Survival was significantly influenced by lymph node status ($p = 0.0001$), number of involved nodes ($p = 0.0001$), number of dissected nodes in pN0 patients ($p = 0.0001$) and application of adjuvant systemic therapy in pN0 patients ($p = 0.0001$). **Conclusions**: The pathological examination of axillary nodes in breast cancer patients offers a valuable information regarding survival. The absence of lymph node metastases of 10 lymph nodes dissection or more, and the application of adjuvant systemic therapy favorably influence the survival. **Keywords**: BREAST CANCER, LYMPH NODES, PROGNOSTIC FACTOR

Cancerul mamar reprezintă o importantă problemă de sănătate publică la nivel mondial, prognosticul acestei afecţiunii fiind influenţat de factori precum dimensiunea tumorii, tipul histologic, gradul de diferenţiere, statusul receptorilor hormonali, statusul limfoganglionilor limfatici şi vârsta pacientei (1). Invazia limfoganglionilor locoregionali constituie principalul factor care condiţionează instituirea terapiei adjuvante (2,3). Tehnica chirurgicală standard presupune disecţia staţiilor limfatice I şi II Berg, procedură cu viză curativă care oferă importante informaţii prognostice. Limfadenectomia axilară este grevată de o rată crescută de morbiditate, fapt care a condus la promovarea investigaţiei limfoganglionului santinelă (4-6).

Studiul nostru are drept obiectiv analiza statusului limfoganglionilor axilari şi investigarea corelaţiilor existente între acest factor prognostic major şi alţi factori (consideraţi minori) care caracterizează tumora, pentru a pune în evidenţă valoarea independentă a invaziei tumorale, în relaţie cu supravieţuirea.

MATERIAL ŞI METODE

Studiul retrospectiv, descriptiv şi analitic a inclus 206 paciente diagnosticate şi tratate pentru cancer mamar în stadiile I-III în Clinica IV Obstetrică Ginecologie, Spitalul Clinic de Obstetrică Ginecologie "Cuza Vodă" Iaşi, în perioada 2002-2005.

Etapele alcătuirii lotului şi analiza foilor de observaţie s-au desfăşurat în conformitate cu principiile eticii cercetării medicale, în baza consimţământului informat al pacientelor pentru folosirea datelor în concordanţă cu obiectivele specifice ale studiului.

Variabilele analizate au fost reprezentate de vârstă, stadiul clinic, prezenţa şi tipul tratamentului adjuvant, dimensiunea tumorii, tipul histologic, gradul de diferenţiere celulară, prezenţa invaziei spaţiului vasculolimfatic, statusul receptorilor hormonali, statusul limfoganglionilor axilari, numărul de limfoganglioni pozitivi în cazul stadiului pN0.

Datele obţinute au fost analizate şi exprimate procentual, diferenţele dintre valorile medii ale parametrilor investigaţi au fost analizate prin intermediul testelor t student şi ANOVA, iar asocierile dintre diferiţi factori au fost interpretate cu ajutorul testelor de corelaţie şi regresie. Influenţa variabilelor analizate asupra supravieţuirii la 5 ani a fost efectuată cu ajutorul metodei Kaplan Meir. Prelucrarea statistică a fost realizată cu programul *Statistica* 8.0.

REZULTATE

Din totalul celor 206 paciente analizate, 160 cazuri (77,7%) au avut vârsta cuprinsă între 40-69 de ani, iar 129 paciente (62,6%) s-au aflat în stadiul II evolutiv. În 39 de cazuri (18,9%) s-a instituit doar tratament chirurgical, în restul de 69,1% din cazuri acesta fiind complementat de radio (71%) şi chimioterapie (69,7%) (tab. I).

TABELUL I

Distribuţia pacientelor funcţie de caracteristicile clinice şi de tratamentul instituit

Parametru	Nr. Cazuri	Procent
Vârsta		
< 40 ani	14	6,77%
40-69 ani	32	15,53%
>70 ani	160	77,7%
Stadiul clinic		
I	45	21,84%
II	129	62,6%
III	32	15,53%
Tratament		
Exclusiv chirurgical	39	18,9%
Chimioterapie	116	69,7%
Hormonoterapie	77	46,3%
Radioterapie	119	71%

Din lotul cuprins în studiu 163 de paciente (79,12%) au prezentat carcinom ductal invaziv (CDI). Majoritatea tumorilor diagnosticate au fost moderat (36,89%) şi slab diferenţiate (34,95%). Invazia spaţiului vasculolimfatic (ISVL) a fost prezent în 93 de cazuri (45,15%). Tumorile identificate au fost de mici dimensiuni (<2 cm) la 116 de paciente (56,31%), între 2-5 cm în 82 de cazuri (39,81%) şi mai mari de 5 cm în 8 cazuri (3,88%). Statusul receptorilor hormonali estrogenici (RE) şi progesteronici (PR) a fost evaluat în 93 de cazuri (45,14%) cu o rată de pozitivitate de 50,5% în cazul RE şi de 37,7% în cazul RP (tab. II).

TABELUL II

Distribuţia pacientelor funcţie de caracteristicile tumorii

Parametru	Valoare	Procent
Tip histologic		
CDI	163	79,12%
CLI	33	16,02%
Alte tipuri	20	9,76%
Gradul histologic		
I	58	28,16%
II	76	36,89%
III	72	34,95%
ISVL		
Da	93	45,15%
Nu	113	54.85%
pT		
<2 cm	116	56,31%
2-5 cm	82	39,81%
> 5 cm	8	3,88%
Statusul receptorilor		
ER +	47	50,5%
PR +	35	37,7%

În 128 (62,13%) de cazuri statusul limfoganglionilor limfatici axilari a fost negativ (pN0), iar în cazul pacientelor cu limfoganglioni pozitivi (37,87%) numărul limfoganglionilor afectaţi a fost de 3 în 52,3% din cazuri, 4-9 în 28,7% din cazuri, şi de ≥ 10 la 19,9% dintre paciente.

Rata de supravieţuire la 5 ani a fost de 88,3% în cazul pacientelor cu limfoganglioni negativi şi de 74,7% în cazul prezenţei de limfoganglioni axilari pozitivi ($p < 0,00001$). În cazul pacientelor cu 3 limfoganglioni pozitivi supravieţuirea a fost de 79,5%, spre deosebire de 53,9% în cazul pacientelor cu 10 sau mai mulţi limfoganglioni invadaţi metastatic ($p<0,00001$) (tab. III).

Stabilirea stadiului pN0 a fost realizată în raport de numărul limfoganglionilor disecaţi, acesta fiind peste 10 în 86,9% din cazuri şi peste 16 în 52% din cazuri. Am remarcat o relaţie directă între numărul limfoganglionilor disecaţi şi supravieţuirea la 5 ani, anume 76,9% în cazul pacientelor cu mai puţin de 10 limfoganglioni excizaţi, 88,7% pentru pacientele cu 10-16 limfoganglioni disecaţi şi 92,3% în cazul celor la care s-au excizat mai mult de 16 limfoganglioni (fig. 1).

TABELUL III

Evaluarea supravieţuirii la 5 ani funcţie de caracteristicile limfoganglionilor axilari

Parametru	Supravieţuire 5 ani	P
Statusul limfoganglionilor		
Negativi	88,3%	0,0001
Pozitivi	77,4%	
Numărul limfoganglionilor pozitivi		
3	79,5%	0,0000
4-9	76,4%	
≥ 10	54,9%	
Numărul limfoganglionilor disecaţi		
<10	76,9%	0,0000
10-16	87,8%	
>16	92,3%	
pN0		
Da	89,3%	0,0000
Nu	71,4%	

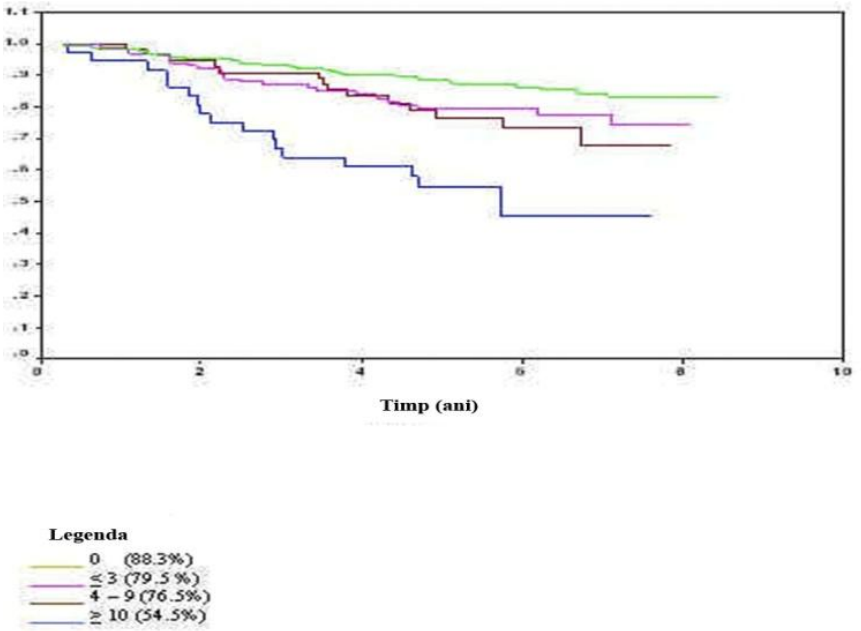

Fig. 1. Supravieţuirea la 5 ani în funcţie de numărul limfoganglionilor invadaţi metastatic

În cazul pacientelor aflate în stadiul pN0 care au primit terapie sistemică supravieţuirea la 5 ani a fost de 88,93%, spre deosebire de 71,4% în cazul celor care nu au beneficiat de acest tratament (p<0,00001).

DISCUŢII

Vârsta reprezintă un factor de risc pentru apariţia cancerului mamar. Un studiu efectuat pe 35769 paciente diagnosticate cu cancer mamar raportează că 81,7% dintre acestea aveau vârsta peste 46 de ani, iar 36,3% peste 65 de ani (7). Lotul investigat a relevat o creştere de 6,7 ori a incidenţei, în raport de vârstă. În ceea ce priveşte dimensiunea tumorilor, leziunile mai mici de 2 cm au fost cele mai frecvente, similar cu raportările din alte publicaţii (8). Procentajul de paciente diagnosticate în stadiul I a fost mai mic în cazul prezentei cercetări (21,84%) comparativ cu date prezentate în literatură (48%, respectiv 34,5%) (7). Carcinomul ductal invaziv a fost tipul histologic predominant, rezultat similar concluziilor altor cercetări (9). Rezultatele noastre referitoare la gradul de diferenţiere au fost însă neconcordante cu literatura, lotul nostru de studiu incluzând un procent mai mare, aproape 30%, de tumori bine diferenţiate.

Toate pacientele incluse în studiu au fost tratate chirurgical, radical sau conservator, iar aplicarea terapiei complementare, adjuvantă sau neoadjuvantă, a fost indicată în raport de: stadiul clinic, evaluarea histopatologică, care a permis stabilirea gradului de extensie al afecţiunii, şi alte caracteristici prognostice (gradul de diferenţiere, ISVL, statusul receptorilor). Prezenţa invaziei spaţiului vasculo-limfatic a fost raportată cu frecvenţe variabile între 20-50% probabil datorită dificultăţii de diferenţiere între vasele sangvine şi artefactele generate de retracţia tisulară, mai ales dacă sunt

folosite strict criteriile morfologice (10).

Invazia spaţiului limfovascular este considerată unul dintre principalii factori de risc pentru recidiva după tratamentul conservator. În ceea ce priveşte frecvenţa metastazelor în limfoganglionii limfatici şi numărul mediu de limfoganglioni invadaţi, rezultatele noastre sunt similare cu cele raportate de alţi autori (6). Supravieţuirea pacientelor diagnosticate cu cancer mamar este redusă considerabil în cazul prezenţei metastazelor limfatice, acesta fiind cel mai important factor de risc de recurenţă. Mai mult decât atât, prognosticul este influenţat de numărul limfoganglionilor limfatici invadaţi, acest parametru fiind considerat cel mai important factor de prognostic al recurenţelor la paciente cu limfoganglioni pozitivi. În majoritatea cazurilor au fost disecaţi minimum 10 limfoganglioni, în conformitate cu indicaţiile din literatură. Necesitatea implementării chimioterapiei sistemice la bolnavele cu limfoganglioni neinvadaţi (pN0) este condiţionată de numărul ganglionilor disecaţi.

În cazul pacientelor cu metastaze ganglionare, numărul limfo-ganglionilor disecaţi nu a influenţat semnificativ supravieţuirea, spre deosebire de pacientele aflate în stadiul pN0, la care numărul limfoganglionilor excizaţi a ameliorat supravieţuirea, mai ales în cazul asocierii terapiei sistemice. Cu excepţia numărului mic de paciente diagnosticate în stadiul I şi rata redusă de utilizare a hormonoterapiei, restul caracteristicilor clinice, morfologice şi terapeutice au fost asemănătoare cu cele regăsite în literatură.

CONCLUZII

Evaluarea corectă a statusului limfoganglionilor axilari a demonstrat rolul de factor prognostic major al acestui parametru morfologic. Acurateţea evaluării precum şi creşterea ratei de supravieţuire la 5 ani este în directă relaţie cu numărul ganglionilor excizaţi. Studiul nostru arată o relaţie directă între numărul limfoganglionilor disecaţi şi supravieţuirea la 5 ani, 76,9% în cazul pacientelor cu mai puţin de 10 limfoganglioni excizaţi, 88,7% pentru pacientele cu 10-16 limfoganglioni disecaţi şi 92,3% în cazul celor la care s-au excizat mai mult de 16 limfoganglioni.

Completarea tratamentului chirurgical cu chimioterapia chiar si la pacientele cu un status limfoganglionar pNo este utilă. Rezultatele studiului nostru demonstrează că pentru grupul pacientelor aflate în stadiul pN0 care au primit terapie sistemică supravieţuirea la 5 ani a crescut cu 17,53%.

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NOUTĂȚI

VITAMINA D ŞI PROTECŢIA ANTICANCER I

Având în vedere studiile recente care menţionează potenţialul antitumoral al concentraţiilor eficiente de vitamina D, metaanaliza publicată de Manson şi colaboratorii, în ultimul număr al New England Journal of Medicine, îşi propune să revizuiască articolele principale care au evidenţiat utilizarea vitaminei D în bolile sistemului articular dar şi în trialurile în care s-au urmărit beneficiile antitumorale şi de stimulare a imunităţii. În ceea ce priveşte beneficiile asupra sănătăţii osoase, doza zilnică recomandată este de 600 UI pentru persoane sub 70 de ani şi de 800 UI la vârste de peste 70 de ani. Autorii metaanalizei au arătat că majoritatea studiilor efectuate până în prezent nu au fost randomizate şi nu au urmărit ca obiectiv principal potenţialul antitumoral. Majoritatea informaţiilor provin din studii de laborator, corelaţii de natură ecologică, sau studii care au dozat nivelul vitaminemiei D la pacienţii diagnosticaţi cu tumori maligne. În literatură au fost evidenţiate trei trialuri principale, inclusiv unul în care administrarea vitaminei D şi calciu a fost comparată cu administrarea izolată a calciului, parametrii urmăriţi fiind numărul de cazuri noi de cancer nou diagnosticate şi mortalitatea prin cancer. În cazul cancerului de sân, administrarea pe termen lung a unor doze suplimentare de vitamina D a fost urmată de creşterea incidenţei tumorale, iar pentru cancerul de colon administrarea de vitamină D a fost urmată de scăderea riscului. În ciuda unor ipoteze patogenice plauzibile, dovezile privind utilizarea vitaminei D pentru profilaxia antitumorală sunt inconsistente. (Manson JE, Susan PH, et al. Vitamin D and prevention of cancer – ready for prime time?, *N Engl J Med* 2011; 364 (15): 1385-7)

Corina Dima-Cozma

EVALUĂRI IMUNOHISTOCHIMICE PRIN MARKERI TUMORALI (Ki67, p_{53} şi Bcl-2) PE O COHORTĂ DE PACIENTE CU CANCER DE COL UTERIN ÎN DIVERSE STADII DE EVOLUŢIE

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ASSESSMENT TUMOR MARKERS BY IMMUNOHISTOCHEMISTRY (Ki67, p53 AND Bcl-2) ON A COHORT OF PATIENTS WITH CERVICAL CANCER IN VARIOUS STAGES OF EVOLUTION (Abstract): In defining new potential prognostic factors in cervical cancer, p53 protein expression, Ki67 or issues related to Bcl-2 oncogene involved in cellular apoptosis process, have been the subject of numerous publications. This paper aims to study with immunohistochemical methods, the way in which these factors are involved in influencing the prognosis of patients with cervical cancer. The study was conducted on 42 pieces operators from the same number of patients diagnosed with this disease, aged between 25-65 years and in different clinical stages. We used an experimental design type 2 (histopathological type squamous cell / adenocarcinoma) x 3 (degree of differentiation weak / moderate / well differentiated) x 4 (stage I / II / III / IV disease). Diagnosis was based on clinical examination and confirmed by histopathology, and hypothesis testing we used Kruskal-Wallis ANOVA test of ranks test, Mann-Whitney U test, χ 2 test, Spearman's correlation test and crosstabs ranking difference. The results on the study group showed the prognostic value of both the proliferation marker Ki67 and p53 oncogene, the presence of tandem Ki67 / p53 in cervical carcinoma meaning more so a poor prognosis, Bcl-2 overexpression was detected in none of the 42 cases investigated. Key words: Ki67, p53, Bcl-2, PROLIFERATIVE ACTIVITY, IMMUNOHISTOCHEMISTRY, PROGNOSIS, CERVICAL CANCER

Deşi neoplasmul de col uterin este o afecțiune ce poare fi prevenită, în România încă reprezintă o problemă de sănătate publică prin lipsa unor programe de scrining, şi o deficiență în educația pentru sănătate a populației. Această situație particulară a incidenței şi mortalității prin cancer de col uterin care se înregistrează în țara noastră, alături de unele posibilități de explorare şi de analiză histopatologică au creat premisele necesare care ne-au determinat să alegem studierea neoplasmului de col uterin drept temă de cercetare.

În ceea ce priveşte investigarea unor biomarkeri, rezultatele studiilor publicate încă sunt contradictorii şi în parte incomplete. Totuşi, pe lângă valoarea lor prognostică, inhibarea acestor biomarkeri ar

putea avea efect terapeutic în mai multe situații.

Dezvoltarea fără precedent a biologiei moleculare în ultimii ani a permis depistarea unor anomalii cromozomice precizând astfel etapele carcinogenezei uterine şi orientând cercetările spre definirea unor noi factori prognostici potențiali. Dintre aceştia expresia proteinelor p_{53}, Ki67 sau aspecte legate de prezența oncoproteinei Bcl-2 cu rol în procesul de apoptoză celulară au făcut obiectul a numeroase publicații.

Studiile publicate au demonstrat semnificația prognostică a fiecăruia din markerii menționați cât şi corelațiile dintre aceştia şi factorii prognostici clasici.

OBIECTIVE

Lucrarea de față are ca obiectiv studierea cu ajutorul metodelor imunohistochimice a modului în care markerii de proliferare celulară (Ki67 şi p_{53}) precum şi oncoproteina Bcl-2 sunt implicați în influențarea prognosticului pacientelor cu cancer de col uterin. S-a încercat identificarea modului în care valorile acestora influențează tipul histopatologic, gradul de diferențiere şi stadiul clinic al tumorii şi dacă există corelații între gradul de exprimare al acestor markeri celulari.

Ne-am aşteptat ca nivelul markerilor de proliferare celulară Ki67 şi p_{53}, precum şi a oncoproteinei Bcl-2 în tumoră să influențeze prognosticul bolii; cu cât nivelul Ki67 şi p_{53} va fi mai crescut, iar cel al oncogenei Bcl-2 mai scăzut în prelevatele tisulare, cu atât prognosticul pacientelor va fi mai grav.

MATERIAL ŞI METODĂ

Cercetarea a fost efectuată pe un număr de 42 piese operatorii provenind de la acelaşi număr de paciente diagnosticate cu cancer de col uterin, internate în cadrul Clinicii Chirurgie – Compartimentul Ginecologie al Spitalului Clinic Militar de Urgență „Dr. Iacob Czihac" Iaşi, în perioada 2007-2010. Pacientele au avut vârsta cuprinsă între 25-65 ani, aflate în stadii clinice diferite şi proveneau atât din mediul urban, cât şi din mediul rural. Modul de selectare a acestor piese a ținut cont de materialul biologic care a trebuit să fie suficient pentru studiul morfologic şi imunohistochimic. Prelevatele tisulare au fost fie piese operatorii, fie biopsii de col uterin efectuate pentru precizarea diagnosticului. Diagnosticul s-a bazat pe examenul clinic şi a fost confirmat de examenul histopatologic al fragmentelor de biopsie a colului uterin şi al pieselor de rezecție chirurgicală. Piesele de rezecție şi prelevatele de biopsie au fost examinate şi prelucrate în cadrul Laboratorului de Anatomie Patologică al Spitalului Clinic Militar de Urgență „Dr. Iacob Czihac" Iaşi.

S-au utilizat ca instrumente de culegere a datelor foile de observație ale pacientelor, registrele de protocoale operatorii şi registrul examenelor anatomo-patologice. S-au studiat datele clinice şi aspectele anatomo-patologice tumorale – macroscopia, microscopia şi stadiul patologic. S-a utilizat, de asemenea, programul de prelucrare statistică a datelor *SPSS 13.0* şi Microsoft Office Excel 2003 pentru realizarea graficelor.

Ca metodă statistică, pentru verificarea ipotezelor, am utilizat tehnicile neparametrice *ANOVA Kruskal-Wallis* al rangurilor, *testul U a lui Mann-Whitney*, *testul chi 2* (hi patrat), *crosstabs* şi *testul Spearman al corelatiei* diferenței rangurilor.

Metoda histopatologică a presupus examinarea anatomo-patologică a pieselor tumorale, descrierea macroscopică, microscopică şi imunohistochimică. Examenul

microscopic s-a realizat utilizând colorația standard (hematoxilină-eozină) şi colorații speciale pentru componentele celulare, tisulare şi a mucusului (Van Gieson, Albastru Alcian). De asemenea, imunohistochimia a permis o analiză structurală şi fenotipică, utilizând criteriul molecular în diagnosticul histopatologic şi oferind date privind diferențierea, histogeneza şi progresia tumorală.

S-a interpretat o reacție *p_{53}* pozitivă colorarea nucleară distinctă, omogenă sau granulară. Produsul de reacție a prezentat culoare brună. Am considerat de asemenea, că tumorile supraexprimă proteina p_{53} numai când am obținut o colorație nucleară intensă, prezentă într-unul din cele 3 pattern-uri. Cazurile cu o colorație nucleară echivocă sau slabă le-am considerat negative. Tot negative am considerat şi cazurile cu nuclei pozitivi extrem de rari sau izolați. Am folosit un sistem de gradare pentru evaluarea reactivității *p_{53}* notând astfel: 0 colorația negativă; + reacție pozitivă în mai puțin de 10% din nucleii celulelor tumorale; ++ reacție pozitivă în mai mult de 10% din nucleii celulelor tumorale dar mai puțin de 33%; +++ reacție pozitivă în mai mult de 33% din nucleii celulelor tumorale. (Ivan, Mihailovici, 2004), (1).

Pentru Ki67 am considerat ca pozitivă orice colorare nucleară detectabilă (punctiformă sau difuză), iar în vederea cuantificării imunoreacției Ki67 am determinat procentul de celule reactive. Pornind de la heterogenitatea în distribuția şi intensitatea colorării, pentru a evita subiectivitatea şi dificultățile în aprecierea rezultatelor, am utilizat un scor semnificativ (corespunzător intensității colorării şi procentului de nuclei reactivi), care a permis clasificarea imunoreactivității Ki67 în trei grupe de scor: scor jos (malignitate joasă) – s-au colorat între 5-20% dintre nucleii celulelor tumorale; scor mediu (malignitate intermediară) - s-au colorat 20-70% dintre nucleii celulelor tumorale; scor înalt (malignitate înaltă) – s-au colorat peste 70% din nucleii celulelor tumorale .

Marcarea imunohistochimică a anti-oncoproteinei Bcl-2, AM287-5M a identificat un pattern de colorare membranară.

REZULTATE

1. analiza vârstei pacientelor a surprins următoarele date: în studiu sunt 31% din paciente cu vârsta cuprinsă între 25-35 ani, 28,6% paciente cu vârsta cuprinsă între 56-65 ani, 26,2% paciente cu vârsta cuprinsă între 46-55 ani şi 14,3% paciente cu vârsta cuprinsă între 36-45 ani.

a) analiza ***tipului histopatologic:*** *$\chi 2$ = 21,36 este semnificativ: p<0,001, la 3 grade libertate.*

- *pentru tipul scuamocelular* 43,3% cazuri cu vârsta cuprinsă între 25-35 de ani, 33,3% cazuri cu vârsta cuprinsă între 56-65 ani şi 23,3% cazuri cu vârsta cuprinsă între 46-55 ani;

- pentru *tipul adenocarcinom*, 50% cazuri la pacientele cu vârsta cuprinsă între 36-45 ani, 33,3% cazuri cu vârsta cuprinsă între 46-55 ani şi 16,7% cazuri cu vârsta cuprinsă între 56-65 ani;

b) analiza ***gradului de diferențiere***: *χ 2=20,33 este semnificativ: p=0,002, la 6 grade libertate;*

- pentru gradul *slab diferențiat:* 58,3% cazuri cu vârsta cuprinsă între 25-35 de ani, 41,7% cazuri cu vârsta cuprinsă între 46-55 ani;

- pentru gradul *moderat diferențiat*: 50% din cazuri cu vârsta cuprinsă între 56-65, 33,3% cazuri cu vârsta cuprinsă între 25-35 ani şi 16,7% cazuri cu vârsta cuprinsă între 46-55 ani.

- pentru gradul *bine diferenţiat*: 33,3% din cazuri cu vârsta cuprinsă între 36-45 şi 56-65 ani, 22,2% cazuri cu vârsta cuprinsă între 46-55 ani şi 11,1% cazuri cu vârsta cuprinsă între 25-35 ani.

c) analiza ***stadiului bolii:*** *χ 2=25,83 este semnificativ: p=0,002, la 9 grade libertate; r = - 0,015, p = 0,92, r^2 = 0,0002.*

- în *stadiul avansat (IV):* 66,7% cazuri cu vârsta cuprinsă între 46-55 ani şi 16,7% cazuri cu vârsta cuprinsă între 36-45 ani, respectiv 25-35 ani;

- în *stadiul III*: 41,7% paciente cu vârsta cuprinsă între 25-35 ani, 33,3% cazuri cu vârsta cuprinsă între 56-65 ani şi 25% cazuri cu vârsta cuprinsă între 46-55 ani;

- în *stadiul II*: 66,7% paciente cu vârsta cuprinsă între 56-65 ani şi 33,3 % paciente cu vârsta cuprinsă între 25-35 ani;

- în *stadiul I*: câte 33,3% cazuri de paciente cu vârsta cuprinsă între 25-35 ani, 36-45 ani şi 56-65 ani.

2) studiul asocierii markerului de proliferare Ki67 cu oncogena p_{53}: *χ 2=3 ,66 este semnificativ: p < 0,001 la 4 grade libertate;* r = 0,837; p < 0,001, r^2 = 0,70.

TABELUL I

Asocierea dintre gradul de malignitate Ki67 si pozitivitatea p53

			P53			
			+ (pozitiv 10%	++ (pozitiv intre 11-33%)	+++ (pozitiv peste 33%)	Total
Ki67	malignitate joasă (5%-20%)	număr cazuri	12	6	0	18
		% din p53	100,0%	50,0%	,0%	42,9%
		% din total	28,6%	14,3%	,0%	42,9%
	malignitate intermediară (21%-70%)	număr cazuri	0	6	12	18
		% din p53	,0%	50,0%	66,7%	42,9%
		% din total	,0%	14,3%	28,6%	42,9%
	malignitate înaltă (>70%)	număr cazuri	0	0	6	6
		% din p53	,0%	,0%	33,3%	14,3%
		% din total	,0%	,0%	14,3%	14,3%
Total		număr cazuri	12	12	18	42
		% din p53	100,0%	100,0%	100,0%	100,0%
		% din total	28,6%	28,6%	42,9%	100,0%

3) studiul asocierii Ki67 cu tipul histopatologic; influenţa tipului histopatologic asupra scorului de malignitate al Ki67: incidenţa lui Ki67 este de 71,4% în cazurile de carcinom scuamocelular şi de 28,6% în cazuile de adenocarcinom; Ki67 cu malignitate înaltă este prezent 100% în cazurile de adenocarcinom, Ki67 cu malignitate intermediară prezent 100% în cazurile de carcinom scuamocelular, iar Ki67 cu malignitate joasă este prezent 66,7% în cazurile de carcinom spinocelular şi 33,3% în cazurile de adenocarcinom; ***Chi-pătrat = 22,400; df = 2, p < 0,001;*** U = 144, p = 0,27; r = 0,17; p = 0,28.

4) studiul asocierii p_{53} cu tipul histopatologic; influenţa tipului histopatogic asupra oncogenei p_{53}: incidenţa p_{53} este de 71,4% în cazurile de carcinom scuamocelular şi de 28,6% în cazurile de adenocarcinom; p_{53}+ este prezent 50% şi în cazurile de carcinom scuamocelular şi în cele de

adenocarcinom, p_{53}++ este prezent 100% în cazurile de carcinoma scuamocelular, iar p_{53}+++ este prezent 66,7% în cazurile de carcinom scuamocelular şi 33,3% în cazurile de adenocarcinom: Chi-pătrat = 7,70; df = 2, p = 0,021; U = 162, p = 0,592; r = - 0,08; p = 0,598.

5) studiul asocierii Ki67 cu gradul de diferenţiere; influenţa gradului de diferenţiere asupra scorului de malignitate al Ki67: incidenţa Ki67 este de 42,9% în cazurile bine diferenţiate şi câte 28,6% în cazurile slab şi moderat diferenţiate; Ki67 cu malignitate înaltă este prezent 100% în cazurile bine diferenţiate, Ki67 cu malignitate intermediară prezent 66,7% în cazurile slab diferenţiate şi 33,3% în cele moderat diferenţiate, iar Ki67 cu malignitate joasă este prezent 66,7% în cazurile bine diferenţiate şi 33,3% în cele moderat diferenţiate: Chi-pătrat = 32,667; df = 4, p < 0,001; ANOVA Kruskal Wallis: Chi-pătrat =4.84, df = 2, p = 0.089; r = - 0,26; p = 0,089.

6) studiul asocierii p_{53} cu gradul de diferenţiere; influenţa gradului de diferenţiere asupra oncogenei p_{53}: incidenţa p_{53} este de 42,9% în cazurile bine diferenţiate şi câte 28,6% în cazurile slab şi moderat diferenţiate; p_{53}+ este prezent 100% în cazurile bine diferenţiate, p_{53}++ este prezent câte 50% în cazurile slab şi moderat diferenţiate, iar p_{53}+++ este prezent câte 33,3% în toate cele trei tipuri de diferenţiere: Chi-pătrat = 25,667; df = 4, p < 0,001; ANOVA Kruskal Wallis: Chi-pătrat =8.61, df = 2, p = 0.014; r = - 0,42; p = 0,006.

7) studiul asocierii Ki67 cu stadiul clinic; influenţa stadiului clinic asupra scorului de malignitate al Ki67: incidenţa Ki67 este de 28,6% în fiecare din stadiile I, III şi IV, iar în stadiul II de 14,3%; Ki67 cu malignitate înaltă este prezent 100% în stadiul IV, Ki67 cu malignitate intermediară prezent 66,7% în stadiul III şi 33,3% în stadiul IV, iar Ki67 cu malignitate joasă este prezent 66,7% în stadiul I şi 33,3% în stadiul II: Chi-pătrat = 56; df = 6, p < 0,001; ANOVA Kruskal Wallis: Chi-pătrat =37,58; df = 3; p < 0,001; r = 0,912; p < 0,001.

8) studiul asocierii p_{53} cu stadiul clinic; influenţa stadiului clinic asupra gradului de pozitivitate al p_{53}: incidenţa p_{53} este de 28,6% în fiecare din stadiile I, III şi IV, iar în stadiul II de 14,3%; p_{53}+ este prezent câte 50% în stadiile I şi II, p_{53}++ este prezent câte 50% în stadiile I şi III, iar p_{53}+++ este prezent 66,7% în stadiul IV şi 33,3% în stadiul III: Chi-pătrat = 45,50; df = 6, p < 0,001; ANOVA Kruskal Wallis: Chi-pătrat =32,59; df = 3; p < 0,001; r = 0,806; p < 0,001.

DISCUŢII

Studiul demonstrează că ***vârsta*** pacientelor din lot se asociază semnificativ cu: tipul histopatologic (la vârsta 25-35 ani predomină carcinomul scuamocelular, faţă de adenocarcinom care predomină în următoarea categorie de vârstă (36-45 de ani), cu gradul de diferenţiere (la vârsta tânără predomină gradul de diferenţiere slab diferenţiat, faţă de cel bine diferenţiat care predomină la vârsta înaintată), cu stadiul bolii (stadiul foarte avansat (IV) predomină în categoria de vârstă 46-55 ani, pe când stadiul incipient al bolii – în categoria premergătoare acesteia, 36-45 ani). Putem spune că pacientele tinere vor avea un prognostic mai rezervat decît cele cu vâstă mai înaintată.

Supraexpresia Bcl-2 nu a fost decelată în nici una din cele 42 de cazuri cercetate, astfel încât a fost imposibil de demonstrat

importanţa ei în ceea ce priveşte prognosticul pacientelor cu cancer de col uterin, deşi unii cercetători susţin că în ceea ce priveşte carcinogeneza cervicală, supraexpresia acestei proteine poate fi determinată în fiecare stadiu al patogenezei, sau că distribuţia şi intensitatea imunoreacţiei Bcl-2 corelează semnificativ cu gradul de diferenţiere al tumorii, acest lucru putând fi un important factor adjuvant în stabilirea unui prognostic corect. Alţi autori găsesc că nu este un factor de predicţie al prognosticului în cancerul cervical.

Analiza studiului markerului de proliferare Ki67 în ceea ce priveşte tipul histopatologic a relevat faptul că a*socierea dintre procentul Ki67 şi tipul histopatologic s-a dovedit a fi statistic semnificativă, expresia Ki67 aflându-se într-un procent semnificativ mai mare în tipul scuamocelular decât în adenocarcinomul de col uterin, dar* cazurile de malignitate înaltă s-au decelat doar în adenocarcinoamele de col uterin. Acest lucru demostrează că prezenţa markerului de proliferare Ki67 cu malignitate înaltă la pacientele cu neoplasm de col uterin va determina un prognostic rezervat.

Analiza studiului markerului de proliferare Ki67 legat de grading-ul tumoral ne permite să concluzionăm că *expresia Ki67 se află într-un procent semnificativ mai mare în tipul bine diferenţiat, comparativ cu tumora de col uterin cu grad de diferenţiere moderat sau slab.* Prin studiul corelaţiei am identificat *existenţa unei corelaţii invers proporţionale, slabe, şi nesemnificative între gradul de malignitate al Ki67 şi gradul de diferenţiere a tumorii.*

De asemenea, analiza stadiului clinic a evidenţiat o a*sociere semnificativă dintre procentul Ki67 şi stadiu bolii: am observat o asociere a scorului crescut şi intermediar de malignitate cu stadiul bolii avansat (stadiul III şi IV) şi invers. Mai mult, s-a putut observa că valorile Ki67 au înregistrat modificări semnificative în funcţie de stadiul bolii, astfel că, în stadiile III şi IV rangurile medii ale Ki67 au fost semnificativ mai mari comparativ cu cele din stadiile I şi II. Totodată sa evidenţiat şi existenţa unei corelaţii pozitive, puternice şi semnificative între gradul de malignitate al Ki67 şi stadiul bolii: pe lotul studiat, cu cât malignitatea Ki67 a fost mai mare, cu atât a crescut stadiul bolii şi invers. Astfel, putem aprecia un prognostic nefavorabil al pacientelor cu valori mari ale lui Ki67.*

În studiile lui Veronese et. al. (1993), (2) detecţia antigenului Ki67 s-a dovedit superioară metodei de determinare a gradului histologic de diferenţiere sau a indexului mitotic şi consideră că, indiferent de tipul histologic, toate tumorile cu peste 5% celule Ki67 pozitive, au un curs clinic nefavorabil. Bouzubar şi colab (1989), (3) susţin că tumorile în care valoarea lui Ki67 este peste 20% au un risc crescut de recădere, Wintzer şi colab (1991), (4), au raportat un prognostic mai sever la pacienţii cu tumori având valori ale lui Ki67 mai mari decât 16%, în timp ce alţi autori notează o probabilitate mai mică de supravieţuire la 5 ani a pacienţilor cu valori ale lui Ki67 mai mari de 13% (Nakano, Oka 1993), (5).

Cu cât activitatea proliferativă a unei tumori este mai mare, cu atât este mai mare potenţialul său malign, iar supravieţuirea (din momentul diagnosticului) mai redusă (Tsamandas et al., 2009, Ivan, Toma 2009), (6), (7). Chen şi colab. (2008), (8), arată că valoarea prognostică a markerului de proliferare Ki67 este redusă .

Analiza studiului oncogenei p_{53} pe lotul nostru de paciente, în ceea ce priveşte ***tipul histopatologic*** a evidenţiat că oncoproteina

p_{53} se află într-un procent semnificativ mai *mare în tipul scuamocelular decât în adenocarcinomul de col uterin. Această asociere este statistic semnificativă. În schimb, în tipul histopatologic scuamocelular valoarea lui p_{53} nu este semnificativ mai mare comparativ cu valoarea medie înregistrată în cazul adenocarcinomului de col uterin şi nici nu s-a evidenţiat existenţa unei corelaţii semnificative între gradul de pozitivitate al p_{53} şi tipul histopatologic.*

În studiul nostru *asocierea dintre procentul oncogenei p_{53} şi* ***gradul de diferenţiere al tumorii*** *este statistic semnificativă: cazurile slab şi moderat diferenţiate s-au asociat cu pozitivitate p_{53} intermediară (++) şi înaltă (+++), iar cele bine diferenţiate cu pozitivitate scăzută (+). Mai mult, valorile oncogenei p_{53} în tumorile slab şi moderat diferenţiate sunt semnificativ mai mari comparativ cu valorile aflate în tumorile bine diferenţiate, ceea ce ne permite să concluzionăm că, cu cât o tumoră va avea aspecte histologice bine diferenţiate, cu atât ne vom aştepta la un prognostic bun şi invers.* De asemenea, s-a evidenţiat *prezenţa unei corelaţii invers proporţionale, medie şi semnificativă între gradul de pozitivitate al p_{53} şi gradul de diferenţiere a tumorii. Pozitivitatea pentru p_{53} a arătat o creştere a intensităţii patternului de colorare de la formele bine diferenţiate, cuantificate cu +, spre formele slab diferenţiate, cuantificate cu +++. Această evoluţie anticipează un comportament agresiv al tumorilor.* Putem spune că p_{53} şi-a dovedit valoarea în aprecierea prognosticului pacientelor cu cancer de col uterin.

De asemenea, ***studiul oncogenei p_{53} legat de stadiul bolii*** a evidenţiat că a*socierea dintre procentul p_{53} şi stadiu bolii este statistic semnificativă: aceasta este explicată prin prezenţa unui procent ridicat de cazuri ce prezintă p_{53} + şi sunt în stadii mici ale bolii (I sau II) şi a unui procent ridicat de cazuri ce prezintă p_{53} +++ şi sunt în stadii avansate ale bolii (III sau IV). Valorile lui p_{53} au înregistrat modificări semnificative în funcţie de stadiul bolii. S-a mai evidenţiat şi existenţa unei corelaţii pozitive, puternice şi semnificative între gradul de pozitivitate al p_{53} şi stadiul bolii: astfel, cu cât pozitivitatea p_{53} a fost mai înaltă, cu atât a crescut stadiul bolii şi invers Putem spune că pacientele cu valori crescute ale p_{53} vor avea un prognostic rezervat.*

CONCLUZII

În studiul nostru determinarea imunohistochimică a oncoproteinei BCL-2 nu este un factor predictiv în stabilirea prognosticului cancerului de col uterin.

Faptul că *markerul de prolifirare tumorală Ki67 poate fi considerat un factor de prognostic* este susţinut astfel: cazurile cu malignitate înaltă s-au înregistrat doar în adenocarcinoamele de col uterin; există *o corelaţie pozitivă, puternică şi semnificativă între gradul de malignitate a lui Ki67 şi stadiul bolii (cu cât malignitatea Ki67a fost mai mare, cu atât a crescut şi stadiul bolii şi invers*); valori crescute s-au înregistrat în formele nediferenţiate de neoplasm de col uterin. *Cu cît valorile lui KI67 vor fi mai mari cu atît prognosticul pacientelor va fi mai sever.*

Oncoproteina p_{53} poate fi un factor de prognostic, întrucât valori mari ale markerului au fost determinate la tipul histologic slab diferenţiat şi în stadiile avansate (lll-lV) ale bolii, deci conduce la un prognostic mai sever a pacientelor.

S-a observat o corelaţie pozitivă, puternică şi semnificativă între intensitatea

reacţiei p_{53} cuantificată cu +, ++, +++ şi numărul de nuclei reactivi Ki67, respectiv scorul de malignitate: cu cât pozitivitatea p_{53} a fost mai crescută, cu atât şi malignitatea Ki67 a fost mai înaltă. Putem susţine că, deşi se încadrează în aceeaşi grupă de malignitate, p_{53} +, ++, şi +++ prezintă o relaţie direct proporţională cu procentul de nuclei reactivi Ki67, sugerând probabil gradul de agresivitate tumorală.

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GREEN SYNTHESIZED SILVER NANOPARTICLES VIA PLANT EXTRACTS AS ANTI-MELANOMA AGENTS

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GREEN SYNTHESIZED SILVER NANOPARTICLES VIA PLANT EXTRACTS AS ANTI-MELANOMA AGENTS (Abstract): Melanoma is one of the most common and aggressive type of cancer. The actual treatment includes excisional surgery, electrochemotherapy and pharmacotherapy. The pharmaceutical treatment has major drawbacks such as severe side effects and development of drug resistance, fact that orientated the research towards the formulation of new drug delivery systems. Nanocarriers represent an innovative and alternative tool which significantly improves the bioavailability and site-specific targeting of pharmaceutical agents. Due to their biological activities, flexibility and reliability, silver nanoparticles (AgNPs) have gained high attention. The green synthesis of AgNPs, mediated by a plant extract, is an eco-friendly, cheap and effective method. Green synthesized AgNPs have been reported to develop cytotoxic effects against different tumor cell lines. With respect to melanoma, few studies have been conducted until now, but with promising results. The antitumor activity depends on the size and shape of AgNPs and relies on the synergistic effect between phytochemicals and nanocarriers. Overall, green synthesized AgNPs represent a promising strategy for melanoma treatment. **Keywords**: ANTI-MELANOMA, SILVER NANOPARTICLES, GREEN SYNTHESIS, PLANT EXTRACTS.

Melanoma is the most dreadful form of skin cancer and one of the most aggressive cancers in its metastatic forms. Lately, the incidence of melanoma in the developed countries has grown more rapidly than any other type of cancer, especially in the population older than 50 years (1). There are two types of factors that are incriminated for a high risk in the development of skin cancer: genetic and environmental factors (exposure to UV radiation) (2). Other major risk factors for melanoma development include family history, fair pigmentation phenotypes, high numbers of melanocytic nevi (over 50) or the presence of dysplastic nevi, exposure to arsenic and other chemicals (polycyclic hydrocarbons, polyvinylchloride, asbestos) and immunosuppressive drugs (azathioprine, cyclosporine A), viral infections (papillomavirus infections) and tobacco consumption (2, 3). Several molecular mechanisms have been associated with melanoma pathogenesis. They are mainly related to

the oncogenic driver mutations in the major signaling pathways that are involved in apoptosis, proliferation and differentiation of melanocytes, such as ERK/MAP-kinase and PI_3-kinase pathways (tab. I) (4).

TABLE I

Molecular changes associated with melanoma pathogenesis

Molecular event	Outcome
Mutations of BRAF and NRAS genes	activation of ERK/MEK pathways uncontrolled cell proliferation tumor development and growth
Activation of PI3K/AKT	activation of anti-apoptotic β-catenin inhibition of pro-apoptotic BAD melanoma proliferation and metastasis
Inhibition of CDKN2A	activation of CDK4 cell proliferation melanoma uncontrolled growth
Mutations/chromosomal duplications of C-KIT	increase of KIT protein expression alteration of melanocyte proliferation tumorigenesis development
MC1R polymorphisms	increase of susceptibility to melanoma impairment of melanin production increase of UV-induced DNA-mutagenesis
Increase of N-cadherin and VE-cadherin expressions	activation of PI3K-AKT pathway metastasis

BAD, Bcl-2-associated death promoter; BRAF, gene that encodes the protein B-RAF, involved in cell growth; NRAS, gene that encodes the protein N-RAS, involved in the regulation of cell division; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDK4, cyclin-dependent kinase 4; C-KIT, receptor tyrosine kinase protein encoded by the KIT gene; ERK/MEK, extracellular signal-regulated kinase/mitogen-activated protein kinase; MC1R, melanocortin 1 receptor; N-cadherin, VE-cadherin, members of type-1 transmembrane proteins; PI3K, phosphatidylinositol 3-kinase/protein kinase B; AKT, serine/threonine kinase known as protein kinase B.

The excisional surgery, electrochemotherapy, pharmacotherapy and immunotherapy are the main therapeutic approaches in melanoma. The modern pharmacotherapeutics are selected after tumor genotyping process and they mainly include BRAF inhibitors (dabrafenib, vemuranifib), MEK inhibitors (cobimetinib, selumetinib, trimetinib), KIT inhibitors (imatinib) and agents targeting the endoplasmic reticulum stress-associated pathways (ruthenium-derived compounds) (4). These compounds possess a rate of response of approximately 50% and develop a rapid effect. The main disadvantage is the resistance of the melanomic cells which appears after seven months of treatment. Besides, these compounds induce cutaneous toxicities (1, 2).

ERK inhibitors such as GDC-0994 and SCH 772984 are currently in clinical trials. Another therapeutic strategy is related to compounds that target non-coding RNAs such as antisense oligopeptides. The monoclonal antibodies (ipilimumab, pembrolizumab, nivolumab) are recommended in the late stage of metastatic melanoma, advanced or unresectable melanomas but they can lead to autoimmune side effects (4). The severe side effects of the conventional chemotherapeutic or immunotherapeutic agents and the development of drug resistance are the major drawbacks of melanoma pharmacotherapy. The identification of drug delivery systems that can ensure site-specific targeting and optimal bioavailability is an important approach in the development of a successful treatment. Nanostructured materials have attracted considerable attention due to their pleiotropic applications in various fields, such as: electronics, medicine, cosmetics, mechanical engineering, applied physics and chemistry, environmental area, and biotechnology (5, 6, 7). In drug delivery field, nanocarriers represent an important tool to develop formulations that can solve some issues affecting the therapeutic efficacy of antitumor agents, such as poor bioavailability, low therapeutic index, lack of targeting, nonspecific bio-distribution, poor water solubility, and low chemical stability (8, 9). The noble metal nanoparticles are of interest due to their exceptional magnetic, optical, and electronic properties (10). AgNPs and gold nanoparticles (AuNPs) are among the most frequently used nanocarriers due to their flexibility and reliability (11). They also have biological activities and promising results have been reported in antitumor assays. In this review we focused on the potential applications of plant extract synthesized AgNPs in melanoma treatment.

Silver nanoparticles: synthesis and properties

In the nano-sized form (1-100 nm), Ag has unique optical, electronic, thermal, chemical, and biological properties, as well as a high electrical conductivity compared to its macroscopic form (12, 13, 14). The spectrum of AgNPs applications is very wide including pharmaceutical industry (diagnostics, therapeutics, drug delivery), orthopedics, medical device coatings (catheters, dentures, surgical masks), wound dressings, food industry, textile industry, cosmetics, catalysis, optoelectronics, photonics, electronics, and production of household, healthcare-related products and antimicrobial nanopaints (8, 14, 15). The synthesis of AgNPs can be accomplished by physical, chemical, and biological (green synthesis) methods. The physical methods include evaporation-condensation, laser ablation, arc-discharge, energy ball milling technique, and direct current magnetron sputtering (13, 16). Although the physical approaches allow the synthesis of shape-controlled AgNPs, they are highly energy consuming (5). The chemical methods include chemical reduction by using organic and inorganic reducing agents, microemulsion techniques, microwave-assisted synthesis, electrochemical techniques, irradiation-assisted techniques, and pyrolysis (13, 16). The main disadvantage of the chemical approaches is the toxicity of reactants and organic solvents (12).

Both microorganisms (bacteria, fungi and Actinomycetes) and plant extracts have been used in the synthesis of AgNPs (12). There is a growing interest for the green synthesized AgNPs. The green synthesis is eco-friendly, cheap, and gives high yields. Plants are easily available and safe to handle. In addition, plants contain a broad

spectrum of metabolites (polyphenols, terpenoids, alkaloids, proteins, carbohydrates) that might act as reducing agents more efficiently than microorganisms (15, 16). These plant metabolites can ensure the stability and biocompatibility of AgNPs (12). Over 200 plants belonging to different families were screened for green synthesis of metallic nanoparticles (Ag, Au, Fe, and Cu nanoparticles). Among them, AgNPs were the most intensively studied (13). The green synthesis of AgNPs involves a plant extract and a solution of Ag salt. The reaction takes place at room temperature and atmospheric pressure and it is completed within a short period of time (12). The green synthesized AgNPs showed better antimicrobial, antioxidant, and antitumor properties compared to bulk Ag, being very promising for the development of new therapeutic agents (11).

Potential application of green synthesized AgNPs via plant extracts in melanoma treatment

AgNPs showed promising effects against a wide variety of tumor cell lines: U251 human glioblastoma, MDA-MB-231, MCF-7, and T47D human breast adenocarcinoma, HCT15, HT29, and Caco-2 human colon cancer, A549 human alveolar adenocarcinoma, HepG2 hepatocellular adenocarcinoma (11, 14, 17), HeLa human cervical cancer (18), Hep2 human epidermoid larynx carcinoma (19), A375 human melanoma, and B16F10 mouse murine melanoma (14) cell lines. Various plant extracts were used to synthesize AgNPs with antitumor properties. Some examples include extracts isolated from *Vitex negundo* (Chinese chaste tree), *Piper nigrum* (black pepper), *Euphorbia nivulia* (leafy milk hedge), *Annona squamosa* (11), *Origanum vulgare* (oregano), *O. heracleoticum*, *Alpinia katsumadai*, *Ficus sp*., *Mentha arvensis* (corn mint), *Taxus sp*., *Panax ginseng* (true ginseng), *Coriandrum sativum* (coriander) (20). Antitumor efficacy of AgNPs is mediated mainly by upregulation of apoptosis and autophagy, cell cycle arrest, activation of caspases, induction of mitochondrial dysfunction, endoplasmic reticulum stress, oxidative stress, and DNA damage (14). Only a few studies have investigated the antitumor effects of green synthesized AgNPs in melanoma. In these studies, AgNPs were obtained using hydroalcoholic and aqueous extracts from several plant species (tab. II).

Flavonoids were the main constituents of plant extracts that serve as bio reducing and capping agents in the green synthesis of AgNPs. The cytotoxic effects were variable depending on the plant species, chemical composition of extracts, concentration and size of AgNPs. It is well-known the fact that the physicochemical properties of AgNPs (particle shape, size, surface chemistry) but also their concentrations are important parameters that influence the antitumor activity (11). Generally, low concentrations of AgNPs induce antiproliferative effects and smaller nanoparticles cause stronger cytotoxic effects (13).

A potent antimelanoma activity was determined for AgNPs synthesized using extracts from the roots of *Gelsemium sempervirens* (IC_{50}=50 μg/mL) and *Phytolacca decandra* (IC_{50}=78 μg/mL) (21). Also, at 100 μg/mL, AgNPs obtained using extracts from peels and leaves of *Citrus maxima* exhibited a significant cytotoxicity (more than 75%) in B16F10 mouse melanoma cells (27). Several mechanisms of AgNPs-induced cytotoxicity in melanoma cell lines have been described,

such as: induction of apoptosis, up-regulation of autophagy, DNA breakage, chromatin condensation, activation of caspase-3 and endonucleases, as well as cell cycle arrest. Caspase-3 plays an important role in the cascade of reactions that induces apoptosis. Endonucleases are enzymes involved in the DNA fragmentation at the internucleosomal linker regions (21, 27).

It has been suggested that the antiproliferative activity of green synthesized AgNPs is based on the synergism between plant constituents and Ag (21). Numerous studies proved the chemo preventive and antitumoral potential of different flavonoids and flavonoid-rich extracts in melanoma. Flavonoids act by targeting different pathways, immune and inflammatory responses involved in melanoma pathogenesis. Thus, flavonoids like apigenin inhibit the signal transducer and activator of transcription (STAT) and AKT pro-survival signaling and activate ERK pro-apoptotic signaling pathways. Kaempferol has an inhibitory activity on ribosomal S6 kinase and stress activated protein kinase (MSK1), which were found to be overexpressed in human squamous cell carcinomas (28). Also, triterpenoids such as oleanolic acid from *Phytolaccca decandra* showed cytotoxic effects in melanoma cells. Oleanolic acid displays antiproliferative effects by suppression of epidermal growth factor receptor (EGFR) activity and inducing mitochondria-dependent caspase 3-mediated apoptosis in A375 human skin melanoma cells (29). It is obvious that the constituents of plant extracts can functionalize silver nanoparticles enhancing their antitumor activity.

TABLE II

The anti-melanoma effects of green synthesized AgNPs

Plant species	Extract	Reducing and capping agents	AgNPs		Cell line	IC_{50} (µg/mL)	Outcome	Ref.
			Size (nm)	Shape				
Phytolacca decandra, Phytolaccaceae, pokeweed	ethanolic, 70%, v/v	flavonoids triterpenes	90.87	spherical	A375	78	antiproliferative activity, apoptosis, activation of caspase 3, cell cycle arrest, DNA fragmentation	21, 22
Gelsemium sempervirens, Gelsemiaceae, yellow jessamine	ethanolic, 70%, v/v	flavonoids alkaloids	112.2	spherical	A375	50		21, 23
Hydrastis canadensis, Ranunculaceae, orangeroot	ethanolic, 70%, v/v	flavonoids alkaloids	111.3	spherical	A375	80		21, 24
Thuja occidentalis, Cupressaceae, northern white cedar	ethanolic, 70%, v/v	flavonoids terpenes	122.8	spherical	A375	120		21,25
Butea monosperma, Fabaceae, flame of forest	aqueous	chalcones flavonoids	20-80	spherical	B16F10	-	no cytotoxicity	26
Citrus maxima, Rutaceae, pomelo	aqueous	flavonoids polyphenolic acids tannins terpenes	2-50	spherical	B16F10	-	oxidative stress, apoptosis, cell cycle arrest	27

CONCLUSIONS

Although the available data fail to define the whole picture of bioactivity of green synthesized AgNPs in melanoma, the results of up to date studies are very promising. The identification of plant extracts having potent intrinsic antitumor properties and being able to generate easily AgNPs with high stability and biocompatibility could be one of the successful approaches to develop new anti-melanoma agents. Also, more research is needed to evaluate the overall impact of AgNPs on human health.

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EVALUAREA CALITĂŢII VIEŢII LA PACIENTELE CU CANCER DE COL UTERIN PRIN ABORDARE HOLISTICĂ PSIHOLOGICĂ ŞI MEDICALĂ

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EVALUATION OF LIFE QUALITY IN PATIENTS WITH CERVICAL CANCER WITH HOLISTIC APPROACHES PSYCHOLOGICAL AND MEDICAL (Abstract): Assessment of quality of life requires a multidimensional measurement of health and wellness, as reported by the patient. The central objective of this research was to capture the phenomenon of impact on cancer diagnosis; disease and treatment have on physical and emotional status of patients with cervical cancer. **Material and method**: The study was realized on a group of 70 patients diagnosed with this disease, aged 25-55 years and from different intellectual categories both in rural and urban. We used an experimental design of type 2 (early disease stage/advanced) x 5 (surgical therapeutic method / surgery with radiotherapy / surgery with chemotherapy / surgery with radiotherapy and chemotherapy / palliative) x 2 (low therapeutic compliance / high) x 2 (low depression / high). As data collection instruments were used histopathological examinations and psychological tests (to measure quality of life SF-36 test and test sensory and affective pain McGill and independent variables Beck Depression Scale and IPQ-R), and to verify hypotheses T test for independent samples and comparing Univariate ANOVA. **Results**: The results showed the importance of emotional state on the development of cancer disease and its quality of life. **Key words**: QUALITY OF LIFE, CERVICAL CANCER, ILLNESS PERCEPTIONS, DEPRESSION, CHEMORADIOTHERAPY

Valoarea oricărei intervenţii, incluzându-i în mod direct pe bolnavii cu cancer, este determinată judecând impactul atât asupra numărului de ani de viaţă câştigaţi cât şi al calităţii vieţii. Acest impact, măsurat din punctul de vedere al costului, determină o anumită valoare. Calitatea vieţii ridică numeroase probleme şi creează suficiente dezbateri. Din punct de vedere istoric timpul de supravieţuire a fost cel mai util indicator de măsurare al eficacităţii tratamentului. Recunoscând că problema timpului, fără calitate are o valoare discutabilă, cercetătorii din domeniul oncologic au avut tendinţa să presupună că adăugarea de timp în supravieţuire ar însemna şi un plus de valoare pentru pacienţii oncologici, deşi în mod frecvent acest lucru nu este întotdeauna adevărat, în special atunci când toxicitatea tratamentului şi efectele întârziate ale acestuia nu sunt luate în considerare. În prezent, prin disponibilitatea recentă a chestionarelor care vizează calitatea vieţii, este oportun a se măsura cele trei dimensiuni: starea de bine fizică, psihică şi socială alături de cantitatea ei. Acest lucru ne permite să

spunem doar că, timpul adăugat de unele tratamente este de o valoare suficientă pentru a justifica costurile lor şi că s-ar impune analiza valorii unor terapii care adaugă timp pentru prelungirea vieţii, dar nu şi a calităţii acesteia. Partea critică a acestui lucru este că nu întotdeauna se poate alege între toleranţa la tratament (efectele lui secundare) şi timpul de supravieţuire ; uneori, un tratament temporar, paleativ al cancerului poate evita simptome greu de suportat, însă fără a prelungi supravieţuirea. Pacienţii pot găsi acest lucru dezirabil chiar şi în faţa competiţiei cu toleranţa. Numai o evaluare atentă, raportată la calitatea vieţii lor, poate permite aprecierea acestor compromisuri, între simptomele relatate şi toxicitate (1).

OBIECTIVE DE LUCRU

Pornind de la premisa că între pacientele cu o dispoziţie afectivă negativă ridicată şi cele cu dispoziţie afectivă negativă scăzută există o diferenţă în ceea ce priveşte calitatea vieţii acestora, am încercat să realizăm o cercetare în care am dorit să stabilim relaţiile între variabilele investigate şi în ce măsură evoluţia unei variabile influenţează celorlalte variabile. Am considerat că este important de evidenţiat stadializarea bolii, a metodei terapeutice urmate de paciente şi implicaţiile acestor proceduri folosite în scop terapeutic asupra dispoziţiei lor afective negative, iar nu în ultimul rând importanţa autocontrolului pacientelor cu cancer de col uterin precum şi percepţia acestora în controlul tratamentului.

Studiul urmăreşte evidenţierea impactului cancerului de col uterin asupra calităţii vieţii pacientelor diagnosticate cu această afecţiune şi a factorilor ce conduc la scăderea calităţii vieţii acestora.

Obiectivele urmărite au fost :

1. Evaluarea influenţei metodei terapeutice asupra stării de sănătate fizică, mentală şi durere.
2. Aprecierea ponderii în care autocontrolul şi controlul tratamentului (complianţa la tratament) influenţează starea de sănătate fizică, mentală şi durerea.
3. Influenţa depresiei asupra stării de sănătate fizică, mentală şi durerii.
4. Înregistrarea unor posibile efecte de interacţiune între stadiul bolii şi depresie, cu consecinţe asupra calităţii vieţii pacientelor cu cancer de col uterin.
5. Cercetarea relaţiei dintre stadiul bolii şi complianţa la tratament, cu importanţă asupra calităţii vieţii pacientelor.

MATERIAL ŞI METODĂ

Pentru a aprecia dacă există diferenţe între grupele de paciente studiate s-a efectuat un studiu transversal, care are ca scop evaluarea diferitelor nivele de durere, de depresie, stare de sănătate fizică şi mentală, stadializare, metodă terapeutică, complianţă la tratament, investigând fiecare subiect (2). Colectarea datelor s-a realizat pe o perioadă de 3 ani (2007 – 2010). Au fost selecţionate diferite grupe în funcţie de aceste variabile, pacientele fiind aflate atât în evidenţa Spitalului Clinic Militar de Urgenţă „Dr. Iacob Czihac" Iaşi, cât şi a Maternităţii „Cuza-Vodă" Iaşi. Subiecţii au fost evaluaţi cu ajutorul instrumentelor de măsurare a variabilelor alese în studiu o singură dată, respectiv la un interval de 3 luni de zile de la intervenţia chirurgicală, sau de la diagnosticare – pentru cele care au ales sa urmeze doar metoda paleativă, iar apoi am verificat în ce măsură aceste variabile se influenţează reciproc.

Lotul de subiecţi a fost alcătuit dintr-un număr de 70 paciente diagnosticate - în urma examenului clinic preoperator coroborat cu examenul histopatologic pre- şi postoperator (aspectele anatomo-patologice tumorale – macroscopia, microscopia şi stadiul patologic) - cu cancer de col uterin, aflate în diferite stadii ale bolii. Pacientele cuprinse în cercetare au avut vârsta cuprinsă între

25-55 ani, de categorii sociale diferite şi provenind atât din mediul rural cât şi din cel urban. O parte dintre subiecţi au beneficiat atât de tratamentele complexe ale cancerului de col uterin (chirurgical cu radioterapie şi chimioterapie), altele au urmat tratamente parţiale (numai chirurgicale, ori chirurgicale cu radioterapie, chirurgicale cu chimioterapie) sau numai tratament paleativ. De asemenea, stadializarea bolii a fost cea decisivă în privinţa recomandării procedurii terapeutice ulterioare pe care pacientele urmau să o efectueze.

S-au utilizat ca instrumente de culegere a datelor testele psihologice (pentru măsurarea calităţii vieţii s-a folosit chestionarul SF-36 şi chestionarul McGill pentru măsurarea durerii senzoriale şi afective ; pentru măsurarea variabilelor independente – Scala de depresie Beck şi Illness Perception Questionnaire R), iar pentru verificarea ipotezelor Testul T pentru compararea eşantioanelor independente, pentru a analiza influenţa variabilelor independente asupra celor dependente şi, de asemenea, ANOVA Univariată, cu interesarea efectelor principale ale variabilelor, cât şi efectele de interacţiune ale acestora. Întreaga metodologie utilizată în cercetare a fost adaptată, şi validat pe populaţia românească. S-au calculat mediile (M) scorurilor obţinute de paciente la testele aplicate, valorile Testului Levene (F) pentru verificarea egalităţii varianţelor, diferenţele dintre medii cu aflarea gradelor de libertate (df) şi a pragului de semnificaţie (p), precum şi Testul de Contrast (Difference) pentru compararea diferitelor grupuri de paciente rezultate din împărţirea lor în funcţie de categoriile variabilelor independente, iar în cazul variabilei ‚metoda terapeutică' Testul Post Hoc Bonferroni. S-a considerat testele statistice ca fiind semnificative dacă pragul de semnificaţie, p, a fost mai mic sau cel mult egal cu valoarea 0,05 (pentru un interval de încredere de 95%).

REZULTATE

1. Pentru realizarea primului obiectiv s-a analizat dacă metoda terapeutică influenţează starea de sănătate fizică, mentală şi durerea. În urma comparării rezultatelor a câte două categorii privind metoda terapeutică 1 (chirurgicală), 4 (chirurgicală + radioterapie + chimioterapie) şi 5 (tratament paleativ) urmată de paciente, am obţinut următoarele date :

a) media scorurilor obţinute pentru starea de sănătate fizică şi mentală :

- *la pacientele care au urmat metoda terapeutică chirurgicală, M=114,40, iar la pacientele care au urmat metoda terapeutica chirurgicală cu radioterapie şi chimioterapie, M=105 ;* F = 47,25 ; p< 0,001 ; t (18) = 30,76 ; p <0,001.
- *la pacientele care au urmat metoda terapeutică chirurgicală cu radioterapie şi chimioterapie, M=105, iar la pacientele care au urmat metoda terapeutică paleativă, M=67,30 ;* F = 647 ; p<0,001 ; t (28) = 14,03 ; p <0,001.

b) media scorurilor obţinute pentru durerea senzorială şi afectivă :

- *la pacientele care au urmat metoda terapeutică chirurgicală, M=8,70, iar la pacientele care au urmat metoda terapeutică chirurgicală cu radioterapie şi chimioterapie, M=7 ;* F = 34,63 ; p < 0,001 ; t (9) = 4,63 ; p = 0,001.
- *la pacientele care au urmat metoda terapeutică chirurgicală cu radioterapie şi chimioterapie, M=7, iar la pacientele care au urmat metoda terapeutica paleativă, M=36,5 ;* F = 51,43 ; p < 0,001 ; t (19) = 51,43 ; p < 0,001.

2. În urma aprecierii ponderii în care autocontrolul şi controlul tratamentului (complianţa la tratament) influenţează starea de sănătate fizică şi mentală şi durerea, se remarcă următoarele :

a) media scorurilor obţinute pentru starea de sănătate fizică şi mentală : la pacientele care au prezentat un control per-

sonal şi al tratamentului scăzut, M=61,80, iar la pacientele cu un control personal şi al tratamentului crescut, M=99,88 ; F = 25,52 ; p < 0,001 ; t (65,90) = 15,92 ; p < 0,001.

b) media scorurilor obţinute pentru durerea senzorială şi afectivă : la pacientele cu un nivel scăzut al complianţei terapeutice, M=31,85, iar la pacientele cu o complianţa crescută,M=16,18 ; F = 39,86 ; p < 0,001 ; t (57,64) = 8,24 ; p < 0,001.

3. În ceea ce priveşte influenţa depresiei asupra stării de sănătate fizică, mentală şi durerea s-au constatat următoarele rezultate :

a) media scorurilor obţinute pentru starea de sănătate fizică şi mentală : la pacientele care au prezentat un nivel al depresiei scăzut, M=106,94, iar la pacientele cu un nivel crescut al depresiei, M=70 ; F = 6,38 ; p = 0,001 ; t (59,13) = 15,52 ; p < 0,001.

b) media scorurilor obţinute pentru durerea senzorială şi afectivă : la pacientele care au prezentat un nivel al depresiei scăzut, M=9,25, iar la pacientele cu un nivel crescut al depresiei, M=32,72 ; F = 0,16 ; p > 0,05 ; t (68) = 17,81 ; p < 0,001.

4. Înregistrarea unor posibile efecte de interacţiune între stadiul bolii şi depresie asupra calităţii vieţii pacientelor cu cancer de col uterin a evidenţiat următoarele rezultate :

a) influenţa stadiului bolii asupra stării de sănătate fizică şi mentală : F (1,69) = 5,25 ; p = 0,02 ; media scorurilor obţinute la pacientele aflate în stadiul 1-2 al bolii, M = 90,35, iar la pacientele aflate în stadiul 3-4 al bolii, M = 85,29 ; p = 0,025 ; ***influenţa depresiei asupra stării de sănătate fizică şi mentală*** : F (1,69) = 278,19 ; p < 0,001 ; media scorurilor obţinute la pacientele cu un nivel scăzut al depresiei, M = 106,22, iar la pacientele cu un nivel crescut al depresiei, M = 69,41 ; p < 0,001 ; ***efectul de interacţiune a stadializării bolii şi depresiei asupra stării de sănătate fizică şi mentală*** : F (1,69) = 980,11 ; p = 0,001 : ***efectul stadializării bolii la fiecare nivel al depresiei :*** în grupul pacientelor cu un nivel scăzut al depresiei, media scorurilor obţinute la pacientele aflate în stadiul 1-2 al bolii este M = 112,70, iar la pacientele aflate în stadiul 3-4 este M= 99,75 ; t (34) = 7,19 ; p < 0,001 ; în grupul pacientelor cu un nivel ridicat al depresiei, media scorurilor obţinute la pacientele aflate în stadiul 1-2 al bolii este M = 68, iar la pacientele aflate în stadiul 3-4, este M = 70,83 ; t (32) = 1,01 ; p > 0,05 (0,321) *;* ***efectul depresiei la fiecare nivel al stadializării bolii :*** în grupul pacientelor aflate în stadiul 1-2 al bolii, media scorurilor obţinute la pacientele cu nivelul depresiei scăzut este M = 112,70, iar la pacientele cu un nivel crescut al depresiei, M= 68 ; t (28) = 83,68 ; p < 0,001 ; în grupul pacientelor aflate în stadiul 3-4 al bolii, media scorurilor obţinute la pacientele cu un nivel al depresiei scăzut este M = 99,75, iar la pacientele cu un nivel ridicat al depresiei, este M = 70,83 ; t (38) = 8,81 ; p < 0,001, (fig. 1,2).

b) influenţa stadiului bolii asupra durerii senzoriale şi afective : F(1,69) = 15,70 ; p <0,001 ; media scorurilor obţinute la pacientele aflate în stadiul 1-2 al bolii este M = 18,17, iar la pacientele aflate în stadiul 3-4 al bolii, M = 23,16, p <0,001 ; ***influenţa depresiei asupra durerii senzoriale şi afective*** : F (1,69) = 311,81 ; p < 0,001 ; media scorurilor obţinute la pacientele cu un nivel scăzut al depresiei este M = 9,55, iar la pacientele cu un nivel crescut al depresiei, M = 31,79, p<0,001 ; ***efectul de interacţiune a stadializării bolii şi depresiei asupra durerii*** : F (1,69) = 0,105 ; p = 0,747 : ***efectul stadializării bolii la fiecare nivel al depresiei :*** în grupul pacientelor cu un nivel scăzut al depresiei, media scorurilor obţinute de pacientele aflate în stadiul 1-2 al bolii la durere este M = 6,85, iar la pacientele aflate în stadiul 3-4, M= 12,25 ; t (34) = 2,29 ; p = 0,008 ; în grupul pacientelor cu un nivel ridicat al depresiei, media sco-

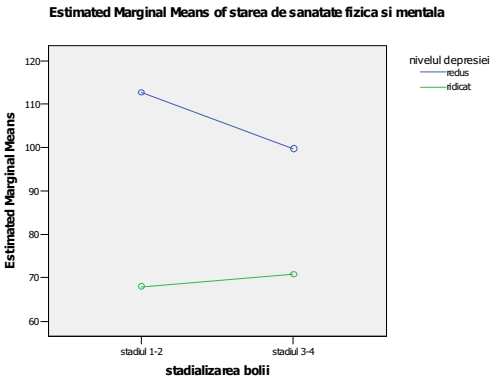

Fig. 1. Efectul de interacţiune a stadializării bolii şi nivelul depresiei asupra stării de sănătate fizică şi mentală

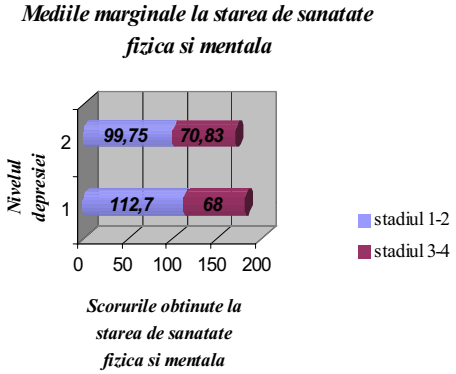

Fig. 2. Mediile scorurilor obţinute la starea de sănătate fizică şi mentală în funcţie de interacţiunea dintre stadializarea bolii şi nivelul depresiei

rurilor obţinute de pacientele aflate în stadiul 1-2 al bolii la durere este M = 29,50, iar la pacientele aflate în stadiul 3-4, M = 34,08 ; t (32) = 3,70 ; p = 0,005 ; ***efectul depresiei la fiecare nivel al stadializării bolii :*** în grupul pacientelor aflate în stadiul 1-2 al bolii, media scorurilor obţinute la durere de pacientele cu nivelul depresiei scăzut este M = 6,85, iar la pacientele cu un nivel crescut al depresiei, M= 29,50 ; t (28) = 83,68 ; p < 0,001 ; în grupul pacientelor aflate în stadiul 3-4 al bolii, media scorurilor obţinute la scala durerii de pacientele cu un nivel al depresiei scăzut, este M = 12,25, iar la pacientele cu un nivel ridicat al depresiei, M = 34,08 ; t (38) = 10,21 ; p = 0,048, (fig. 3,4).

5. Cercetarea relaţiei dintre stadiul bolii şi complianţa la tratament cu importanţă asupra calităţii vieţii pacientelor a relevat următoarele :

a) influenţa stadiului bolii asupra stării de sănătate fizică şi mentală : F (1,69) = 75,68 ; p < 0,001 ; media scorurilor obţinute la starea de sănătate fizică şi mentală de pacientele aflate în stadiul 1-2 al bolii este M = 90,35, iar la pacientele aflate în stadiul 3-4 al bolii, M = 73,46, p < 0,001 ; ***influenţa complianţei la tratament asupra stării de sănătate fizică şi mentală*** : F (1,69) = 429,44 ; p < 0,001 ; media scorurilor obţinute la starea de sănătate fizică şi mentală de pacientele cu un nivel scăzut al complianţei este M = 61,80, iar la pacientele cu un nivel crescut al complianţei, M = 102,01, p < 0,001 ; ***efectul de interacţiune al variabilei stadializarea bolii şi variabilei complianţa la tratament asupra stării de sănătate fizică şi mentală :*** F (1,69) = 5,33 ; p = 0,024 ; ***efectul stadializării bolii la fiecare nivel al complianţei terapeutice :*** în grupul pacientelor cu un nivel scăzut al complianţei, media scorurilor obţinute la starea de sănătate fizică şi mentală de pacientele aflate în stadiul 1-2 al bolii este M = 68, iar la pacientele aflate în stadiul 3-4, M= 55,60 ; t (18) = 33,40 ; p < 0,001 ; în grupul pacientelor cu un nivel ridicat al complianţei, media scorurilor obţinute de pacientele aflate în stadiul 1-2 al bolii este M = 112,70, iar la pacientele aflate în stadiul 3-4, M = 91,33 ; t (48) = 10,53 ; p < 0,001 ; ***efectul complianţei terapeutice la fiecare nivel al stadializării bolii :*** în grupul pacientelor aflate în stadiul 1-2 al bolii, media scorurilor obţinute la starea de sănătate fizică şi mentală de pacientele cu nivelul complianţei scăzut este M = 68, iar la pacientele cu un nivel crescut al complianţei, M= 112,70 ; t (28) = 83,68 ; p < 0,001 ; în grupul pacientelor aflate în stadiul 3-4 al bolii, media sco-

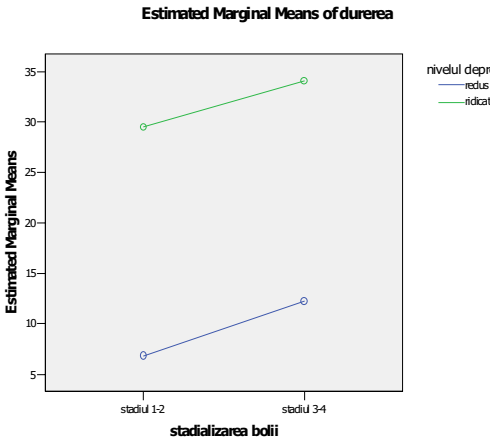

Fig. 3. Efectul de interacţiune a stadializării bolii şi nivelul depresiei asupra durerii afective şi senzoriale

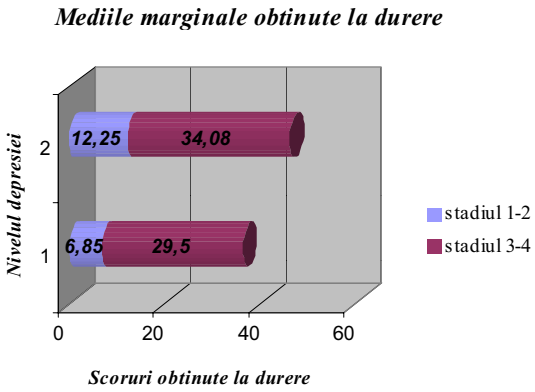

Fig. 4. Mediile scorurilor obţinute la durerea afectivă şi senzorială în funcţie de interacţiunea dintre stadializarea bolii şi nivelul depresiei

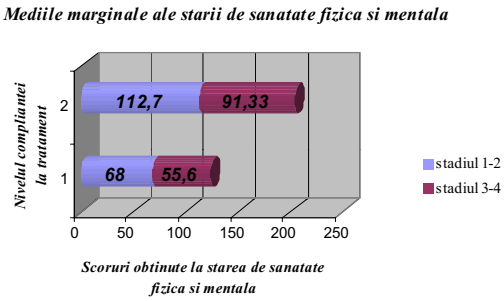

Fig. 5. Mediile scorurilor obţinute la starea de sănătate fizică şi mentală în funcţie de interacţiunea dintre stadializarea bolii şi nivelul complianţei la tratament

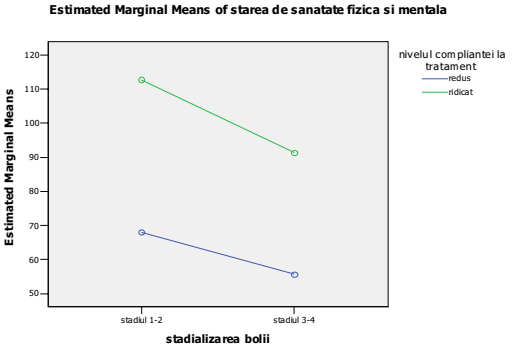

Fig. 6. Efectul de interacţiune a stadializării bolii şi nivelul complianţei la tratament asupra stării de sănătate fizică şi mentală

rurilor obţinute de pacientele cu un nivel al complianţei scăzut este M = 55,60, iar la pacientele cu un nivel ridicat al complianţei, M = 91,33); t (38) = 17,93; p < 0,001, (fig. 5,6).

b) influenţa stadiului bolii asupra durerii afective şi senzoriale: F (1,69) = 18,44; p < 0,001; media scorurilor obţinute la testul durerii de pacientele aflate în stadiul 1-2 al bolii este M = 18,17, iar la pacientelor aflate în stadiul 3-4 al bolii, M = 28,30, p < 0,001; ***influenţa complianţei la tratament asupra durerii afective şi senzoriale:*** F (1,69) = 53,37; p < 0,001; media scorurilor obţinute la durere de pacientele cu un nivel scăzut al complianţei este M = 31,85, iar la pacientele cu un nivel crescut al complianţei, M = 14,62, p < 0,001; ***efectul de interacţiune a stadializării bolii şi complianţei la tratament asupra durerii afective şi senzoriale***: F (1,69) = 5,29; p = 0,025: ***efectul stadializării bolii la fiecare nivel al complianţei terapeutice:*** în grupul pacientelor cu un nivel scăzut al complianţei, media scorurilor obţinute la durere de pacientele aflate în stadiul 1-2 al bolii este M = 29,50, iar la pacientele aflate în stadiul 3-4, M= 34,20; t (18) = 13,39; p

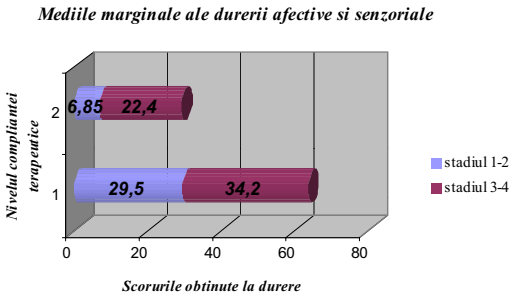

Fig. 7. Mediile scorurilor obţinute la durerea afectivă şi senzorială în funcţie de interacţiunea dintre stadializarea bolii şi nivelul complianţei

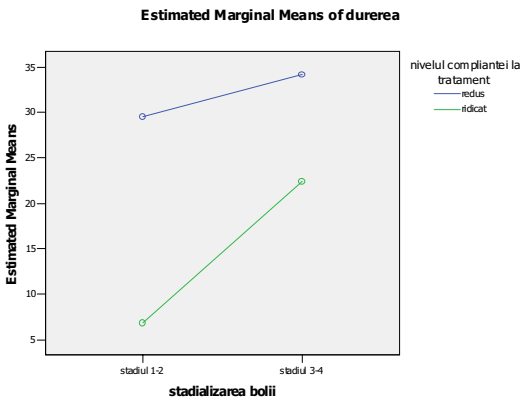

Fig. 8. Efectul de interacţiune a stadializării bolii şi nivelul complianţei asupra durerii afective şi senzoriale

< 0,001 ; în grupul pacientelor cu un nivel ridicat al complianţei, media scorurilor obţinute de pacientele aflate în stadiul 1-2 al bolii este M = 6,85, iar la pacientele aflate în stadiul 3-4, M = 22,40 ; t (48) = 6,31 ; p < 0,001 ; ***efectul complianţei terapeutice la fiecare nivel al stadializării bolii :*** în grupul pacientelor aflate în stadiul 1-2 al bolii, media scorurilor obţinute la durere de pacientele cu nivelul complianţei scăzut este M =29,50, iar la pacientele cu un nivel crescut al complianţei, M= 6,85 ; t (28) = 23,45 ; p < 0,001 ; în grupul pacientelor aflate în stadiul 3-4 al bolii, media scorurilor obţinute de pacientele cu un nivel al complianţei scăzut este M = 34,20, iar la pacientele cu un nivel ridicat al complianţei, M = 22,40 ; t (38) = 17,93 ; p < 0,001, (fig. 7,8).

DISCUŢII

În cazul tratamentului pentru cancerul de col uterin, oboseala, care este un factor de scădere a stării de sănătate fizică şi mentală şi care, de altfel, a fost măsurat în studiu, se asociază mai frecvent radioterapiei şi chimioterapiei ; în schimb, după aplicarea doar a intervenţiei chirurgicale fie diagnostice, fie terapeutice, oboseala tinde să dispară în timp ; nu se întâmplă acest lucru în cazul terapiilor combinate ; chimioterapia are cele mai multe inconveniente dintre toate tratamentele din cauza numeroaselor efecte secundare, deşi ele pot varia de la individ la individ şi într-un oarecare grad ; s-a observat că pe termen mai lung durerea la pacientele tratate chirurgical a scăzut semnificativ comparativ cu durerea preoperatorie, în schimb dacă tratamentul cancerului nu a fost continuat, aceasta a recidivat în intensitate şi frecvenţă (3,4) ;

Complianţa la tratament este legată mai ales de percepţia de către pacientă a stării ei de boală, de atitudinea faţă de tratament şi de conştientizarea bolii, de costurile medicamentelor, efectele secundare ale tratamentului şi tratamentul inadecvat al simptomelor de boală (5) ; convingerile personale despre durere pot fi predictive pentru nivelul durerii percepute de paciente, suferinţele emoţionale şi credinţa că durerea semnalează o înrăutăţire a stării de sănătate au reprezentat predictori ai raportării durerii ; complexitatea tratamentului a putut fi un factor cu influenţă directă asupra aderenţei terapeutice, o influenţă negativă fiind determinată de numărul mare de medicamente, dozele frecvente, durata lungă a tratamentului, efecte secundare numeroase şi forme diferite de administrare ; aderenţa terapeutică este cu atât mai mică cu cât remisiunea bolii este de durată mai lungă ; în toate cazurile, beneficiile terapeutice trebuie să contra-balanseze

disconfortul (cu cât este mai mare beneficiul perceput, cu atât e mai mare dorinţa de a tolera un oarecare disconfort) ;

Prin progresia cancerului, apariţia durerilor se asociază cu simptome depresive mai severe, în timp ce depresia comorbidă se asociază cu diminuarea funcţionării şi o calitate a vieţii mai scăzută (6) ; cele mai multe complicaţii depresive au apărut cu probabilitate mai mare la pacientele cu durere şi invers (7) ;

Cele mai mici scoruri la starea de sănătate fizică şi mentală au fost obţinute de pacientele aflate în stadiul incipient al bolii (1-2) dar cu un nivel ridicat al depresiei, ceea ce demonstrează influenţa nu atât a stadializării bolii cât mai important a depresiei asupra calităţii vieţii ; rezultatele acestora sunt comparative cu cele ale pacientelor aflate în stadiul 3-4 şi cu depresie ridicată, care au obţinut scoruri mici la calitatea vieţii, dar diferenţe semnificative nu există între aceste două grupe de paciente.

Este o mare diferenţă între problemele cu care se confruntă şi trebuie să le facă faţă o bolnavă cu o leziune incipientă la nivelul colului uterin, faţă de alta, cu o tumoră invazivă, cu posibile metastaze, aici fiind vorba de un stres particular asociat diagnosticului care poate influenţa reacţia la problemele cu care se confruntă, implicit starea psihică ; s-au întâlnit în clinică paciente cu tumori în stadiu incipient având şanse mari de vindecare, dar care au reacţionat cu neîncredere faţă de eficacitatea tratamentului, prin stare depresivă şi senzaţie de neputinţă şi lipsă de speranţă, în timp ce bolnave aflate în stadii avansate, cu un prognostic mai rezervat, s-au angajat să-şi asume boala, iar între curele terapeutice s-au străduit să ducă o viaţă cât mai apropiată de cea dinaintea îmbolnăvirii (8) ;

Cele mai mici scoruri la starea de sănătate fizică şi mentală le-au obţinut pacientele aflate în stadiul 3-4 al bolii şi cu un nivel scăzut al controlului personal şi al tratamentului, iar cele mai bune scoruri - cele aflate în stadiul 1-2 al bolii, cu un autocontrol şi control al tratamentului ridicat ; între cele două grupe de paciente, unele aflate în stadiul 1-2 al bolii, iar altele în stadiul 3-4, diferenţele semnificative în ceea ce priveşte starea de sănătate fizică şi mentală au fost date de modul în care aceste paciente şi-au gestionat autocontrolul şi s-au adaptat tratamentului oncologic recomandat.

Analizând grupul pacientelor aflate în aceleaşi stadii ale bolii, diferenţele semnificative pentru scala durerii sunt date de gradul lor de aderenţă la tratamentul oncologic ; pacientele cu o încredere în sine extrem de puternică au exprimat scepticism faţă de cadrele medicale ; în schimb pacientele care au dezvoltat un control ridicat personal au fost mai puţin deprimate, mai puţin ostile şi s-au adaptat mai bine din punct de vedere psihosocial decât cele cu un control scăzut ; aşteptările negative privind boala au fost asociate cu mai multe complicaţii în timpul spitalizărilor şi cu o probabilitate mai mare de a dezvolta episoade de depresie ; dacă pacientele au înţeles scopul tratamentului şi motivul precis al administrării medicamentelor, s-a obţinut un grad superior de aderenţă ; cel mai important factor de influenţare a complianţei ar fi nu neapărat stadializarea bolii, cât perceperea de către pacientă a interesului acordat de către medic, aceasta fiind ameliorată atunci când îi sunt oferite explicaţii clare, încurajare, susţinere şi o urmărire sistematică a evoluţiei.

CONCLUZII

Pacientele care au urmat doar metoda chirurgicală au prezentat un nivel al stării de sănătate fizică şi mentală, precum şi un nivel al durerii semnificativ crescut comparativ cu a celor care au urmat metoda chirurgicală + radioterapie + chimioterapie. Pacientele care au urmat doar metoda paleativă au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ scă-

zut comparativ cu a celor care au urmat metoda terapeutică complexă ; apar diferenţe semnificative şi între nivelul durerii la pacientele care au urmat metoda terapeutică complexă şi celor care au urmat doar un tratament paleativ ; pacientele care au urmat metoda chirurgicală complexă au prezentat un nivel al durerii semnificativ scăzut comparativ cu a celor care au urmat doar terapia paleativă.

Pacientele care au prezentat un control personal şi al tratamentului crescut au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ crescut şi un nivel al durerii semnificativ scăzut comparativ cu a celor cu un grad redus al complianţei. Pacientele cu un nivel al depresiei crescut au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ scăzut şi un nivel al durerii semnificativ crescut comparativ cu pacientele care au avut un nivel al depresiei scăzut.

Pacientele aflate în stadiul 1-2 al bolii şi cu un nivel redus al depresiei au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ crescut decât pacientele aflate în stadiul 3-4 al bolii ; în schimb nu există diferenţe semnificative între nivelul stării de sănătate fizică şi mentală la pacientele cu un nivel crescut al depresiei, indiferent de stadiul bolii. Pacientele aflate în stadiul 3-4 al bolii şi cu un nivel redus al depresiei au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ mai mare comparativ cu cele cu un nivel crescut al depresiei ; în ceea ce priveşte durerea, pacientele aflate în stadiul 1-2 al bolii şi cu un nivel redus al depresiei au prezentat un nivel al durerii afective şi senzoriale semnificativ scăzut comparativ cu pacientele aflate în stadiul 3-4 al bolii.

Pacientele aflate în stadiul 1-2 al bolii şi cu un nivel redus al controlului personal şi autocontrolului au prezentat un nivel al stării de sănătate fizică şi mentală semnificativ mai mare decât pacientele aflate în stadiul 3-4 al bolii. Pacientele aflate în stadiul 1-2 al bolii şi cu un nivel redus al controlului personal şi autocontrolului au prezentat un nivel al durerii semnificativ mai mic decât pacientele aflate în stadiul 3-4 al bolii ; nivelul durerii a crescut invers proporţional cu nivelul autocontrolului şi controlul tratamentului.

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THE EVALUATION OF SOME ANTIBACTERIAL DRUGS INFLUENCE ON EXPERIMENTAL-INDUCED ACUTE INFLAMMATION IN RATS

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THE EVALUATION OF SOME ANTIBACTERIAL DRUGS INFLUENCE ON EXPERIMENTAL-INDUCED ACUTE INFLAMMATION IN RATS (Abstract): In present study we aimed to investigate the effects of some antibiotics on carrageenan-induced rat paw inflammation. **Material and methods:** The experiment was performed on white male Wistar rats, randomly distributed into 7 groups of 6 rats each. The animals were treated intraperitoneally with saline solution, prednisone (PDN), indomethacin (IND); cefotaxime (CEF), gentamicin (GEN) and sulfamethoxazole (SMT), 30 minutes before carrageenan sub-plantar injection. Control group contains rats with intra-paw injection of saline. Prednisone and indomethacin were administered in a single daily injection, and the antibiotics every 12 hours, for 3 days. The paw volume was assessed plethysmographycally before, and 1, 3, 6, 24 and 72 hours after the administration of carrageenan. We studied the effects of the tested antibiotics in acute inflammation, by evaluating their influence on leukocyte formula, erythrocyte sedimentation rate and fibrinogen levels, before and at 3, 24 and 72 hours in the study. Experimental protocol was implemented according our University Committee for Research and Ethical Issues guidelines. **Results**: The treatment with CEF, GEN, SMT was associated by an important suppression of the edema development, in all time moments of the investigation, but less intense than those of PDN and IND. **Conclusions:** CEF manifested the most potent anti-inflammatory activity, compared to GEN and SMT at 3 hours and 6 hours in this acute of paw`s inflammation test. **Keywords:** CEFOTAXIME, GENTAMICIN, SULFAMETHOXAZOLE, CARRAGEENAN, RAT.

Inflammation is an essential part of the body's immune response. It is the organism's attempt to heal after an injury; to protect against foreign invaders such as viruses, bacteria or fungi; but also repairing damaged tissues (1). The inflammatory response is a defense mechanism that has evolved to the organs to protect them from infection and wound. Its purpose is to locate and remove the harmful agent, to eliminate the damaged tissue components, helping the organism`s healing process. The response consists of changes in blood flow, an increase in vessels permeability, the

migration of fluids, proteins, and leukocytes from the circulation to the tissue lesion (1, 2). An inflammatory response lasting only a few days is called acute inflammation, while a continuing response is defined as chronic inflammation.

For many years, it has been thought that acute and chronic inflammatory processes have different causes, with the involvement of various cells and numerous mediators of inflammation, which could be responsible for distinct clinical manifestations. Over time, however, several hypotheses have been suggested, among which, a more modern vision postulates, that these processes would be interconnected. Moreover, in the evolution of acute inflammation, tissue healing processes may progress in another direction and lead to the development of a chronic inflammatory state, in which fibrotic changes predominate (4). Acute inflammation has a rapid onset and immediately becomes severe. Signs and symptoms are present for only a few days, but in some situations, inflammation may persist for several weeks. A series of biochemical events propagate and contribute to the evolution of the inflammatory response, involving the local vessels, the immune system, and various cells in the wound tissue. As characteristic elements of acute inflammatory process are mentioned: the protein exudate and the presence of neutrophils (5, 6). Chronic inflammation has a prolonged evolution for several months or even years. Continued inflammation leads to a progressive change in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and tissue healing. As characteristic elements of chronic inflammation are considered: the presence of macrophages and lymphocytes, few neutrophils; blood vessel proliferation, fibrosis and tissue necrosis (2, 6).

The current trend involves the performing of multiple researches, to evaluate the influence of antibiotics on the mediators of inflammation, on the body`s immune defense parameters and on the mechanisms responsible for oxidative stress. All these investigations include complex studies to define the pathophysiological mechanisms involved, the evolution, consequences on the body, and to find new methods for the prevention and treatment of infectious diseases.

The analysis of data obtained from the experimental researches, will open up the prospects of completing, theoretical and practical information about the influence of antibacterial chemotherapies on the acute inflammatory markers.

We aimed to investigate the effects of some antibacterial chemotherapeutic agents (from the groups of cephalosporins, aminoglycosides, sulfonamides) on carrageenan-induced rat paw inflammation.

MATERIAL AND METHODS

White male Wistar rats (200-250 g) purchased from the bio-base of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, were used for the researches.

The animals were randomly distributed into 7 groups of 6 rats each, according to the following protocol: Group 1 (Control): rats with intra-paw injection of saline - control group without inflammation. For the following groups, carrageenan-induced paw inflammation was induced. Group 2 (SS): saline solution - 0.3 ml/100g body – control group with inflammation; Group 3 (PDN): prednisone 5 mg/kg b.w. /day; Group 4 (IND): indomethacin 150 mg/ kg b.w. /day; Group 5(CEF): cefotaxime 200 mg/ kg b.w. /day; Group 6 (GEN): gentamicin 20 mg/ kg b.w. /day; Group 7 (SMT): sulfamethoxazole 250 mg/ kg b.w. /day. The substances used in the experi-

ment were purchased from Sigma-Aldrich Chemical Co. Germany.

In order to familiarize with the experimental environment, the animals were housed for 7 days in laboratory, under standard conditions (constant temperature of 21 ± 2°C, relative humidity of 50-70% and alternating illumination regimen, with a light/dark ratio of 12 hours/12 hours). Rats received granulated diet and water *ad libitum* except of the day of investigations. To avoid the chronobiological influences, experimental researches were performed in the interval between 8.00-13.00 a.m.

The animals were treated intraperitoneally with the tested substances, 30 minutes before 1% carrageenan sub-plantar injection, and for 3 consecutive days. Two known anti-inflammatory drugs have been used as positive control substances, in the experiment: prednisone (corticosteroid with marked anti-inflammatory and immunosuppressive effects) and indomethacin (non-steroidal anti-inflammatory with strong anti-inflammatory effects), in a single daily injection, for 3 days. The antibiotics were administered every 12 hours, for 3 days.

Study design

Carrageenan-induced acute paw inflammation model

The experimental induced paw inflammation (using carrageenan, oil of turpentine), is a standardized and internationally recognized model of acute inflammation, being an easy and convenient method for studying the anti-inflammatory effects of different substances, the evaluation being based on their ability to prevent or reduce the development of the animal`s paw inflammatory process (7,8). The local subcutaneous injection of 0.2 ml 1% carrageenan solution, on the rat`s paw, induces a biphasic response: an immediate inflammatory reaction and neurogenic pain, lasting for 6 hours, and a late response phase (inflammatory hyperalgesia), with a maximum intensity after 72 hours, that gradually decreases over time to 96 hours (9).

Plethysmometer assessment

The paw volume was measured before injection of the chemical agent (at baseline), 1 hour, 3 hours, 6 hours, 24 hours and 72 hours after the inducing of inflammation, using a Digital Water Plethysmometer (PanLAB Apparatus).

Blood tests

Under general anesthesia with isoflurane, blood samples (2 ml) were collected from the rat`s retro-orbital plexus and hematological determinations were performed before inflammation, as well as, after 3 hours, 24 hours and 72 hours in the experiment. Using the ACCENT 200 Analyzer, the leukocyte formula was determined. Measurement of erythrocyte sedimentation rate (ESR) was performed by micro capillary technique in EDTA (ethylene-di-amine tetra-acetic acid) anti-coagulated blood. The whole fibrinogen levels were assessed by coagulometry method in citrated venous blood. The both ESR and the acute-phase protein fibrinogen were considered to be markers of inflammatory reaction. The evaluation of ESR value is a nonspecific test for inflammation assessing indirectly its presence and evolution. It is well known that, ESR, as well as fibrinogen raise in response to inflammatory processes.

Data were centralized and statistically processed using the t-student test and analysis of variance (ANOVA) method for *Microsoft Office Excel* 2010. The results were expressed as arithmetic mean of values ± the standard deviation (SD). The p-values (representing the probability), lower than the 0.05 and 0.01 levels were considered to be statistically significant, respec-

tively very significant, comparing with control group.

The experimental protocol was approved by the Ethic Committee on Research of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania, in concordance with EU Directive 2010/63/EU, regarding the handling of laboratory animals (10, 11).

RESULTS

The statistical analysis of the data revealed that, intra-paw administration of carrageenan 1%, induced local acute inflammation, manifested by an increase in rat paw volume, statistically significant comparing with baseline volume. The local pain was suggested by flinching and licking of the injected paw, redness and swelling of inflamed paw were observable in direct examination.

Inflammation has progressively developed, with a maximal intensity at 6 hours, after which it has slowly diminished, being persistent for 72 hours, in the experiment. These results are consistent with the data communicated in the literature, about the evolution of carrageenan paw edema in rats (12, 13). No evidence of acute inflammatory manifestations, nor significant changes in paw volume and skin color, have been observed, in control group with intra-paw injection of saline solution.

The administration of PDN and IND determined a substantial inhibition of the paw local edema, evidenced in all time points of testing, after the administration of carrageenan. These observations are in accordance with the literature data, regarding the effects of different non-steroidal anti-inflammatory drugs, in the experimental model of carrageenan-induced paw inflammation in rats (14, 15, 16). For CEF, GEN, SMT groups, an important suppression of the local inflammation, was observed, at all the moments at which paw edema was evaluated.

The use of PDN resulted in the most accentuated diminution of the paw volume, statistically significant compared to control group with local inflammation. The weakest reduction in the volume of the inflamed paw, was induced by SMT. The effects of the tested substances on the decreasing of paw edema volume, were in the descending order, the following: PDN > IND > CEF > GEN> SMT (fig. 1).

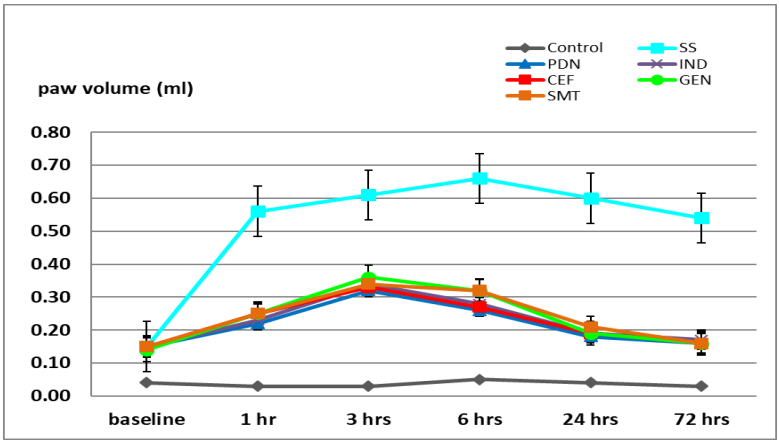

Fig. 1. The effects of CEF, GEN and SMT on carrageenan-induced paw inflammation in rats. Each point represents the mean ± S.D. of paw volume for 6 animals in a group.

The use of PDN, respectively of IND resulted in a considerable decreasing of the neutrophils percentage in peripheral blood, statistically significant compared to saline group, in each moment of determinations, in carrageenan-induced paw inflammation model in rats. Intraperitoneal injection of the tested antibiotics was associated by a slight diminution in the percent of the serum neutrophils, statistically non-significant compared to the group treated with saline solution, during the experiment (fig. 2). Due to the absence of acute paw inflammation, control group has not been used in the blood tests.

No significant changes in the values of the ESR, between GEN, SMT groups and SS group were shown, during the experiment. The use of PDN, IND and CEF was associated by a decrease in ESR, statistically significant comparing with SS group at 24 hours and 72 hours (fig. 3).

The treatment with PDN, IND, CEF, GEN and SMT was associated with a significant decrease in plasma fibrinogen values, statistically significant comparing with control. The use of PDN, IND and SMT showed the strongest effects after 3 hours; of CEF at 3 hours and 72 hours; and of GEN after 72 hours in carrageenan-induced paw inflammation test in rats (fig. 4).

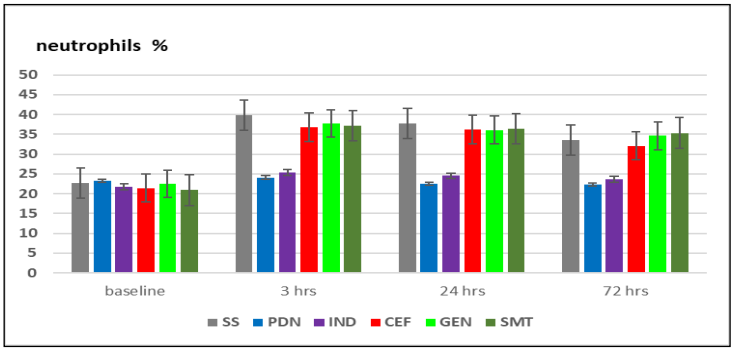

Fig. 2. The effects of CEF, GEN and SMT on the neutrophil's percentage in carrageenan-induced paw inflammation in rats.
Each point represents the mean ± S.D. of mean for 6 animals in a group.

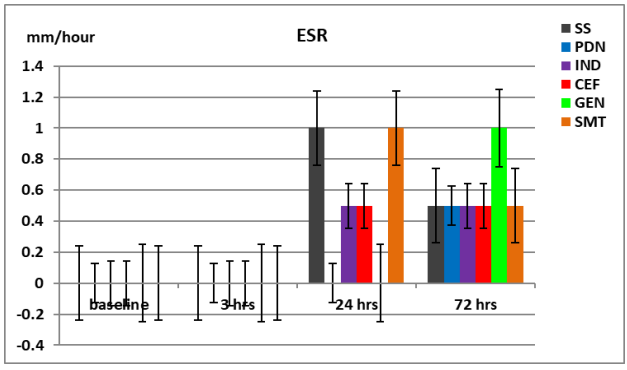

Fig. 3. The effects of CEF, GEN, SMT on the ESR (mm/hour) in carrageenan-induced paw inflammation model in rats.
Data are expressed as mean values ± S.D. of mean for 6 animals in a group.

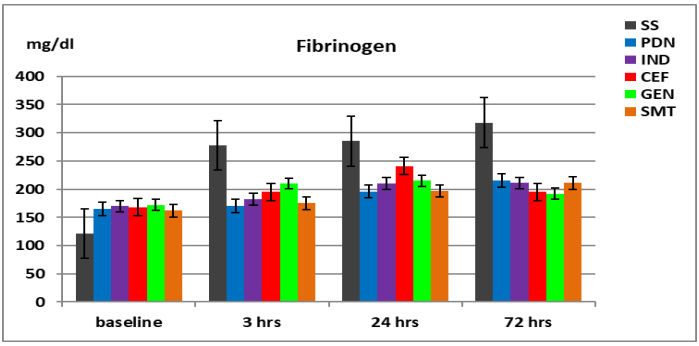

Fig. 4. The effects of CEF, GEN, SMT on the serum fibrinogen levels (mg/dL). Data are expressed as mean values ± S.D. of mean for 6 animals in a group.

DISCUSSION

Inflammatory reaction is of particular interest in immuno-pharmacology, the evaluation of the influence on the inflammatory response, being a key step in the testing of pharmacological agents. The inflammatory process is a complex of biochemical and cellular phenomena, evolving in cascade, that are activated in response to tissue damage or to the presence of foreign substances in the body. The observed effects of these two anti-inflammatory drugs PDN and IND, reinforce the idea of white blood cells involvement in the immune defense (17). Literature data (18) suggests that, the inflammatory process, can significantly influence the effects of chemotherapeutic drugs. Also, the various types of antibiotics interact with the cellular and/or humoral components of the immune system.

In terms of effects on the immune system, antibacterial substances have been classified into four groups: chemotherapeutic agents that do not alter the immune system: β-lactam antibiotics (with the exception of 3rd, 4th and 5th generation cephalosporin); aminoglycosides (depending on the dose); chemotherapeutic agents depressing the immune system: tetracyclines, rifampin, erythromycin, sulfonamides; chemotherapeutic drugs, that act synergistically with the immune system: macrolides, roxithromycin, quinolones; chemotherapeutic agents, that stimulate immune system functions: isoniazid, 3rd, 4th and 5th generation cephalosporin, aminoglycosides (depending on the dose) (19). Usually, the immune-compromised hosts (for example, in the case of human immunodeficiency virus infections) have difficulty in healing after the antibacterial treatment of opportunistic infections. Furthermore, acute or chronic infections or inflammatory processes, in these patients are resistant to many antimicrobial therapy schemas (20, 21).

In our experimental conditions, the local injection of carrageenan caused a visible inflammation, after about one hour with important edema, which increased the volume of the paw. Acute inflammation of the paw has progressively evolved, with a maximum intensity at 6 hours, which then has gradually diminished and maintained up to about 72 hours, after local administration of the irritant chemical agent.

The treatment with PDN and IND was associated with a reduction in the volume of the inflamed paw, statistically significant compared to the group receiving SS;

the anti-inflammatory effects of PDN being more intense than those of IND at any time of determination in the experiment. All three investigated antibacterial agents: CEF, GEN and SMT produced significant anti-inflammatory effects by reducing the volume of swelling that persist throughout the experiment. The most pronounced decrease in paw inflammation was produced by CEF, its effects being however lower than those of PDN and IND. The anti-inflammatory effects of the tested are positive correlated with the modifications of the white blood elements, which are specific for the assessing of the inflammatory process evolution.

The treatment with PDN, IND, CEF, GEN and SMT was associated with a significant reduction in plasma fibrinogen levels, statistically significant comparing with control group, in this experimental model of acute paw inflammation. The serum fibrinogen profile is directly linked with the changes in neutrophils percent values, during the progress of the inflammatory reaction.

CONCLUSIONS

We can appreciate that, in our experimental conditions, CEF manifested the most potent anti-inflammatory activity, compared to GEN and SMT at 3 hours and 6 hours, in this acute of paw`s inflammation test. The anti-inflammatory effects of CEF were less pronounced than those of PDN and IND, at each time point of the investigations, in the acute inflammatory edema test induced with carrageenan.

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NEWS

PRENYLATED COMPOUNDS FROM LEGUMES INDUCE ANTIBACTERIAL AND MEMBRANE PERMEABILIZATION OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI*

Background: Under (a)biotic stress, plants from Fabaceae family, produce prenylated phenolic compounds as a defense mechanism, which have shown very good antibacterial activity. Aim of the study: To study the antibacterial proprieties and the effects on the permeability of the bacterial membrane. Materials and methods: *L. angustifolius*, *A. hypogaea* and *G. max* underwent germination, CC extraction, RP-UHPLC-UV-MS analysis, antibacterial susceptibility and cell membrane permeability tests using fluorescent propidium iodine. Results: Pools contained cca. 165-350 mg prenylated phenolics/g. Chain prenylated isoflavones (*L. angustifolius*) were the most active against *L. monocytogenes* (10 μ/ml) compared to controls. None of the pools showed activity against *E. coli* alone. However, cIsf realized the most efficient permeabilization in combination with efflux pump inhibitor (EPI) PaβN, in a dose-dependent manner. Conclusion: Co-administration of prenylated isoflavones with EPI in-creased bacterial membrane permeabilization and an antimicrobial effect has been shown. Further experiments with different phenolic classes are necessary to confirm the antibacterial activity (Carla Araya-Cloutier, Jean-Paul Vincken, Roan van Ederen *et al*. Rapid membrane permeabilization of *Listeria monocytogenes* and *Escherichia coli* induced by antibacterial prenylated phenolic compounds from legumes *Food Chemistry* 2018; 240: 147-155).

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THE ASSESSMENT OF RELATIONS BETWEEN MAIN HISTOLOGIC TYPE OF OVARIAN CANCER AND SOME RISK AND PROGNOSTIC FACTORS

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THE ASSESSMENT OF RELATIONS BETWEEN MAIN HISTOLOGIC TYPE OF OVARIAN CANCER AND SOME RISK AND PROGNOSTIC FACTORS. (Abstract) : The authors have highlighted some aspects regarding the domestic risk and prognostic factors for different types of ovarian cancer. **Material and method** : The study has included 249 women diagnosed with ovarian cancer. **Results** : The assessment of age group distribution of patients with serous carcinoma (150 cases) underlying that the age group of 60-69 years was more frequent compared with the other age groups (OR = 0.20 ; IC 95% = 0.12–0.33 ; $p < 0.01 \times 10^{-5}$) and the mean of age was 61 years, 8 months, 19 days (DS = 11 years, 11 months, 22 days). The most frequent risk factor for serous type was the ovulation lifetime over 30 years (78.66%) ; followed by early menarche (under 12 years) (13.33%) ; smoking (12%) ; late menopause (over 52 years) (10%) and hormone replacement therapy (HRT) (2.67%), compared with mucinous carcinoma for which the most frequent was the ovulation lifetime over 30 years (65.21%) ; followed by early menarche (under 12 years) (34.78%) ; late menopause (over 52 years) (26.08%) and smoking (17.39%). **Conclusions** : The ovulation lifetime over 30 years was identified as the main risk factor for both histological types, but it was significant more frequent among women with serous type. The smoking was more frequent among those who had mucinous carcinoma compared with serous type. No significant differences were identified among women with both histological types regarding the age at first menstrual bleeding and parity. **Key words** : OVARIAN CANCER, RISK FACTORS, PROGNOSTIC FACTORS, HISTOLOGICAL TYPE

The ovarian cancer comprises a family of malignant entities presenting a great histo-pathological and biological diversity (1,2). All these histo-pathological types differ by the cell line from which they grow, frequency, risk factors, the rate of progression, the possibility of early detection, prognosis and response to treatment (4,5,6,7,8,9,10,11,12).

The epithelial cancers account for 90% of ovarian cancers, and, among these, the most common histological types are serous (75-80%), mucinous (10-15%), endometrioid (9-25%) and with clear cells (5-13%). The uncommon types are *Brenner tumors*, those with *small cells* and the *undifferentiated* cancers (3,4,5,11).

In many epidemiological studies and clinical investigations on confirmed and well

established risk factors, such as the nuliparity or not using oral contraceptives (OC), ovarian malignancies have been treated as a single condition, without distinguishing between histological types. The first reporting of differences in risk factors for epithelial ovarian cancer according to histological type was that of Rich et al., 1996 (13) identifying a protective role of reproductive factors, such as parity (multiple births) and the use of O.C., regarding non-mucinous tumors, but not to the serous tumors.

The diverse potential impact of ovarian cancer risk factors on different histological types of disease has not been well investigated, some studies suggesting that the mucinous ovarian neoplasia differs in several respects from other types, that involving a different approach of these malignancies in the aspects of carcinogenesis, diagnosis, prevention, treatment and prognosis (7,8,9,10,12,14, 15,16,17).

In these circumstances we considered appropriate to realize a survey on the influence of risk factors on different histological types of ovarian cancer.

The purpose of this study was to evaluate the relationship between the main histological types of ovarian cancer and some risk or prognostic factors in the studied population.

Study objectives were: to highlight the characteristics of sublots with serous and mucinous histologic type; to characterize of risk factors for the two most common histological subtypes identified in the studied population; to analyze relationships between identified factors and histological subtypes of interest.

MATERIAL AND METHODS

The study included 249 patients diagnosed with ovarian cancer. The patients with ovarian cancer selected from the Outpatient Oncology Department (Oncology County Office) and the Department of Oncology from the University Hospital St. Spiridon Iaşi, in 1999-2009, were divided in two subgroups according to their appurtenance to a particular histopathological type, which were compared then in terms of risk factors. Epidemiological data were collected via a questionnaire validated in a previous study (18), they were included in a database in Excel, which was then processed using the program *Epi Info 3.5.1*. In interpretation the results, values of $p < 0.05$ were considered significant.

RESULTS

The distribution of cases after histological types reveals the predominance of serous carcinomas. Most of them (70%) were serous and mucinous carcinomas (60.24%, and 9.24%, respectively), 6.02% were un-

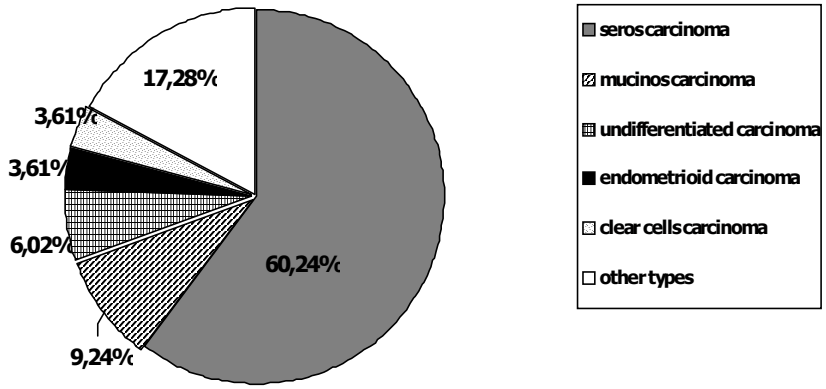

Fig. 1. The distribution on histological types of the patients with ovarian cancer

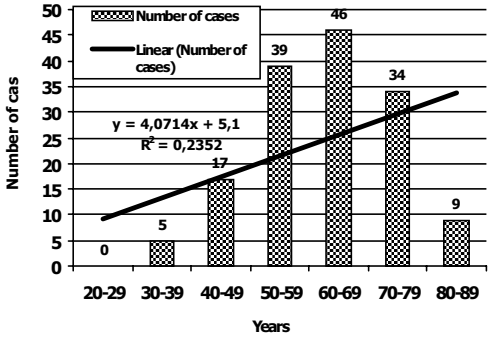

Fig. 2. The groups distribution trend of diagnosed patients with serous ovarian carcinoma

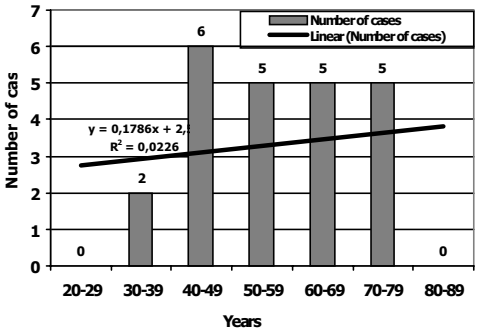

Fig. 3. Age distribution of cases diagnosed with ovarian mucinous carcinoma

differentiated carcinomas, endometrioid and clear cell carcinomas were 3.61% each, and the rest were histological types, in small proportions (Krukenberg tumor, squamous carcinoma, small cells carcinoma, disgerminom, granulosa carcinoma, teratoma, sarcoma, endodermal sinus carcinoma)(Fig. 1).

The evaluation of age-group distribution of patients with serous carcinoma (150 cases) shows the prevalence of the age group 60--69 years, compared with the other age groups ($p < 0.01$ x10-5) (Fig. 2) with an average age of 61 years (SD = 12 years). The average age at the time of diagnosis was slightly higher compared with women hospitalized for mucinous ovarian carcinoma (serous - 56 years versus mucinous - 51 years).

In Table I there is collated information on risk factors for serous type of ovarian cancer. We can notice that between risk factors for this histologic type, the most common was ovulatory period more than 30 years (78.66%), followed by early menarche (under 12 years) (13.33%), smoking (12%), late menopause (over 52) (10%) and hormone replacement therapy (HRT) (2.67%).

For patients with mucinous ovarian carcinoma (23 cases), the most affected age group was 40-49 years (as the scientific literature specificates) ($p = 0.003$) (Fig. 3), with an average age of 57 years (SD = 13 years).

In Table II there is collated information on risk factors for mucinous type of ovarian cancer. It notes that between risk factors for this histologic type, the most common was the ovulatory period more than 30 years (65.21%); followed by early menarche (under 12 years old) (34.78%); late menopause (more than 52 years old) (26.08%)

TABLE I

The relation of studied factors with the risk of serous ovarian cancer in the analysed population studied

No.	Studied factor	Number of patients	Frequency (%)	OR	IC	p
1.	Ovulatory period > 30 years	118	78.66	13.6	7.56 – 24.62	$<0.01 \times 10^{-5}$
2.	Early menarha (under 12 years)	20	13.33	0.02	0.01 - 0.05	$<0.01 \times 10^{-5}$
3.	Smoking	18	12	0.02	0.01 – 0.04	$<0.01 \times 10^{-5}$
4.	Late menopause (over 52 years)	15	10	0.01	0.01 – 0.03	$<0.01 \times 10^{-5}$
5.	THS	4	2.67	0.00	0.00 – 0.00	$<0.01 \times 10^{-5}$

* for values < 5, we applied the Yeats correction

TABLE II
The relations of studied factors with the risk for mucinous ovarian cancer in the studied population

No.	Studied factor	No. of patients	Frequency (%)	OR	IC	*p*
1.	Ovulatory period > 30 years	15	65.21	3.52	0.89 – 14.83	0.076
2.	Early menarha (under 12 years)	8	34.78	0.20	0.07 - 1.12	0.070
3.	Late menopause (over 52 years)	6	26.08	0.12	0.03 – 0.55	0.003
4.	Smoking	4	17.39	0.04	0.01 – 0.25	$<0.01x10^{-5}$

* for values < 5, we applied the Yeats correction

and smoking (17.39%).

Regarding the comparison between serous and mucinous carcinoma, applying t-Student significance test, we noticed the following (Table III) :

- the average age of patients with serous carcinoma is significantly higher than the average age of patients with mucinous carcinoma (p = 0.035) ;
- the average age for diagnosis of serous carcinoma is significantly higher compared with the average age for diagnosis of mucinous carcinoma (p = 0.021) ;
- the average age of installing of menopause in patients with serous carcinoma is significantly higher than the average age of patients with mucinous carcinoma (p = 0.025) ;
- the average duration of exposure to estrogen (ovulatory period) in patients with serous carcinoma is significantly higher than the average duration of exposure to estrogen of patients with mucinous carcinoma (p = 0.038).
- on the number of births and age at first menstruation, the differences between patients in the 2 subgroups were insignificant.

TABLE III
Characteristics of studied factors that have statistic significance in serous carcinoma compared with the mucinous

No.	Risk factor	*t-Student Test*	*p*
1.	Ovulatory period > 30 years	2.08	0.021
2.	The average age at time of diagnosis	2.32	0.025
3.	The average age at menopause	2.25	0.038

If analysing the serous carcinoma compared with mucinous, applying the significance test χ^2 we have found the following results :

- patients whose menarche occurred before 12 years are more common among those with mucinous compared to serous carcinoma (p = 0.009) ;
- patients whose menopause installed after the age of 52 years are more common among those with mucinous compared to serous carcinoma (p =0.028) ;
- patients who smoked had a higher frequency among those with mucinous carcinoma compared with the serous (p = 0.046) ;
- in terms of personal history (polycystic ovary, endometriosis, infertility) we ob-

TABLE IV
Characteristics of studied factors that have statistic significance for serous carcinoma compared with the mucinous

No.	Risk factor	χ^2	*p*
1.	Ovulatory period > 30 years	6.72	0.009
2.	The average age at time of diagnosis	4.81	0.028
3.	The average age at menopause	3.96	0.046

served no statistically significant differences between the two histologic types (Table IV).

DISCUSSIONS

Histopathologic distribution observed in this study is consistent with published data (8,9,15). With regard to potential sources of error, the selection should not have a big influence on the results, because cases and controls were selected from hospitals covering the same region, the participation in the study was almost complete, and women with chronic, gynecological or hormonal diseases have been excluded.

In an Italian study (9) parity was inversely associated with risk for non-mucinous cancers, but not for mucinous ones. Other studies show that pregnancy is associated with reduced risk for all histological types of cancer. In the current study, for a number of 249 cases, the pregnancy was not significantly associated with reduced risk for ovarian cancer for either of the two major histological types identified in the population studied.

A protective role of O.C. use in serous and endometrioid tumors, but not for mucinous ones was reported by Modugno (19). Risch et al. (13) have also identified a stronger protection of O.C. in non-mucinous cancers (OR = 0.89) compared with mucinous (OR = 0.98).

Hormone replacement therapy (HRT) (non-contraceptive estrogens) increases the risk of endometrioid ovarian cancer but not for other types (13). Chiaffarino et al (9) found no difference between the different histological types.

In their study, Kurian et al. (8) found a positive association of *serous ovarian carcinoma* and a family history of breast, ovarian or colorectal cancer (OR = 2.9, CI = 2.2 to 3.7) and an inverse association with body mass index (BMI) (OR = 0.72, CI = 0.59 to 0.88), educational level (OR = 0.76, CI = 0.64 to 0.90) and tubal ligation (OR = 0.68 , CI = 0.50 to 0.95). The authors also showed that histological type is not associated with smoking (OR = 1, CI = 0.77 to 1.3). We have identified also similar results in our current research, in terms of BMI and educational level. As for *mucinous carcinoma*, this was the only one positively associated with smoking (OR = 2.4, CI = 1.5 to 3.8). Another significant positive association is with family history of breast, ovarian or colorectal cancer (OR = 1.8, CI = 1.1 to 3.2) and insignificant with BMI (OR = 1.3, CI = 0.88 - 2.00). Cited research found an inverse association of mucinous histologic type with the educational level (OR = 0.69, CI = 0.49-0.98) and no association with tubal ligation (OR = 0.89, CI = 0.49 -1.6) (8), a situation also found in the current study.

In contrast to the results from Kurian et al. (8), who found, in their research, no association with age at first menstruation, age at menopause, number of incomplete pregnancies (abortions), duration of HRT use or alcohol consumption, this study has identified early menarche, late menopause and smoking as risk factors for mucinous ovarian cancer. For all histological types we observe a descending trend with increasing parity : serous (OR = 0.87, CI = 0.83 to 0.91), mucinous (OR = 0.85, CI = 0.77 to 0.93), endometrioid (OR = 0.72, CI = 0.66 to 0.77) and with clear cell (OR = 0.56, CI = 0.49-0.65). A number of 3-4 births protects against ovarian cancer risk, endometrioid histologic and with clear cells type (8).

All histological types of ovarian cancer, except that with clear cells, showed an inverse association with lactation : serous (OR = 0.98, CI = 0.97-0.99), mucinous (OR = 0.97, CI = 0.95 to 1), endometrioid (OR = 0.98, CI = 0.96 to 1) and with clear cells (OR = 1, CI = 0.97 to 1.03). An inverse association with CO was observed for all histological types : serous (OR = 0.66, CI

= 0.55 to 0.80), mucinous (OR = 0.60, CI = 0.43 to 0, 84), endometrioid (OR = 0.70, CI = 0.52 to 0.93) and with clear cells (OR = 0.65, CI = 0.41 to 1) (8).

Another study investigating risk factors and histological types of ovarian cancers is that of Tung et al (15). The authors state that : women with mucinous type of ovarian cancer had a lower education level compared with those who have non-mucinous types ; those in postmenopausa had a risk of developing malignant ovarian tumors 2 times higher than those in premenopausal, the association being stronger in those with non-mucinous invasive cancer (particularly serous and endometrioid). Our results were in contrast with the findings of Tung team.

In terms of total number of years the woman has menstrual cycle, it was correlated with an increase of the risk for non-mucinous tumors, but not for mucinous ones (p = 0.001) (15), data which this research were consistent. We noticed a significant positive association with risk for non-mucinous carcinomas, but not for mucinous ones, with increasing number of lifetime ovulatory cycles (p = 0.005).

Pregnancy was associated with reduced risk for all histological types - women who had at least one pregnancy had a significantly lower risk for invasive endometrioid cancer or with clear cell than those without any birth ; except for mucinous cancers, breast--feeding by suppressing ovulation, also reduces the risk for non-mucinous types, especially endometrioid and with clear cells (p = 0.03), consumption of CO was associated with decreased risk for all types (15). For these factors, our study has not identified differences between histological types.

In the Tung et al study (15), ovulation factors (age at menopause and at first menstruation) had little influence on the risk for all histological types. Neither in our study we showed no strong relationship between these factors and the risk for ovarian cancer.

CONCLUSIONS

1. The average age of patients, the mean age at diagnosis clarification, and the average age of menopause instauration in patients with serous carcinoma is significantly higher than that of subjects with mucinous carcinoma.
2. The ovulatory period (median duration of exposure to endogenous estrogens) in patients with serous carcinoma is significantly higher than during the ovulatory period at patients with mucinous carcinoma.
3. There are no significant differences between patients with both histological types in terms of age at first menstruation and parity.
4. Patients who had their first menstruation before the age of 12 are more common among those with mucinous carcinoma, compared to serous carcinoma ; those with menopause installed after the age of 52 years are more common among those with mucinous carcinoma compared with the serous.
5. Patients who smoked were more common among those with mucinous carcinoma, compared to serous carcinoma, and in terms of personal history (uterine polyps, polycystic ovary, infertility) there are no statistically significant differences between the two histological types.
6. Ovulatory period of more than 30 years was identified as the main risk factor for both histological types.

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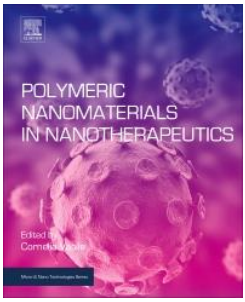
BOOK REVIEW



POLYMERIC NANOMATERIALS FOR NANOTHERAPEUTICS

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eBook ISBN: 9780128139332; Paperback ISBN: 9780128139325:
Elsevier, Published Date: 2nd November 2008; 558 pages In Micro and Nano Technologies Series.

Polymeric Nanomaterials in Nanotherapeutics describes how polymeric nanomaterials are used in the obtainment of nanosensors and nanorobotics as biomedical instrumentation, surgery, diagnosis and controlled and targeted drug delivery for cancer, improved pharmacokinetics, monitoring of diabetes and other healthcare problems. Polymeric nanomaterials (synthetic and/or natural) used for these purposes described in this book are: polymers as drugs, polymer drug conjugates, polymeric nanoparticles, micelles, nanogels, nano emulsions, viruses, dendrimers, polyplexes, multi-functional nanoparticles, polymersomes, viral nanoparticles, stimuli-responsive nanomaterials, etc. Various ways of drug administration and to create drug formulations for controlled and targeted delivery and release of active agents (drug molecules) to non-healthy tissues and cells as new nanotherapeutics are described. As methods of administration described are included oral, dental, topical and transdermal, pulmonary and nasal, ocular, vaginal, and brain drug delivery. In different chapters it is demonstrated how these nanotherapies are applied to dental work, wound healing, cancer, cardiovascular diseases, neurodegenerative disorders, infectious diseases, chronic inflammatory diseases, metabolic diseases, and more.

In most cases are presented new drugs existing in various stages of development as: FDA or EU-approved polymeric or polymers containing "nanopharmaceuticals", commercial state as *Abraxane case* or in different clinical trials or in research stage. The applications are depended on physicochemical properties of nanomaterials, and because of the novelty of both drugs and carrier systems the risk assessment is necessary to be established. Therefore, the toxicity and regulatory status of therapeutic polymeric nanomaterials are also examined. All the 15 chapters are written by experts in the field.

As key features of the book it can be mentioned: it is demonstrated how the properties of polymeric nanomaterials can be used to create more efficient medical treatments/therapies; the potential and range of applications of polymeric nanomaterials in disease prevention, diagnosis, drug development, and for improving treatment outcomes, it is explained in detail how nanotherapeutics can help in solving problems in the field through the latest technologies and formulations.

The book gives a broad perspective on the topic for researchers, postgraduate students and professionals in the fields of nanomaterials, biomaterials, biotechnology and applications in biomedical fields and medicine.

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PSA CA MARKER ÎN EVOLUŢIA CANCERULUI DE PROSTATĂ

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PSA AS A MARKER FOR PROSTATE CANCER PROGRESSION (Abstract): Monitoring the evolution of prostate cancer has crucial importance since this tip of neoplasia has a variable biology, ranging from latent cancers to extremely aggressive tumors. At the moment, PSA (prostate specific antigen) values point out to either successful or unsuccessful prostate cancer therapy. Depending on therapeutic strategies, biochemical recurrence (BCR) is differently defined. After radical prostatectomy, the PSA declines to undetectable levels in 3-4 weeks; BCR: PSA = 0.2-0.5 ng/mL for a single measurement or two consecutive values which surpass 0,2-0,4 ng/mL. PSADT < 10 months is a predictor for the progression of the disease. After external beam therapy, PSA is slowly decreasing reaching up the nadir of 0.2-0.5 ng/mL in months or years; BCR: PSA = nadir + 2 ng/mL. In the case of brachytherapy, the nadir is reached in 2-4 years; BCR: nadir + 2 ng/mL. After hormonal therapy, PSA declines in 3-6 months, maintains low values for 18-24 months, then increases, settling for hormone independency. After chemotherapy, PSA is considered normal at values < 2 ng/mL; the response of PSA represents a confirmed decreasing from the second test at 4 or more weeks after the initial decline; the length of the response is the period between first decline with 50 % of PSA to 50 % increase from nadir; the progression of PSA is shown in the increase with 25 % in comparison to the basic level. In the case of active monitoring, a PSADT < 2 years is an indication for radical prostatectomy, but there are cancers which can evoluate despite a stable PSA. The „PSA bounce" phenomenon and the „PSA surge postchimiotherapie" syndrome can complicate the assessment of biochemical recurrence. **Key words**: PSA, PROGRESSION OF PROSTATE CANCER, BIOCHEMICAL RECURRENCE/ RECURRENCE OF TUMORAL MARKERS

Cancerul de prostată se remarcă prin eterogenitate la nivel clinic, histologic şi biologic. În prostată pot coexista în acelaşi timp celulele epiteliale canceroase în diferite pattern-uri arhitecturale şi diferite grade de diferenţiere (care se reflectă în scorul Gleason), celule stromale fibromusculare, multipli foci de neoplazie intraepitelială benignă (PIN), leziuni atrofice, noduli de hiperplazie prostatică benignă (BPH) şi glande normale. Clasic, se consideră că multe cancere umane se dezvoltă printr-o serie de etape genetice secvenţiale care culminează în carcinom şi boala metastatică, iar acestea se reflectă la nivel genetic, histologic şi clinic. Multe aspecte din biologia cancerului de prostată nu se potrivesc acestui model. Tehnicile genetice clasice au adus informaţii limitate cu privire la procesul progresiei cancerului de prostată (1).

Cel mai utilizat marker, în prezent, pentru urmărirea evoluţiei cancerului de prostată este PSA (antigenul specific prostatic). PSA este eliberat în sistemul circulator când

membrana bazală prostatică este compromisă, cum se întâmplă în cancer, şi, prin urmare, este utilizat ca marker seric sensibil pentru screening-ul, diagnosticul şi progresia cancerului de prostată. (În lucrarea noastră nu ne vom referi la rolul determinant al PSA în screening-ul şi diagnosticul neoplaziei prostatice, ci doar la rolul său în urmărirea evoluţiei cancerului, în cursul sau după diferite terapii). De fapt detecţia recidivei bolii a fost aplicaţia clinică iniţială a PSA în cancerul de prostată (2).

PSA, o serinprotează produsă de celulele epiteliale secretorii, este codificat de gena kalicreinei umane 3 (hK3). Expresia PSA este AR (receptor androgenic) - dependentă, iar nivelele transcripţionale sunt semnificativ reduse în absenţa androgenilor (3). Alături de PSA total, în urmărirea evoluţiei cancerului de prostată s-au remarcat a fi utile derivatele acestuia, în principal PSADT (timpul de dublare al PSA) şi PSAV (velocitatea PSA).

Aproximativ o treime până la jumătate din pacienţii trataţi, indiferent de metoda terapeutică, vor experimenta recidivă biochimică (creşterea valorilor PSA după normalizarea lor iniţială)(4). Semnificaţia recidivei biologice este, ea însăşi, neclară, întrucât nu toţi pacienţii cu recidivă biochimică vor experimenta boala metastatică. Se defineşte astfel recurenţa biochimică agresivă, pentru valori ale PSADT < 6 luni (5).

Recurenţa biochimică reprezintă un marker al progresiei spre boala metastatică şi al mortalităţii cancer-specifică (6). Momentul recurenţei biochimice postterapie este crucial în urmărirea evoluţiei cancerului prostatic, deoarece permite administrarea precoce a terapiei de salvaj în cazul pacienţilor cu recurenţă locală.

RECURENŢA BIOCHIMICĂ DUPĂ PROSTATECTOMIA RADICALĂ

Nivelul seric al PSA ar trebui să scadă la valori nedetectabile ($< 0,1$ ng/mL) în 3-4 săptămâni după prostatectomia radicală. Definiţia recurenţei biochimice variază de la valori cuprinse între 0,2 ng/mL şi 0,5 ng/mL, pentru o singură măsurătoare, la două valori consecutive care depăşesc 0,2 sau 0,4 ng/mL. Un nivel al PSA $\geq 0,4$ ng/mL este asociat puternic cu progresia bolii şi este considerat că defineşte recurenţa biochimică, care poate avea loc după mai mulţi ani de la prostatectomia radicală (7). 27% din recidivele biochimice apar la mai mult de 5 ani de la prostatectomie radicală, iar riscul de recurenţă continuă până la 15 ani postoperator, ceea ce impune urmărirea prelungită a valorilor PSA (6).

În prezent pacienţii sunt monitorizaţi, în ce priveşte valoarea PSA, o dată la 3 luni în primul an, la 6 luni pentru următorii 4 ani, şi apoi anual. Intervalul de timp dintre recurenţa biochimică şi metastaze depinde de stadiul patologic preoperator, de scorul Gleason şi de PSADT postoperator. Un PSADT mic (< 10 luni) este cel mai puternic predictor pentru progresia bolii. În cazuri rare, pacienţii pot dezvolta metastaze la distanţă postprostatectomie fără creşterea nivelului PSA.

Pound et al. au raportat o recurenţă biochimică în 15 % din cazuri şi metastaze osoase în 5 % din cele 1997 cazuri de pacienţi cu stadiu clinic T1-2 trataţi prin prostatectomie radicală, după o monitorizare de 5 ani (8). Timpul median de la recurenţa PSA la metastaze a fost de 8 ani. După dezvoltarea metastazelor, timpul median până la deces a fost de 5 ani. Factorii de risc pentru progresia către metastazele osoase, după prostatectomia radicală, au inclus un scor Gleason ≥ 7, prezenţa invaziei veziculelor seminale, sau atingerea ganglionară, sau recurenţa biochimică < 2 ani de la prostatectomie.

Recurenţa biochimică agresivă poate fi, din fericire, identificată precoce în cursul bolii pe baza PSADT scurt postoperator (< 6 luni). Timpul până la recurenţa bio-

chimică se corelează cu supravieţuirea cancer specifică, dar el nu este un indicator pentru moartea prin cancer. Dimpotrivă, un PSADT foarte scurt este un indicator pentru moartea prin cancer (5). Pacienţii cu PSADT < 3 luni prezintă un risc extrem de crescut pentru rezultate clinice negative. Dimpotrivă, bărbaţii cu PSADT > 15 luni au un risc foarte mic pentru moarte cancer-specifică (9).

Conform unui studiu canadian, numai 30% din pacienţii cu recurenţă biochimică după prostatectomia radicală au prezentat metastaze la distanţă, iar 20% au murit de cancer de prostată la 15 ani după recurenţa biochimică. 40% dintre pacienţii cu metastaze osoase mor de cancer la 5 ani după apariţia lor. Mortalitatea prin cancer la 5 ani după diagnosticarea metastazelor este de 40% (8).

RECURENŢA BIOCHIMICĂ DUPĂ RADIOTERAPIA EXTERNĂ

Nivelul PSA descreşte lent şi e posibil să nu atingă niciodată nivele nedetectabile după radioterapia externă. Uneori se atinge nadirul (0,2-0,5 ng/mL) (4) după luni sau chiar ani, în funcţie de doza de iradiere, mărimea prostatei şi valoarea PSA pretratament. Recurenţa biochimică după radioterapia definitivă a fost iniţial definită de către American Society for Therapeutic Radiology and Oncology (ASTRO) drept trei creşteri consecutive ale PSA după ce se atinge nadirul (10). Ulterior, conform definiţiei Phoenix (7), recurenţa biochimică a fost definită drept creşterea absolută cu ≥ 2 ng/mL faţă de nadir (creştere continuă, fără reducere). Totuşi, ca şi în cazul prostatectomiei radicale, eşecul clinic postiradiere poate fi apreciat mai bine de către PSADT decît de valorile absolute ale PSA. Ca şi în cazul pacienţilor trataţi chirurgical, bărbaţii cu PSADT < 3 luni (calculat după substracţia nadirului PSA) prezintă un risc extrem de crescut, cu mortalitate cancer-specifică atingând 100% (9).

Există un fenomen – *saltul PSA (PSA bounce)* – observat la 10-30% dintre pacienţii trataţi prin radioterapie externă, care apare de obicei în primele 9 luni postterapie, dar care poate să apară până la 60 de luni după tratament. Este o creştere tranzitorie a PSA de cel puţin 0,4 ng/mL sau o creştere de 15% faţă de PSA precedent, care ulterior dispare spontan. Etiologia fenomenului nu este cunoscută. Pentru a distinge între recurenţa biochimică şi *"saltul PSA" (PSA bounce),* se consideră că are loc recurenţa biochimică dacă se înregistrează valori nadir plus 2 ng/mL (4).

RECURENŢA BIOCHIMICĂ DUPĂ BRAHITERAPIE

După brahiterapie, regresia valorilor PSA este lentă, atingându-se nadirul la 2-4 ani (11). Supravieţuirea fără recurenţă biochimică la 5 şi 10 ani a fost raportată ca fiind 71-93%, respectiv (65-85%) (12).

"Saltul PSA" este frecvent după brahiterapie (32-46 %). În unele studii, pacienţii care au experimentat "saltul PSA" au prezentat o probabilitatea mai redusă pentru recurenţa biochimică (4).

Prognosticul pentru pacienţii trataţi prin brahiterapie şi care nu prezintă recurenţă biochimică la 5 ani este excelent. Este improbabil ca pacienţii cu valori < 0,2 ng/mL la 5 ani posttratament să prezinte o recurenţă biochimică ulterioară (13).

Standardul "nadir + 2", valabil pentru recurenţa biochimică postradioterapie externă, este valabil şi pentru pacienţii trataţi prin implant radioizotopic (2).

PSA ÎN MONITORIZAREA ACTIVĂ ŞI *WATCHFUL WAITING*

Monitorizarea activă este o alternativă terapeutică pentru pacienţii cu cancer de prostată cu risc mic de progresie. Bărbaţii cu cancer de prostată bine diferenţiat au o supravieţuire la 20 de ani de 80-90%. Moni-

torizarea activă implică urmărirea regulată a evoluţiei bolii (prin PSA, DRE şi, posibil, repetarea periodică a biopsiei) în vederea instituirii terapiei curative, când există indicii că boala progresează (14).

Monitorizarea activă este indicată în cazul cancerelor localizate (T1c-T2), de grad mic (< 3) şi de volum < 0,5 cm^3, cu valori ale PSA < 10 ng/mL, cu scorul Gleason = 6 (12). Acestea sunt cancerele considerate, conform nomogramelor, cu potenţial evolutiv redus. Dozajul PSA se realizează la fiecare 6 luni timp de 4 ani, apoi anual (15). PSADT este un indicator important în strategia de monitorizare a acestor cancere, pentru a indica necesitatea unui tratament. Se consideră că un PSADT < 2 ani constituie indicaţie terapeutică de prostatectomie totală, dar există cancere care pot progresa în ciuda unui PSA stabil (11). Un studiu recent al Universităţii *John Hopkins* susţine că cinetica PSA postdiagnostic nu reprezintă un test prognostic valid şi nu ar trebui utilizată pentru înlocuirea biopsiei anuale pentru urmărirea bărbaţilor prin monitorizare activă (16).

Spre deosebire de monitorizarea activă, *watchful waiting* implică o urmărire mai puţin agresivă şi, în general, absenţa tratamentului până la apariţia bolii simptomatice (14).

Această alternativă terapeutică este aplicată în cazul pacienţilor cu boală incurabilă. Terapia de privare androgenică, recomandată acestor pacienţi, este aplicată fie în cazul creşterii valorii absolute a PSA, fie când PSADT < 6 luni (4).

PSA ÎN CAZUL PACIENŢILOR TRATAŢI HORMONAL

După tratamentul hormonal, nadirul PSA este un factor prognostic important, care se corelează cu timpul de supravieţuire fără recidivă. Valoarea PSA descreşte în 3-6 luni şi se menţine la valori scăzute timp de 18-24 de luni, după care survine o creştere a PSA, care defineşte hormono-independenţa. Când PSA continuă să crească după a doua linie de hormonoterapie, este vorba de hormono-rezistenţă (11). Recidiva metastatică este asociată întotdeauna cu creşterea PSA, în afara cazurilor rare de tumori nediferenţiate, când PSA poate rămâne nedetectabil.

EVALUAREA RĂSPUNSULUI LA CHIMIOTERAPIE

Normalizarea PSA este considerată la valori < 2ng/mL. Un răspuns al PSA este demonstrat de descreşterea PSA confirmată de un al doilea test, la 4 sau mai multe săptămâni după declinul iniţial. Durata răspunsului PSA reprezintă perioada dintre prima reducere cu 50% a PSA până la 50% creştere de la nadir. PSA este considerat că progresează când creşte cu 25% faţă de nivelul de bază sau când există o creştere progresivă în valoare absolută a PSA de cel puţin 5ng/mL (17).

Thuret et al. au studiat modificările iniţiale ale PSA în cursul chimioterapiei la pacienţii cu cancer de prostată hormono-refractar şi au corelat aceste modificări cu răspunsul ulterior la terapie. S-a observat că PSA poate creşte iniţial (în 20% din cazuri), apoi descreşte, la pacienţii cu un răspuns bun la chimioterapie. Acest fenomen – numit sindromul *PSA surge post-chimioterapie* – a fost observat în primele 1-8 săptămâni după iniţierea chimioterapiei. Aceeaşi observaţie este valabilă pentru chimioterapia second line. Cunoscând acest fenomen, clinicienii vor evita întreruperea precoce, inoportună, a chimioterapiei (18).

TRATAMENTUL METASTAZELOR OSOASE

O constatare neaşteptată în studiul lui Porter et al. a fost o reducere semnificativă a valorilor PSA în grupul tratat cu ^{89}Sr. S-a sugerat astfel că tratamentul cu radiofarmaceutice cu tropism osos ar putea exercita, dincolo doar de ameliorarea durerii,

un efect tumoricid important (19).

DIFERENŢIEREA ÎNTRE RECIDIVA LOCALĂ ŞI RECIDIVA METASTATICĂ

După prostatectomia radicală sau radioterapie pentru un stadiu localizat, intervalul mai scurt de un an înaintea recidivei biologice, creşterea rapidă a valorilor PSA cu o velocitate crescută (în ng/mL pe an) sau un PSADT scurt sunt în favoarea unei recidive metastatice la distanţă, în timp ce o creştere lentă în mai mult de un an a PSA ar sugera mai curând o recidivă locală (11). Caracteristicile anatomopatologice şi biologice în favoarea unei recidive locale, după prostatectomia radicală, sunt scorul Gleason ≥ 7 (3+4), stadiul pT3, pN0, reascensiunea PSA după mai mult de un an şi un PSADT $>$ 10 luni. În favoarea unei recidive metastatice pledează un PSA detectabil în mai puţin de un an postoperator, un PSADT inferior la 4-6 luni, un scor Gleason = 7 (4+3) şi un stadiu pTxN1.

După radioterapie, valoarea nadirului PSA $<$ 2 ng/mL, timpul până la nadir de 12-18 luni şi un PSADT de 11-13 luni sunt în favoarea unei recidive locale. Un nadir al PSA $>$ 2 ng/mL, obţinut în mai puţin de 12 luni şi un PSADT de 3-6 luni pledează pentru o recidivă la distanţă (11).

PROGRESE ÎN URMĂRIREA EVOLUŢIEI CANCERULUI DE PROSTATĂ

Modificările moleculare care determină progresia cancerului de prostată de la glandele benigne, la leziunile precursoare, cancerul local şi în final boala metastatică sunt puţin înţelese. Tehnicile *ADN microarray* au permis studiul unor aspecte ale progresiei cancerului de prostată. În această progresie sunt implicate mai multe gene şi căi, precum *GSTP1*, *PTEN*, *NKX3.1*, *AR*, *c-MYC* şi calea de semnalizare *hedgehog* (1). Studiile s-au concentrat pe identificarea profilului expresiei genice caracteristic pentru diferite scoruri Gleason. *SPARC* a fost asociată cu un scor Gleason mare. Alte studii au avut drept scop identificarea genelor diferenţial exprimate în cancerul local comparativ cu cel metastatic. De exemplu într-un model Cox care a inclus statusul marginii chirurgicale, mărimea tumorii, scorul Gleason şi valoarea PSA preoperator, expresia crescută a proteinei EZH2 a fost cel mai bun predictor al recurenţei (1). Câteva studii s-au concentrat pe identificarea profilului care anticipează care dintre cancerele localizate sunt mai predispuse la recurenţa PSA după prostatectomie. Astfel pierderea genei *TRPM8* constituie un predictor semnificativ pentru recurenţa biochimică.

CONCLUZII

Detecţia recidivei cancerului de prostată a fost prima aplicaţie clinică a PSA. În prezent, PSA continuă să fie cel mai utilizat marker pentru urmărirea evoluţiei bolii. Recurenţa biochimică este diferit definită, în funcţie de diferitele strategii terapeutice :

1. După prostatectomia radicală, PSA descreşte la valori nedetectabile în 3-4 săptămâni. BCR : PSA = 0,2-0,5 ng/ pentru o singură măsurătoare sau două valori consecutive care depăşesc 0,2-0,4 ng/mL.
2. După radioterapia externă, nadirul de 0,2-0,5 ng/mL se atinge în luni sau ani. BCR : PSA = nadir + 2 ng/mL.
3. În cazul brahiterapiei, nadirul este atins în 2-4 ani. BCR : PSA = nadir + 2 ng/mL.
4. După terapia hormonală, PSA descreşte în 3-6 luni, se menţine la valori scăzute 18-24 de luni, după care creşte, instalându-se etapa de hormono-independenţă.
5. După chemoterapie, PSA este considerat normal la valori $<$ 2 ng/mL.
6. În cazul monitorizării active, un PSADT $<$ 2 ani constituie indicaţie pentru prostatectomia radicală.

Conform consensului ASTRO, este important de notat că definiţiile propuse preci-

zează succesul sau eşecul terapeutic în contextul unei populaţii, nu al unui individ (2). Ele oferă un context în care fiecare caz clinic va fi judecat individual.

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VARIANTE ANATOMICE ALE REPERELOR DIN CHIRURGIA ENDOSCOPICĂ A SINUSURILOR PARANAZALE EVIDENŢIATE IMAGISTIC PRIN RMN

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ANATOMICAL VARIATIONS OF LANDMARKS IN ENDOSCOPIC SURGERY OF THE PARANASAL SINUSES REVEALED BY MRI IMAGING (Abstract): Continue development of endoscopic techniques of rinosinusal approach involves knowledge about the anatomy of this region, with a three-dimensional orientation before surgery, a therapeutic plan well thought, anticipating the gesture controller, taking into account the particularities of each case. Endoscopic surgical anatomic variants parts at this level can be well identified by imaging methods (CT, MRI). CT study of patients who are candidates for surgery Fess is on top, as the technique of choice. MRI is the imaging method to supply the CT data, providing a study carried out the normal anatomy and standard deviation of the numerous variants, single or in combination. This paper aims a randomized retrospective study of 120 MRI of the head and face region, to investigate and highlight the anatomical variations of structures at this level of impact and those with sinusitis etiopatologia, as well as those with operative risk (bleeding complications, ocular, vital risk) to determine the prevalence in the general population and have to compare the data with the literature. Using all sections (axial, sagittal and coronal) with the aid of different sequences (T1, T2, Flair and diffusion) allowed study of three-dimensional images of whole rhino-sinus-related pathology. Anatomic variations of surgical landmarks have investigated a wide range of expression, which are unique or associated with a complex architecture, with sufficient accuracy and visible on MRI. Their prevalence in the group studied was generally consistent with the literature studied. **Key words**: MRI, ANATOMIC VARIANTS, PARTS RHINO-SINUS SURGERY

THE RELATIONSHIP BETWEEN SOIL QUALITY, SECONDARY METABOLITE CONTENT AND ANTIOXIDANT EFFICACY IN *CARTHAMUS TINCTORIUS* L. AND *CALENDULA OFFICINALIS* L.

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CARTHAMUS TINCTORIUS L. AND CALENDULA OFFICINALIS L.: NATURAL SOURCE OF ANTIAGING AGENTS FOR COSMETIC FORMULATIONS (Abstract): Asteraceae plants are commonly used for their antibacterial, anti-inflammatory and anti-aging effects. They are widely utilized in therapeutic and cosmetic formulations, while also serving decorative purposes. Safflower also serve a range of purposes, from medicine, food coloring and ornaments. *Calendula officinalis* is widely cultivated for medicinal and cosmetic purposes, especially for its therapeutic effects in topical formulations. Thus, the **aim** of our study was to investigate the influence of soil fertility on the secondary metabolites profile and its antioxidant activity. **Materials and methods:** To achieve this, *Calendula officinalis* and *Carthamus tinctorius* were cultivated in Romania under two different conditions: local soil, and enriched soil respectively. After collection, fragments of dried leaves and inflorescences were extracted with either ethanol or acetonitrile and analyzed. Semiquantitative analysis was performed using the HPLC method and he total phenolic content of each sample was quantified with Folin-Ciocalteu reagents. For the *in vitro* tests, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was used to evaluate the radical scavenger activity, and reaction with ferrozine was used to evaluate the ferrous iron chelation capacity. **Results**: The quantitative analyses showed a clear influence of soil fertility on polyphenolic profile, impacting the antioxidant activity. **Conclusions:** The timing of harvest and the soil quality should be taken into consideration in order to ensure optimal pharmacological effects. **Keywords**: *CARTHAMUS TINCTORIUS, CALENDULA OFFICINALIS,* PHENOLS, ALLERGENIC RISK, ALANTO-LACTONE.

INTRODUCTION

Secondary metabolites (SMs) identified in Asteraceae plants possess proven pharmacological effects, which are attributed to their phytochemical compounds, such as polyphenols, phenolic acids, flavonoids, acetylenes and triterpenes (1). The specific SMs profile is related to local environmental conditions and agro-techniques of cultivated plants (2). The Asteraceae are also a source of sesquiterpene lactones (SLs). Of those, alantolactone has been documented as one of the principal allergenic and sensitizing compounds in such species (3). Alantolactone (helenin, eupatal, inula camphor), an eudesmanolide derived from *Inu-*

la helenium, also has antibacterial, anti-helmintic, anti-inflammatory properties and has been studied as a novel antineoplastic agent (4-6). The plant-related allergy symptoms involve contact dermatitis, hay fever, asthma or even anaphylaxis. The typical routes of exposure include skin contact, eye contact, ingestion or inhalation (4). The evidence of cross-reactivity of Asteraceae species with other plants and anaphylactic reactions has been reported (4). Cross-reactivity can occur between plant allergens and food and pollen allergens, especially in patients sensitive to Asteraceae (4).

Species of this plant family are now widely utilized in cosmetic formulations (creams, soaps, shampoos), aromatherapy or massage, in professional care or folk medicine. This high usage leads to speculation that sensitization to Compositae will increase. Compositae containing cosmetics are thought to provoke allergic reactions in susceptible individuals because they contain SLs (4).

Carthamus tinctorius (Safflower) has been grown for various purposes: medicine, food coloring, ornamental, fabric dye, biofuel. It is adaptable to arid conditions worldwide (7). It has many beneficial effects, such as: anti-inflammatory, antioxidant, antitumor effects. Injectable formulas have been widely applied in clinics. Although this species is considered non-toxic and has negligible side effects, anaphylactic reactions to safflower injections have been reported. This annual plant is not very used in modern cosmetic products (8). Studies of extracts from seeds, stems, buds and flowers suggest the potential cosmetic of safflower has not yet been completely uncovered (9).

Calendula officinalis, usually known as common marigold, is widely cultivated and used for medicinal and decorative purposes (8, 10). Much scientific research has established that marigold has a wide spectrum of pharmacological effects (11, 12). Phenolic acids and flavonoids have been identified mainly from inflorescences (8, 13). Compounds of a sesquiterpene nature were also detected or isolated (13). Calendula cream and products have shown very few allergic and side effects (14). The European Commission accepted calendula as a safe cosmetic ingredient (15). Although sensitization to calendula and allergic contact reactions are observed, anaphylaxis incidents were only reported in the context of infusion of marigold after gargling (14). Special care must be taken by people who are allergic to species from the Asteraceae family when using or consuming preparations containing marigold extract or oil (14). Only flowers are commonly used, while the rest of the biomass is considered waste. Unfloral parts of the plant should also be taken into consideration (9).

This study aims to evaluate *Carthamus tinctorius* and *Calendula officinalis* as sources of antiaging agents for cosmetics related to their allergenic potential in cosmetic formulations. The allergenic risk factor considered consisted in assessing the presence and quantity of alantolactone in the plant. Total phenolic content was evaluated as a marker of antioxidant capacity. The two species were cultivated in local and enriched soil conditions, to evaluate their biosynthesis capacity for SLs and antiaging secondary metabolites. Aerial parts of plants were investigated, floral and unfloral, to study the potential of "waste" vegetal material as a source of natural products.

MATERIALS AND METHODS

Plant material. *Calendula officinalis*

and *Carthamus tinctorius* were cultivated in north-eastern Romania in the year 2015 under two nutritional statuses: local (V1) and enriched soil conditions (V2) with N-P-K (Mg) 15: 10: 12. The fertilizer was administered just before planting (75g/m^2). Rooted cuttings were planted at a density rate of 4 plants/m^2. Aerial parts of the plants were collected: fully bloomed inflorescences (F), leaves and stems, defined as unfloral aerial parts (L). The samples were codified as follows: V1F, V2F, V1L, V2L. Fresh aerial parts of plants were dried in a thermostatic oven at 35°C until three consecutive measurements turned out identical. Voucher specimens are deposited as DE-8425906 (marigold) and NL-62850040 (safflower) at the Department of Pharmacognosy ("Grigore T. Popa" University of Medicine and Pharmacy Iasi) for ready reference.

Extraction. Fragments (2 g) of dried leaves and inflorescences were extracted with methanol or acetonitrile R in the Laboratory of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. For polyphenol quantification, the methanolic extract was obtained (1:10, m/m) by repeated sonication for 15 minutes.

For alantolactone evaluation by HPLC, the test solutions were obtained in acetonitrile R (1.0 g powdered drug in 3 mL solvent, three times, sonicated for 15 minutes). The filtrates were reunited and brought to 10 mL in a volumetric flask. A quantum of 0.20 µL was used for HPLC analysis.

Total phenolic content determination. The total phenolic content of each sample was quantified with Folin-Ciocalteu reagent according to the Singleton and Rossi method (1965), using gallic acid as a standard (16). The measurements were performed on ABLE-JASCO V 550 UV-VIS spectrophotometer. The absorbance was measured at 765 nm versus blank samples. The blank consisted of samples and all reagents and solvents except sodium carbonate. The results were expressed as mg gallic acid equivalents (GAE)/100 g dried sample.

HPLC analysis. Semiquantitative analysis of alantolactone was performed using the HPLC method described by Ivănescu (2015) with modifications (18). The method was adapted for a Thermo-Fischer UltiMate 3000 system coupled with DAD detector used to assess the profile of sesquiterpene lactones. The working conditions were: Accucore XL-C18 column (4.6 x 150 mm, 4 Wm); column temperature: 34 °C; detection wavelength was set at 225 nm and the flow rate was 1.2 mL/min. The mobile phase consisting of two eluents as A (water) and B (methanol) used the following linear gradient elution: 0-3 min 38% B; 3-20 min 45% B; 20-30 min 45% B; 30-55 min 55% B; 55-57 min100% B; 70 min 100% B; 90 min 38% B. As standard, alantolactone was used in the amount of 20 µL of a 5 mg/mL solution. Five consecutive injections were done for repeatability purposes. Samples UV spectra registered at 225 nm were automatically compared by Chromeleon 7.2 software and the concentration was expressed as % of the standard's area/concentration.

Antioxidant activity. For the *in vitro* tests, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was used to evaluate the radical scavenger activity. The test was performed using the method of Hatanao *et al.* For each sample EC50 value (expressed in µg extract/mL) was calculated by plotting the radical scavenger activity variation and the concentration of the extract. Gallic acid

was posed as positive control (20). The scavenger activity was determined according to the formula:

$$\% \text{ activity} = (A_{DPPH}-A_P) \cdot 100/A_{DPPH}$$

where ADPPH represents the absorbance of alcoholic DPPH solution, AP represents the absorbance after 10 minutes of DPPH solution treated with methanol extract.

The ferrous iron chelation capacity was also evaluated. When reacting with ferrozine, Fe^{2+} forms a pink-colored complex, exhibiting maximum absorbance at 562 nm. The presence of a chelating agent in the reaction medium results in reduced absorbance of the complex, causing a decrease in the solution's color intensity (21).

All reagents were of analytical purity and were purchased from Sigma Aldrich (St. Louis, MO, USA). All measurements and analyses were performed in triplicates. The variants were compared with their average. Data for quantitative and semi-quantitative analytical determinations were presented as mean values ± standard deviations.

RESULTS AND DISCUSSION

Total phenolic content. Flavonoids and polyphenolic derivatives are proven natural antioxidants. Phenolic content reflects the potential of investigated Asteraceae to have important value as a source of anti-ageing compounds. Analysis of samples from different nutritional plant status pointed out the capacity of plant biosynthesis for SMs depending on local and enriched soil conditions.

The literature data confirm the polyphenolic contents in marigold flowers (11), especially in safflower oils, worldwide (10, 11, 17). As it can be seen from Table I, the amount of phenols differs between samples.

Carthamus has approximately double phenolic content in leaves and stems of unfertilized plants in comparison with flowers. Enriched soil conditions will increase total phenols in flowers, but decrease their total phenols in the aerial parts level. Total phenols in *Carthamus* are similar with quantities found in *Echinacea purpurea* cultivated in similar biotic conditions (18), but lower than the amounts of phenols found in *Tagetes erecta* and *Rudbeckia hirta* (19). The results point out the important role of biomass (leaves, stems) as a source of phenolic compounds from unfertilized cultivated safflower in circular economy.

TABLE I.

Total polyphenols determined in *Carthamus tinctorius* and *Calendula officinalis* (mg GAE/100 g dried sample)

Sample	V1F	V2F	V1L	V2L
Carthamus	145.79± 0.25	228.91± 0.52	276.07± 0.43	204.39± 0.50
Calendula	160.62± 0.35	189.41± 0.22	198.77 ± 0.40	124.36± 0.38

Similar to that of *Carthamus*, the polyphenolic content of marigold leaves and stems is greater in comparison with content determined in flowers in unfertilized plants. This emphasizes the economic importance of "vegetal waste" obtained from crops cultivated especially for flowers. The fertilization increases phenolic content in flowers but not in the aerial parts.

Alantolactone content. Proven aller-

gen, alantolactone presence is confirmed in marigold and safflower in our study (tab. II). The quantity of alantolactone detected in samples is greater in unfertilized plants, both in flower tops and in the aerial parts. On a general note, flowers have smaller quantities of alantolactone in comparison with leaves and stems in safflower and marigold. That could explain the low allergenic potential of flower tops. The risk of allergen reactions still exists in patients with known allergies.

TABLE II.

Semiquantitative determination of alantolactone in *Carthamus tinctorius* and *Calendula officinalis* (µg %)

Sample	V1F	V2F	V1L	V2L
Carthamus	0.1740	0.1561	0.6568	0.5333
Calendula	0.2414	0.0213	6.0045	0.0438

Marigold aerial parts of unfertilized plants have large amounts of alantolactone (V1L). This data suggests that the leaves and stems could be a potent source of alantolactone. The quantities found in our study are superior to data published on other ornamental Asteraceae cultivated in eastern Romania (19). On the other hand, these data could explain the low allergenic profile of marigold flower, but also point out a high allergenic risk for people who handle the crops or vegetal material.

Antioxidant capacity of the investigated samples. The obtained results for the antioxidant assays are given in third table and indicate that the nutritional status of the soil influences the biosynthetic capacity of the plant. Moreover, the accumulation pattern of secondary metabolites with antiradical potential is similar for both species, and increases in the flower tops of the fertilized group.

TABLE III.

The antioxidant potential of *Carthamus tinctorius* and *Calendula officinalis* samples against DPPH and iron radicals

Sample	V1F	V2F	V1L	V2L
DPPH inhibition (IC50, µg/mL)				
Carthamus	105.2±1.84	37.4±0.56	43.1±0.96	89.5±1.13
Calendula	147.3±0.96	100.2±1.04	128.2±0.67	213.7±0.07
Iron chelation (IC50, µg/mL)				
Carthamus	460.1±0.87	310.3±0.04	242.6±1.04	712.3±0.24
Calendula	228.2±1.02	184.2±0.16	237.1±0.82	378.6±0.09

According to our results, the investigated samples have a good effect against free radicals, but much lower if compared to gallic acid that had a value of IC50 at 1.15 µg/mL for DPPH assay, and 6.65 µg/mL for iron chelation assay. Also, the variation of the activity is directly correlated to the polyphenolic content. Interestingly, for *Carthamus* flowers the iron chelation inhibition is much lower than the capacity to

quench DPPH radicals.

Considering that the oxidative stress is a multifactorial complex process cell membrane are exposed to, the use of cosmetic applications that include natural components with antioxidant potential should prevent the degradation induced by such factors (2, 7, 22, 23). Polyphenols are compounds recognized for their antioxidative and protective activities, therefore they have been used for various preparations (22). Both marigold and safflower represent important raw materials for pharmaceutical and cosmetics industries and the quality of the plant products have a direct impact on the benefits given by the final products (2, 22). This is why the chemical composition and the antioxidant capacity of the plant material is extremely important for further development of different preparations.

Another important aspect is shown by the low quantity of alantolactone in the flower tops of the both investigated species, which indicates that harvesting should be done at the complete maturity when terpene lactones decrease and total phenols accumulate in higher amounts. This will ensure a low allergenic profile and a good protective potential of the formulations obtained from the flower material.

CONCLUSIONS

The studied Asteraceae cultivated in NE Romania are a potent source of polyphenols and could be a natural source of anti-aging agents in cosmetics. The allergenic risk of calendula and carthamus flowers extracts is low as confirmed by HPLC analysis. The allergic potential is greater in the unflower aerial parts of the plants, especially in the marigold. The use of flowers as raw materials for cosmetic formulation is generally safe. The biomass of nonflower aerial parts has very important economic value as a natural source of polyphenols (*Carthamus*) and alantolactone (*Calendula*). Our data confirm that vegetal "waste" is an important source for secondary metabolites. Other studies are needed to extend these findings.

CONFLICTS OF INTEREST AND FUNDING

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RELATIONSHIP BETWEEN LEVELS OF STREPTOCOCCUS MUTANS AND SOCIOECONOMIC STATUS OF SCHOOLCHILDREN IN IASI, ROMANIA

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RELATIONSHIP BETWEEN LEVELS OF STREPTOCOCCUS MUTANS AND SOCIOECONOMIC STATUS OF SCHOOLCHILDREN IN IASI, ROMANIA (Abstract). **Aim:** To evaluate the dental and salivary level of *Streptococcus Mutans* among Iasi schoolchildren in relationship with their family socioeconomic status. **Material and methods**: This cross-sectional study included 533 schoolchildren aged 7-12 years from Iasi town. The level of *Streptococcus Mutans* on dental surfaces (mesial and occlusal surfaces of the molars 1.6 and 3.6) and in saliva was assessed using Dentocult® SM Strip mutans kits (Orion Diagnostica Oy) according to manufacturer's instructions. Subjects were given one of the following scores: 0 = $<10^4$ CFU/ml; 1 = $<10^5$ CFU/ml; 2 = 10^5–10^6 CFU/ml; 3 = > 10^6 CFU/ml. Parental occupation, income and number of children in the family were considered for the assessment of the socioeconomic status. **Results**: Score 0 was predominant both in saliva and on the mesial and occlusal surfaces of first permanent molars of children of high socioeconomic status. Scores 2 and 3 were predominant in children of medium socioeconomic status, while score 3 was predominant on dental surfaces and in the saliva of children of low socioeconomic status. **Conclusions**: Children from families with low socioeconomic status have a higher prevalence of increased levels of *Streptococcus Mutans*, as compared to children from families with high socioeconomic level, underlining the need for complex prevention programs that should reduce inequalities in children oral health status caused by socioeconomic factors. **Keywords**: SCHOOLCHILDREN, STREPTOCOCCUS MUTANS, SOCIOECONOMIC STATUS.

Pointing out the risk factors for oral diseases enables the identification of their role in oral health. Knowledge about the population distribution of those factors is an essential step in developing preventive strategies and in planning preventive oral healthcare services.

Numerous studies have shown the correlation between parental social status and oral health status of children. In Romania, despite the fact that all children receive free dental services through the National Health Insurance, there are marked inequalities in oral health. Besides the social aspects, parents' oral health education level plays an important role.

The aim of the present study was to comparatively evaluate the dental and salivary level of *Streptococcus Mutans* (*SM*) among schoolchildren in Iasi town,

depending on the socio-economic status (SES) of their families.

MATERIAL AND METHODS

The cross-sectional, clinical and microbiological study was conducted between May 2015 and September 2016. The following criteria were considered for the selection of the study sample: children aged 7 to12 years; children attending schools in various districts of Iasi town; schools in which a dental office was available. The sample was selected using the probabilistic method and included 533 children (50.47% males and 49.53% females). Clinical examinations were performed in the dental offices of the selected schools.

The level of *SM* on dental surfaces (mesial and occlusal surfaces of the molars 1.6 and 3.6) and in saliva was assessed using Dentocult® SM Strip mutans kits (Orion Diagnostica Oy), according to manufacturer's instructions. For the assessment of dental *SM* level, special tips were used to obtain bacterial plaque samples, which were subsequently spread on the four sites of the square-tipped strips. For salivary *SM* evaluation, the patient was invited to chew a paraffin pellet for 1 minute, then the rough surface of the round-tipped strip was pressed against the saliva remaining on patient's tongue. The two strips were then placed in the selective culture broth; the vials were incubated at 35-37°C for 48 h; after incubation, the density of the grown colonies was compared to the chart provided by the producer, according to the following scores: 0 = $<10^4$ CFU (colony-forming unit)/ml; 1 = $<10^5$ CFU/ml; 2 = $10^5 – 10^6$ CFU/ml; 3 = > 10^6 CFU/ml.

Tests were performed at least 1 h after eating and toothbrushing, in a distinct dental visit and not during a treatment session; patients on antibiotic therapy during the last month before testing were excluded.

The socioeconomic status of the family (SES), established as low, medium or high, was assessed considering parents' occupation, income and number of children in the family. A balanced distribution of the subjects belonging to the three socioeconomic levels was ensured in the study sample.

Children participation in the study was conditioned by parental written consent. Children also had the opportunity to accept or refuse participation.

Data privacy as well as the impossible identification of subjects in the case the results are published were guaranteed.

SPSS 17.0 was used for the statistical analysis of data. The statistical significance threshold p was set at 0.05; chi-square test was used for data comparison and correlation analysis was performed using Spearman test.

RESULTS

Analysis of data obtained from the bacteriological tests for the evaluation of dental levels of *SM* indicated that score 3, meaning a density of more than 10^6 CFU/ml was predominant both on the mesial surfaces of 1.6 and 3.6 and on the occlusal surfaces of the same teeth.

Comparative evaluation depending on SES indicated significant differences in *SM* number; on the dental surfaces, such differences were observed particularly on the mesial surfaces of first permanent molars (1.6) (tab. I). Score 0 was predominant in children of high SES (48.8%), while score 2 prevailed in children of medium SES (35.1% for upper teeth 1.6 and 36.8% for lower teeth 3.6) and score 3 - in the group of low SES (56.2% and 63.6%, respectively).

TABLE I

Children distribution (%) depending on *Streptococcus Mutans* scores on mesial surfaces of the teeth and socioeconomic status

Score	*Situs*					
	Mesial surface 1.6			Mesial surface 3.6		
	Low SES	Medium SES	High SES	Low SES	Medium SES	High SES
0	10.8	21.1	48.8	10.8	21.1	48.8
1	4.5	12.4	20.3	4	14.6	20.3
2	28.4	35.1	27.3	21.6	36.8	27.3
3	56.2	31.4	3.5	63.6	27.6	3.5

Chi-square statistical test showed that the differences were statistically significant at a threshold of 0.01, and the correlation between *SM* number on the mesial surfaces of first permanent molars (1.6) and SES was strongly inversely proportional (Spearman coefficient r = -0.510 and r = -0.539, respectively).

Such differences, but slightly reduced, were observed in the number of *SM* on the occlusal surfaces (tab. II): score 0 was predominant in children of high SES (47.1%), while score 3 prevailed in children of low and medium SES (58.5%, 56.2% and 44.3%, 35.7%, respectively).

Differences remained statistically significant ($p < 0.01$) and the correlation between the number of *SM* on the occlusal surfaces of first permanent molars (1.6) and SES was still inversely proportional, but moderate (r = -0.456 and r = -0.471, respectively).

TABLE II

Children distribution (%) depending on *Streptococcus Mutans* scores on occlusal surfaces and socioeconomic status

Score	*Situs*					
	Occlusal surface 1.6			Occlusal surface 3.6		
	Low SES	Medium SES	High SES	Low SES	Medium SES	High SES
0	10.8	21.1	47.1	10.8	21.1	47.1
1	1.7	15.1	20.3	4.5	14.6	20.9
2	29	19.5	20.9	28.4	28.6	23.8
3	58.5	44.3	11.6	56.2	35.7	8.1

Among the most important differences in caries risk factors between children of different socioeconomic levels were those in the number of salivary *SM* (tab. III); nearly 50% of the subjects of high SES presented score 0, while in those of low SES scores 2 and 3 were predominant (29.5% and 54%, respectively).

TABLE III

Children distribution (%) depending on *Streptococcus Mutans* salivary scores and socioeconomic status

Score	Low SES	Medium SES	High SES
0	10.8	21.1	48.8
1	5.7	15.1	24.4
2	29.5	38.9	23.8
3	54	24.9	2.9

Chi-square analysis indicated statistically significant differences ($p < 0.01$). Spearman correlation coefficient showed an inverse, strong correlation: *SM* salivary level increases as SES decreases ($r = -0.521$).

DISCUSSION

One of the greatest challenges of public health systems in the XXI century is represented by detecting and reducing inequalities in oral health status of the population (1, 2). Reasons for disparities in oral health are complex. There are differences caused by biological factors (e.g. gender, age), their existence being normal and unavoidable in a balanced society.

However, there are avoidable inequalities that are unacceptable in modern society, caused mainly by socioeconomic differences (3). Studies have shown that, over the last decade, differences in health between individuals of high and low socioeconomic level have increased (4).

Given these considerations, the present study analyzed the influence of the family socioeconomic level on the salivary and dental level of *SM*. Comparative assessment and use of statistical correlation tests showed that there were statistically significant inequalities in terms of *SM* density caused by SES. Among high SES children, score 0 of *SM* density was predominant both in saliva and on the mesial and occlusal surfaces of first permanent molars (1.6). Scores 2 and 3 were predominant in medium SES children, while score 3 of dental and salivary *SM* prevailed among low SES subjects.

Of the four major categories of factors that influence oral health (environmental factors, biological factors, behavioral factors and dental care system), social environmental factors have the greatest impact (5). The results of the present study confirm it by the evident differences in *SM* levels according to the socioeconomic level.

The most plausible explanation for these discrepancies is the behavioral differences related to oral hygiene habits and healthy diet. Although behaviors towards oral health, including various types of behavior, cannot be influenced by the same social and economic variables, two of the most important behaviors in terms of etiology of dental caries, i.e. brushing and sugar consumption are directly correlated with income level, educational level and occupational status (6). High SES individuals usually have a higher level of oral health knowledge and good oral health behaviors, mainly due to the improved access to information means (7). In addition, their income allows them to access more expensive hygiene products and healthy foods.

Results consistent with those obtained in this study were highlighted by other studies in the literature. In a study that assessed oral colonization of small children with *SM* it was found that mothers of children infected with this microorganism showed increased levels of salivary *SM*, poor oral hygiene and a low socioeconomic level (8, 9).

Parental educational level plays an important role in oral hygiene status of their children and, thus, in the level of cariogenic microorganisms. Studies conducted in this regard have shown higher *SM* levels among adolescents from families with low education level (7).

The impact of SES on the level of cariogenic microorganisms is further reflected in the level of dental caries. Numerous studies showed that lower-class children experience more caries. Children of highly educated, professional and high income parents are at lower risk for dental caries (10).

Socioeconomic level impacts on many of the healthy oral behaviors. The more likely they visited a dentist for routine checkup, the higher the SES, the less frequently they had sweets, and the lower the SM level, the more likely they were caries-free and the less likely they were to have a high DMFT (11).

Contamination with *SM* increases the risk of caries development. However, this risk can be partially offset by other factors, with protective role, including good oral hygiene and non-cariogenic diet. On their turn, these factors can counterbalance each other, meaning that cariogenic diet does not necessarily lead to the emergence of caries, provided that rigorous oral hygiene is ensured (12).

CONCLUSIONS

Children from low SES families have a higher prevalence of increased *Streptococcus Mutans* levels, as compared to children from high SES families, underlining the need for complex prevention programs that should reduce inequalities in children oral health status caused by socioeconomic factors.

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THE ANALYSIS OF THE PERSONAL VARIABLES AMONG THE ROAD TRAFFIC CASUALTIES TAKEN OVER BY THE IASI COUNTY AMBULANCE SERVICE, ROMANIA

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THE ANALYSIS OF THE PERSONAL VARIABLES AMONG THE ROAD TRAFFIC CASUALTIES TAKEN OVER BY THE IASI COUNTY AMBULANCE SERVICE, ROMANIA (Abstract): In last three years (2013-2015) the Ambulance Service of Iași, the largest county from the North-East region of Romania, provided emergency care to 4,028 road traffic injured persons, after the 112 Single Emergency Call Number has been dialed. **Aim**: This paper describes the main personal characteristics of the road traffic casualties taken over by the Ambulance Service, to design a profile of the injured persons, on whose foundation, specific protective measures regarding the road traffic injuries could be developed. **Materials and methods:** The survey is based on a descriptive analysis of the 4,028 cases forwarded by the central dispatch to the Iasi County Ambulance Service. The data source was provided by the Iasi County Ambulance Service and conceived of the emergency care files of the injured persons. The analysis of the personal variables was based on: demographic issues, the categories involved in road accidents and the patient's status at the crash scene. **Results:** More male (61.7%) were injured than female, the male: female ratio of injuries being 5/3. Of the total 4028 cases recorded along the three years, 806 were young people aged 20-29 years and of these, male subjects represented 74.2%. A strong negative correlation was found between age and the number of subjects by age groups (r = -0.96). More than two thirds (68.7%) of the total sample are living in urban area, the urban/rural ratio being 2/1. Car occupants represented 91.7% of the total injured cases, followed by the motorized 2-wheelers drivers (4.7%), pedestrians (1.5%), wagon drivers (1.1%) and cyclists (1%). Approximately two persons of one hundred casualties (1.9%) were found deceased at the scene of the crash. The severe unconscious cases and the deceased combined accounted 6% of all cases. An analysis of the fatalities by gender pointed out a higher death rate among male (2.9%), compared to 0.4% among female, the difference being strong significant ($p<0.001$). The repartition of the total deaths by road user category placed on the first place the car occupants (52.6%), followed by motorized 2-wheelers drivers (23.1%), pedestrian (20.5%) and wagon drivers (3.8%). The severity of the injury has been assessed and revealed four risk factors: male gender, pedestrian, cyclist and motorized 2-wheelers. The odds ratio values ranged from 8.19 for pedestrians to 2.81 for motorized 2-wheelers drivers. **Conclusions**: All these findings allow a profile of the people affected most frequent by the road traffic injuries, representing casualties taken over by the Iasi County Ambulance Service in the interval 2013-2015: young male, aged 20-29, urban resident, car occupant. The most severe cases were found among male of three road user categories: pedestrian, cyclist and motorized 2-wheelers. **Keywords**: AMBULANCE SERVICE, ROAD TRAFFIC INJURY, PERSONAL VARIABLES.

In the road traffic injuries, the access to the emergency medical system is most frequently made by telephone, dialing 112, the Single Emergency Call Number. The next step in the post-impact chain interventions from crash scene to hospital is delivering of the emergency care at the place of the collision and getting victims as soon as possible to a health-care facility (1). Because of many road crashes are avoidable, the descriptive epidemiology data are essential for the preventive measures related to the human safety (drivers, passengers, pedestrians), and as severity predictors in a crash injury (2).

This paper describes the main personal variables of the road traffic casualties taken over by the Ambulance Service of Iasi, the largest county of the North-East Romania, along an interval of three years (2013-2015). The analysis of these data allows the elaboration of the profile of the injured persons, on whose foundation, protective measures regarding the accidents and the traumas could be developed, to reduce complications, but mainly to prevent other deaths.

MATERIALS AND METHODS

The survey is based on a descriptive analysis of the cases forwarded by the central dispatch to the Iași County Ambulance Service, after the 112 Single Emergency Call Number has been dialed and the provision of emergency care to people involved in road traffic accidents in Iași County has been requested.

The data source was provided by the Iași County Ambulance Service and conceived of the 4028 emergency care request files of persons involved in road traffic accidents and who received emergency care during 2013-2015. The analysis of the personal variables was based on: demographic issues (age, gender and residence area), the categories involved in road accidents (pedestrians, cyclists, wagon drivers, tram drivers, car drivers, motorcyclists) and the patient's status (deceased, conscious, unconscious).

The database and the statistical analysis were done in *EpiInfo TM 7*.

RESULTS

During 2013-2015, the Iasi County Ambulance Service reported a total number of 392,267 calls, of which 4,028 represented road traffic injuries. Each year, the share of the road crashes casualties was approximately 1% of the total. The number of injured persons increased with 52.07%, from 1,085 in 2013 to 1650 in 2015.

The burden of injury by gender was unequal (tab. I). More male (61.7%) were injured than female, the male: female ratio of injuries being 5/3.

Of the total 4,028 cases recorded along the three years, 806 were young people aged 20-29 years (fig. 1) and of these, male subjects represented 74.2%. This proportion corresponds to a male: female ratio of 3/1 and in an analysis by decennial age groups, is the highest value in" favor" of male. On average, in Iasi County, yearly, the Ambulance Service delivered emergency care to 268 young people belonging to the third decade of life and who sustained a road crash injury (tab. II).

The average age for all injured persons was 35.6±19.6 years (range 1-91years). A strong negative correlation was found between age and the number of subjects by age groups (r = -0,96).

Another personal variable was the residence area of the road traffic injured cases. The annual average of the injuries in rural was 419.6 ± 118.5, whereas in urban was 923 ± 167.5. More than two thirds (68.7%) of the total sample are living in urban area, the urban/rural ratio being 2/1.

TABLE I

Data on the breakdown by gender

Year	Male		Female		Total
	No.	%	No.	%	
2013	641	59.07	444	40.93	1085
2014	777	60.09	516	39.91	1293
2015	1068	64.7	582	35.3	1650
Total	2486	61.7	1542	38.3	4028

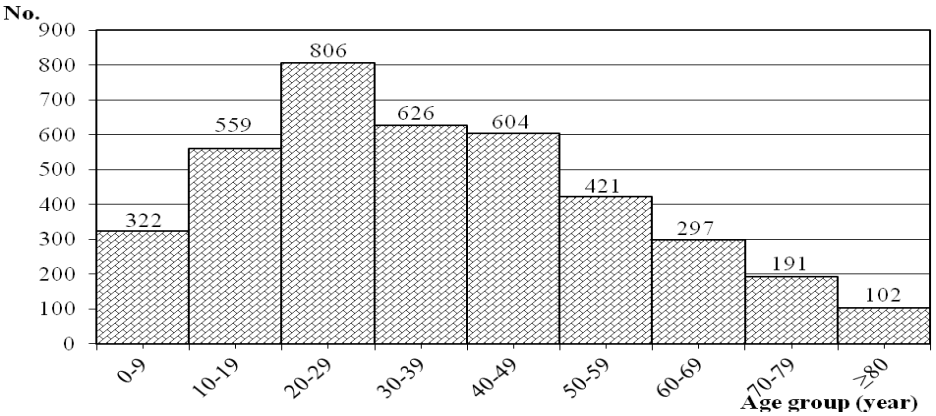

Fig. 1. Age group distribution of the total sample of road traffic injuries

TABLE II

Distribution by year and age group

Age group (yrs.)	2013	2014	2015	Annual average ± SD
0-9	93	105	124	107.3 ± 15.6
10-19	126	208	225	186.3 ± 52.9
20-29	254	288	264	268.6 ± 17.4
30-39	165	194	263	207.3 ± 50.3
40-49	143	200	261	201.3 ± 60.5
50-59	122	131	168	140.3 ± 24.3
60-69	76	78	143	99.0 ± 38.1
70-79	76	50	65	63.6 ± 13.0
≥ 80	31	40	31	34.0 ± 5.1

The profile of the people affected by road traffic injuries was assessed due to important differences between various road user groups. On average, each year, 1270 people traveling with a 4-wheeled car sustained a road crash injury (tab. III).

They represented 91.7% of the total, followed by the motorized 2-wheelers drivers (4.7%), pedestrians (1.5%), wagon drivers (1.1%) and cyclists (1%).

TABLE III
Data on the breakdown by road user category

Road user category	Cumulative number	Annual average ± SD
Car occupants	3695	1270.3 ± 204.2
Motorized 2-wheelers	188	60.6 ± 6.5
Pedestrians	61	15.6 ± 4.2
Wagon drivers	45	14 ± 5.4
Cyclists	39	13 ± 4.1

When delivering emergency care at the scene of the crash, the ambulance professionally trained staff make firstly a complete evaluation of every injured person, to identify life-threatening injuries. Approximately two persons of one hundred casualties (1.9%) were found deceased at the scene of the crash. The severe unconscious cases and the deceased combined accounted 6% of all cases (tab. IV).

TABLE IV
Patient's condition at the scene of the crash

Patient's condition	Cumulative number	Annual average ± SD
Conscious	3785	1263.6 ± 280.4
Unconscious	165	55 ± 13
Deceased	78	26 ± 4.5

An analysis of the fatalities by gender pointed out a higher death rate among male (2.9%), compared to 0.4% among female, the difference being strong significant ($p<0.001$). A similar situation has been recorded for the unconscious cases (male 5.5%, female 1.8%, $p<0.001$).

The repartition of the total deaths by road user category placed on the first place the car occupants, followed by motorized 2-wheelers drivers, pedestrian and wagon drivers, as shown in fig. 2. Car occupants were the first largest group of road users killed and found by the ambulances at the scene of the crash.

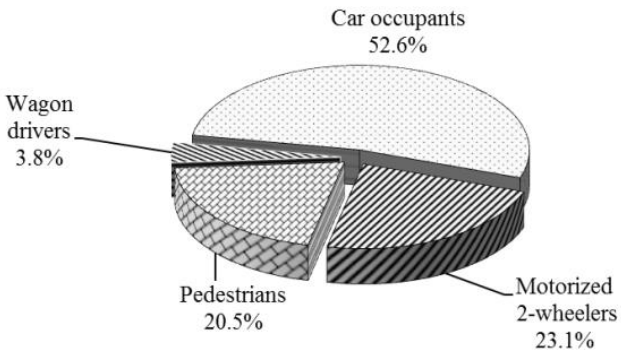

Fig. 2. Distribution of the total deaths by the road user category

The fatality rate among different road user groups was 26.2% for pedestrians, 9.6% for motorized 2-wheelers drivers and 6.6% for wagon drivers. The lowest fatality rate (1.1%) was found at the vehicle occupants. No deaths were recorded in the cyclists group.

The severity of the injury has been assessed by comparing the personal variables among the conscious cases with the unconscious and deceased cases. The analysis revealed four risk factors for a severe prognosis: male gender, pedestrian, cyclist and motorized 2-wheelers. The odds ratio values ranged from 8.19 for pedestrians to 2.81 for motorized 2-wheelers drivers (tab. V).

TABLE V

Risk factors for a severe prognosis among the injured road users

Risk Factor	Unconscious +	Conscious	Odds	IC_{95}	p value
Pedestrians	20	41	8.19	4.71-14.21	<0.001
Male gender	209	2277	6.54	4.19-10.19	<0.001
Cyclists	7	32	5.19	2.25-11.95	0.001
Motorized 2-wheelers	27	161	2.81	1.83-4.32	<0.001

DISCUSSION

In the interval 2013-2015, the number of injured persons involved in road traffic accidents and taken over by the Iasi County Ambulance Service increased with 52.1%.

Injury rates and mortality rates are higher in men than in women across all age groups. The gender difference may be related to both exposure and risk-taking behavior.

Adults aged between 20 and 49 years' account for more than 50% of all road traffic injuries. In an analysis by decennial age groups the highest value was recorded among young people in their third decade of life (20%). The number of cases for older persons over 60 years was, on average, less than 100 per year, due to the lower rate of exposure they experience.

Road traffic crashes are more frequent in urban areas, as urbanization increases (3,4). According to this fact, in this study, people from urban area sustained twice more often road trauma compared with rural area.

Among the various categories involved in road accidents, car occupants (drivers and passengers) accounted more of 90% of injured persons and more of 50% of deaths found by the ambulance at the scene of the crash. Despite these values, the fatality rate among car occupants was low (1.14%), compared to the motorized 2-wheelers drivers, pedestrians, wagon drivers and cyclists, they often bearing the greatest burden of the injury.

Crashes between vehicles and pedestrians are responsible for many deaths in this category of road users (5,6). In terms of fatality rate, one of four injured pedestrians were found dead at the scene of the crash (26.2%). This was the highest fatality rate, followed by the motorized 2-wheelers (9.5%) and wagon drivers (6.6%). They represented the most vulnerable groups, compared to the vehicle occupants, being far the most exposed group.

All these findings allow a profile of the people affected most frequent, but nonfatal, by the road traffic injuries, representing casualties taken over by the Iasi County Ambulance Service in the interval 2013-

2015: young male, aged 20-29, urban resident, car occupant.

In the same time, by calculating the Odds Ratios for various personal variables, a profile of the most severe case was designed: male gender, pedestrian, cyclist or a 2-wheels vehicle rider.

CONCLUSIONS

Along an interval of three years (2013-2015), the Iasi County Ambulance Service provided emergency care to 4,028 road traffic injured persons. The number of injured persons increased from 2013 to 2015 with 52.07%

The proportion of males exceeded females (61.7%), the male: female ratio for injuries being 5/3.

One of two injuries were an adult aged between 20 and 49 years and one of five was in the third decade of life.

More than two thirds (68.7%) of the total sample are living in urban area, the urban/rural ratio being 2/1.

Car occupants (drivers and passengers) accounted more of 90% of injured persons and more of 50% of deaths found by the ambulance at the scene of the crash, but the fatality rate among car occupants was low (1.14%).

The highest fatality rate was recorded among pedestrians (26.2%), followed by the motorized 2-wheelers (9.5%) and the wagon drivers (6.6%).

The typical road traffic injury to which the Iasi County Ambulance Service provided emergency care was: young male, aged 20-29, urban resident, car occupant.

The analysis of the injuries' severity based on the Odds Ratios, allowed the identification of the following personal variables: male gender, pedestrian or a 2-wheels vehicle rider.

Various protective measures, such as making walking safer or paths for wagons, are critical to decrease the number of road traffic injuries, to reduce complications and to prevent deaths.

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NEW NITRIC OXIDE DONORS WITH THERAPEUTIC POTENTIAL

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NEW NITRIC OXIDE DONORS WITH THERAPEUTIC POTENTIAL (Abstract): Nitric oxide (NO), formerly known as the endothelium-derived relaxing factor, is a mediator with a key role in the body, both in the central nervous system and in periphery. NO is synthesized by several cell types, where it acts as an autocrine and paracrine signaling molecule. Harnessing the impressive therapeutic potential of nitric oxide (NO) remains an ongoing challenge. In order to overcome the limitations linked with the use of nitric oxide and specially to increase the release of the radical in the targeted area, promising therapeutic strategies have been implemented, based on specific technologies which create releasing agents and vehicles for nitric oxide. Organic nitrites are the most known NO donor drugs, used especially in the treatment of cardiovascular diseases. In recent years, technological advances have allowed obtaining variations synthetic derivatives (such as diazeniumdiolates, S-nitrosothiols), which can generate NO in a controlled mode in the body and to chemically stabilize it; these compounds were studied with promising results in various animal models of vasospasm and pulmonary hypertension. Another high value therapeutic path is represented by the development of hybrid drugs (new nonsteroidal anti-inflammatory NO donor agents), with practical applications in inflammatory disorders accompanied by pain. Also, there is increasing evidence of the existence of NO donors with important antioxidant and hepatoprotective effects. **Keywords:** NITRIC OXIDE, NO DONORS, DIAZENIUMDIOLATES, *S*-NITROSOTHIOLS.

During the 1980s, the free radical nitric oxide (NO) was discovered to be a crucial signaling molecule, with a variety of pharmacological activities in the cardiovascular, nervous and immune systems. In 1992, the free radical nitric oxide was awarded the title 'molecule of the year' (1).

Formerly known as the endothelium-derived relaxing factor, nitric oxide (NO) has been intensively studied over the years, its importance in the body being fully recognized in 1998 when the Nobel Prize for medicine was awarded to a team of researchers for their studies on the biological effects of this molecule (2). Despite its structural simplicity, NO plays an active role in complex and varied biological processes. An important fact is that its sphere

of influence is likely to extend to only ~100 μm from its origin. This property has important consequences: first, it means that NO must be rapidly synthesized on demand in response to stimuli and second, it is a local mediator that does not require complex metabolism for clearance; it is simply oxidized to nitrite/nitrate as it diffuses away from its source (3). Therefore, an important property of NO, which distinguishes it from other mediators of the central nervous system, is diffusivity. This is increased in both the aqueous and the lipid media, providing a three-dimensional spreading signal, regardless of the presence of membranes (4).

The most studied actions of NO are on the cardiovascular system, where it is continuously produced by the endothelial cells that line the lumen of blood vessels. Here, L-arginine is converted into NO by the endothelial isoform of the enzyme NO synthase, in response to mechanical and chemical stimuli that act by mobilizing intracellular calcium.

NO diffuses away from the cell of origin, passing easily through membranes of neighboring cells, bringing on a range of physiological effects. NO participates in maintaining and regulating blood pressure, due to its role in adjusting systemic vascular resistance (5, 6).

NO acts almost exclusively in vascular smooth muscle cells (3) via the soluble enzyme guanylate cyclase, stimulating cyclic guanosine-3',5'-monophosphate (cGMP) production and elicit vasodilation via cGMP-dependent protein kinases. Also, NO has an impact on circulating platelets, where, in response to endothelium and platelet-derived NO, both cGMP-dependent and independent mechanisms play a part in its powerful inhibitory effect on aggregation and adhesion (7, 8).

Considering that a characteristic of many cardiovascular diseases is the reduced level of NO in the soluble guanylate cyclase pathway, an intake of exogenous NO would be a possible and attractive therapeutic choice (6, 9).

NO donors in the cardiovascular disease

The translation of therapeutic potential of NO to bedside has been slowed down by its short biological lifetime, instability during storage and potential toxicity. Taking these into account, new drugs have been obtained, with the role of molecular NO vehicles (also named *NO donors*), created for stabilizing this radical, until the moment when it needs to be released (10). Increased understanding of the essential role of nitric oxide (NO) in various physiological processes and diseases has stimulated the development of multiple pharmacologic strategies to release NO at the involved level of pathogenic pathways (11). These approaches include therapies that directly or indirectly release or “donate” NO and the use of existing drugs, such as antihypertensive agents (e.g. nebivolol) and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) which enhance NO bioactivity in the body (12).

Capitalizing on the biological actions of NO, these therapeutic strategies are providing new insights into the pathogenesis and treatment of various diseases, including hypertension, atherosclerosis, cancer, Alzheimer's disease, colitis and asthma (3, 13).

The organic nitrites are the most commonly used NO donor drugs: glyceryl trinitrate (GTN; also, known as nitroglycerin) has been the most studied nitrate, used mainly in the relief of acute pain associated with angi-

na, whereas other slower release preparations, such as isosorbide mononitrate, are used for the treatment of chronic angina. The other clinically relevant NO donor in current use is sodium nitroprusside, known to provide rapid lowering of blood pressure in severe hypertensive crises (14, 15).

A great number of synthetic compounds (e.g., N-diazeniumdiolates, S-nitrosothiols, nitrosohydroxylamines and nitrosyl metal complexes) have been developed to chemically stabilize and release NO in a controlled manner, and have been exploited in many biomedical applications (3, 16). Diazeniumdiolates represent another class of NO donors to which increased attention has been paid recently for their ability to generate predictable amounts of NO (17). The biological activity of diazeniumdiolates was tested with promising results in various experimental models, such as vasospasm, pulmonary hypertension, platelet adhesion/aggregation (11, 3).

In addition, S-nitrosothiols are considered an excellent source of NO. S-nitrosothiols-based therapeutics can be considered more efficient than NO-based therapeutics, due to its more prolonged release of NO, as well as a more facile transnitrosating capability (16). These donors were proven to induce more prolonged vasodilation than the other NO donor types tested. Moreover, lipophilic S-nitrosothiols induce even longer vasodilation than the conventional S-nitrosothiols (11).

NO donors with antioxidant and hepatoprotective effects

From another point of view, there is an increase of evidence in support of the fact that NO reduces the arachidonic acid-induced oxidative stress. Also, it was demonstrated that antioxidants can prevent the l-N^G-Nitroarginine Methylester (l-NAME) potentiation of arachidonic acid toxicity. Toxic effects to mitochondria induced by arachidonic acid were demonstrated by a decline in the mitochondrial membrane potential. L-NAME potentiated this decline in mitochondrial membrane potential, this being linked to its increase in arachidonic acid-induced oxidative stress (18).

NO donors decrease the decline in mitochondrial membrane potential, due to their decrease in arachidonic acid-induced oxidative stress (19). These results indicate that NO can exert hepatoprotective effects against CYP2E1-dependent toxicity, preventing arachidonic acid-induced oxidative stress (20). Published research data sets show that NO contributes to liver homeostasis, its production playing an important role in both cytoprotectant and cytotoxicity processes (21, 22).

Experimental data shows that NO seems to be beneficial to hepatocytes, having cytoprotective effects against inflammation and tissue damage and direct cytotoxic effects on invading microorganisms and tumor cells (23, 24).

NO donors with a beneficial role in reducing inflammation and pain

Another high value therapeutic path is represented by the development of NO donor hybrid drugs. These are the new nonsteroidal anti-inflammatory NO donor drugs (NO-NSAIDS) that keep the pharmacodynamic actions of the basic compound (analgesia, inhibition of inflammation) as well as have other benefits (assigned to NO release in body) such as the protection of gastric mucosa against the irritating action of NSAIDS (25).

These compounds are prepared by adding a radical such as nitrobutyl or nitro-

sothiol by short-chain ester linkage of an NO-releasing moiety to conventional NSAIDS, such as aspirin (*NO-Aspirin*), flurbiprofen (*NO-flurbiprofen*), naproxen (*NO-naproxen*), diclofenac (*Nitrofenac*), Ibuprofen (*NO-ibuprofen*) and indomethacin (*NO-indomethacin*) (25, 26). Experimental and clinical data sets show that the capacity of NO-NSAID to release NO appears to reduce the gastrointestinal toxicity compared with NSAIDs alone (27).

The various mechanisms that underline the protective effect of NO on the stomach include vasodilation of the gastric mucosa, inhibition of caspase enzymes activity and inhibition of leukocyte adhesion (25).

CONCLUSIONS

We concluded that multiple new NO donors (such as: diazeniumdiolates, S-nitrosothiols, nonsteroidal anti-inflammatory NO donor compounds) have been developed in the last years. They were proven to have beneficial pharmacodynamics effects in various experimental models of induced vasospasm, pulmonary hypertension, inflammatory states, acute and chronic liver diseases. As we have outlined in this material, these compounds appear to be promising agents that may be used in the treatment of cardiovascular maladies, inflammatory conditions, liver function disturbances, or of neoplastic diseases.

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NEWS

NEXT GENERATION SEQUENCING (NGS) AS A PROMISING TOOL IN FORENSIC GENETICS

The biggest challenge for a forensic geneticist is to obtain sufficient information from trace samples, and thus being able to sequence every DNA molecule in a sample is a very intriguing idea. At the present, the forensic markers are typed using PCR-CE and there are individual assays for autosomal STRs, Y-chromosome STRs, X-chromosome STRs, indels, mtDNA SNPs, autosomal SNPs, Y-chromosome SNPs etc. The most important advantage of NGS is that all these assays could be combined into a single NGS assay. This will save time when dealing with cases where supplementary investigations are necessary and reduce the overall time needed for processing a sample. Another advantage is that the DNA fragments do not need to be separated by lengths in CE, so all the analyzed fragments can be designed to be as short as possible, increasing the chance of typing degraded DNA (Claus Børsting, Niels Morling. Next generation sequencing and its applications in forensic genetics. *Forensic Sci Int Genet* 2015; 18: 78-89. doi: 10.1016/j.fsigen.2015.02.002).

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THE HARMFULNESS OF ALCOHOL AND TOBACCO SMOKING IN CARDIOVASCULAR PATHOLOGY AND THE PERSPECTIVE OF SPIRITUAL THERAPY

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THE HARMFULNESS OF ALCOHOL AND TOBACCO SMOKING IN CARDIOVASCULAR PATHOLOGY AND THE PERSPECTIVE OF SPIRITUAL THERAPY (Abstract): A holistic approach to the human person, from a somatic, psychological and spiritual point of view, with reference to the harmfulness of alcoholism and smoking in cardiovascular pathology, highlights several therapeutic variants over time. Among them are: the current somatic therapy, which could not offer optimal solution in eradicating this vice, and psychiatry which, using will power, could not solve man's contradiction with himself, caused by a tempting and treacherous irrationality of senses, instinctive-affective desires, turned into vices, as a *"Quasi altera natura",* devoid of any bright and creative horizon. As such we propose an alternative that entails changing one's lifestyle to eliminate the harmful effect of alcoholism and smoking on cardiovascular pathology, through the perspective of spiritual therapy, accessible to contemporary society, at no cost and with no adverse events. **Keywords:** SPIRITUAL THERAPY, ALCOHOLISM, TOBACCO ADDICTION.

Tobacco smoking is a major risk factor for coronary, carotid and peripheral atherosclerotic disease; passive smoking also shows an increase in cardiovascular risk. The impact of smoking on metabolism consists of increased total cholesterol and decreased HDL cholesterol in platelet and leukocyte activation, increased circulating fibrinogen, endothelial dysfunction with the promotion of fissure in vulnerable plates, increased heart rate and blood pressure, as well as other effects aggravating myocardial ischemia due to inhaled carbon monoxide (1,2).

Smoking, together with increased blood pressure, is a strong cardiovascular risk factor; it causes cardiovascular events: stroke, myocardial infarction and peripheral vascular disease. The most effective measure to prevent cardiovascular disease is smoking cessation and lifestyle changes. In particular cases, nicotine replacement therapy might be considered. A detailed research on 36 studies comparing long-term smoking cessation rate using bupropion showed a relative success of 1.69% on control; whereas the combination of nicotine replacement therapy with varenicline yielded no results. Recently, the *Food and Drug Administration* (FDA) issued a warning regarding the safety profile of varenicline, in the sense that spreading smoke substances in the blood, across respiratory surfaces, acts on the nervous system, caus-

ing shivers, decreased appetite and decreased visual acuity. Acting on the skin, it alters its color and causes wrinkles; it harmfully influences the heart and blood vessels, increasing heart rate, creating vasoconstriction, as well as arterial hypertension. The above-mentioned show that smoking is an addiction with destructive consequences on the human being. A solution to the problem would be to detect the cause of smoking; however, so far research has not discovered a "smoking gene". Although drug therapy for smoking cessation has proven effective, its use is limited, on account of side effects and high prices; these drugs are not reimbursable in most countries, and there is a lack of information among the population on the risks smokers are exposed to. The most effective and inexpensive treatment in smoking cessation therapy is one's own will, the awareness of the harmful effects of smoking and the desire to live a healthy life (3, 4, 5).

Alcohol is regarded as the fourth risk of health problem after cardiovascular diseases. Alcohol is the most common risk factor in the occurrence and evolution of cardiovascular risk. The excessive long-term alcohol consumption increases hypertensive risk and, in parallel, the risk of strokes, or causes supra-arachnoid hemorrhage.

Physiologically speaking, alcohol passes entirely in the blood at the level of digestive surfaces; it creates irritation and necrosis in the mucous membranes, and eventually cirrhosis of the liver. Influencing the circulatory system, alcoholism creates and aggravates, like smoking, myocardial infarction, arteritis, as well as other metabolic disorders. On the nervous system it creates disturbances in reflexes, manifested paradoxically.

The alcohol can be eliminated, like smoking, through one's own will, by becoming aware of the danger it poses on health and wishing to have a healthy life, while also considering the resources wasted to the detriment of well-being. In the following lines we will attempt to present an alternative to eliminate the harmfulness of alcoholism and smoking, through the perspective of spiritual therapy (6, 7, 8).

Spiritual therapy. In principle, any desire that is inconsistent with human nature, as well as excesses of any kind, have been regarded since the oldest times as vices or enslaving passions, owing to their insatiability. In particulars, *stoics* believed that vices, which they called "*κακία"* were diseases of the soul, was mainly aimed at the spiritual part of the human person, and especially the rational factor, thought of as the divine in man. This integral, holistic, psychosomatic view, applied to ancient medical practice, becomes increasingly more topical in our times, when stress and anxiety help proliferate diseases barely heard of in the past, such as diabetes, arterial hypertension, coronary heart disease, tumors, or strokes; and smoking, drug use and alcoholism keep claiming more victims, including depression, which is triggered by specific energetic imbalance, all sorts of somatic pathologies (9, 10, 11, 12).

To reference ancient thought again, we will say that, having the main concern of continuously developing and perfecting psychosomatic human nature, it was believed that any natural thing is not bad in and of itself, but becomes bad through its defective or unnatural use, due mostly to excess (13,14). Suggestive in this sense are the words of Apuleius of Madaurus, who established that "*The first cup is for thirst, the second for mirth, the third for delight,*

the fourth for madness" (15). Hence, the need to apply moderation, after good rational *counsel;* defining the health of human nature itself by avoiding the two extremes that are harmful to it, i.e. *lack or insufficiency,* which does not satisfy necessity; and *excess* or *abuse,* which exceeds necessity. Resulting from the insatiability of lusts, alcoholism, along with other vices, generates all kind of suffering, both to the soul, by dominating it and canceling its authentic freedom of expression, and to the body, through all sorts of diseases, which ruin its health, bringing suffering, degradation and death. This is why, in order to foster health, as a normal and necessary way to develop and perfect integral (psychosomatic) human nature, education is promoted in the culture of all peoples across the world, that is**,** as a culture of the spirit, as shaping or polishing human personality according to principles intended to bring its accomplishment or perfection.

Nature comprises native predispositions, flaws and qualities, most often passed from parent to child. *Reason,* referring to learning, comprises instruction made based on arguments, to create a certain *mentality* likely to cultivate qualities and eliminate flaws contained in each person's nature. *Habits* mean practicing what was learned to create a stable attitude. Thus, habit can become a second nature (*quasi: altera natura*), when set free from the strict rule of heredity, becoming the result of environment or the imitation of new forms, freely and consciously accepted by man (15).

However, delving deeper into these three factors, essential differences were noted, both in their structure, and in their functionality. Thus, as Plato said, there are people with a *nature of gold,* willing to receive and cultivate fundamental values, summarized in: *truth, goodness* and *beauty;* but there are also coarse, difficult natures in the path towards the rational order of life. *Reason* can, in its turn, be fair, but most of the times it lacks the lucidity of a correct decision. *Habits* depend on the *power of will,* which fulfill an essential role in transforming rational decision into constant action. This leads to a contradiction between man and himself, i.e. a contradiction between vulgar pleasures, turned into habits, as blind necessities, which shut and unravel the bright horizon of life, and the light of reason or judgment of conscience, oriented towards real values intended to fulfill the aspirations of human nature, through health and authentic assertion. Thus, ancient scholars were right to deplore failure, saying: *video meliora proboque, sed deteriora sequor* (I see and approve of the better, but I follow the worse – Ovid, *Metamorphoses*, VII – 20, 21); and later, Paul the Apostle stated "*For what I want to do I do not do, but what I hate I do... I do not do the good I want to do, but the evil I do not want to do*" **(***Romans 7*, 15-19). And if this contradiction exists, it only means that human nature is suffering, as simple education as therapy of the soul, meant to find the optimal solution in its quest for accomplishment or perfection, cannot achieve everything, being most of the times destined for failure (15).

We can see that spiritual therapy as a work of divine grace brings forth in the soul the *power of faith able to convert*, to change mentalities and feelings, guiding them towards a new behavior. A concrete model in this context is given by Saint Paul the Apostle who said, referring to his own conversion: "*But whatever were gains to me I now consider loss for the sake of*

Christ ... for whose sake I have lost all things. I consider them garbage that I may gain Christ" (*Philippians 3*, 7-8). Given that excessive alcoholic drink also generates disturbances in conscience by inciting other vices, it opposes the cultivation of spiritual life, for which reason, one must nurture self-control, according to the apostolic urge, as a *virtue of moderation* and as a *norm of Christian life*: "*And be not drunk with wine, wherein is excess; but be filled with the Spirit"* (*Ephesians* 5, 18). This apostolic urge has become the *golden rule of Christian behavior*, as it was constituted as a holy tradition originating in the words of the Lord: "*And take heed to yourselves, lest at any time your hearts be overcharged with surfeiting, and drunkenness, and cares of this life, and so that day come upon you unawares."* (*Luke* 21, 34). On these grounds, Clement of Alexandria laid down in writing the appropriate manner in which Christians should use wine, as a *drink of moderation*: *"It is proper, therefore, that boys and girls should keep as much as possible away from this medicine. For it is not right to pour into the burning season of life the hottest of all liquids—wine—adding, as it were, fire to fire. For hence wild impulses and burning lusts and fiery habits are kindled [...] I however think that then especially ought reason to be introduced to mix in the feast, to act the part of director (pedagogue) to wine-drinking[...] Drink not wine to drunkenness [...] For the gurgling occasioned by the drink rushing with violence[…], is a shameful and unseemly spectacle of intemperance […].The Scythians, the Celts, the Iberians, and the Thracians, all of them warlike races, are greatly addicted to intoxication, and think that it is an honorable, happy pursuit to engage in. But we, the people of peace, feasting for lawful enjoyment, not to wantonness, drink sober cups of friendship that our friendships may be shown in a way truly appropriate to the name."* (*Pedagogues*, Book II, Chapter II, 19,2; 20,3; 25,1; 31,1) (15).

A concrete example of application of spiritual therapy, especially with regard to alcoholism, tobacco addiction and other lusts, is apparent in the action specially organized by the Transylvanian Church, after the First World War, through the work of the *missionary priest Iosif Trifa, founder of the "Army of the Lord", as movement of spiritual awakening from the death of lusts.* In the *"Foreword*" to his books, suggestively entitled according to the model of Christian hermits "*Alcohol – a spirit of the devil", Sibiu, 1936*, the following are said: "*This book is the fruit of work and concern lasting for 14 years. Around 1921, I started a movement to combat alcoholism in our people. This is a plague others have dealt with. However, unlike others, we started the fight in the name of the Lord and with the power of the Lord. We dragged our enemy alcohol where he alone cannot win: on the battlefield of the Gospel. We laid the foundations of the Great Crusade of religion against alcohol – the 'Army of the Lord' movement – and we harvested unexpected victories. The Army of the Lord now counts more than 100,000 fighters and marches victorious across the entire country. Once again it was proven that alcoholism, tobacco addiction and other lusts cannot be fought with advice and conference, but only with the power of the Gospel […] Tens of thousands of souls were delivered by this movement from the chains of devil – alcohol"* (15). Of course, faced with this success in applying spiritual therapy to combat the harmfulness of alcoholism and

smoking, we should not display an exclusivist triumph, but in reaching a common goal we ought to make a synergy of all alternatives that have proved to be efficient. Paul the Apostle urged us to this sort of sound counsel, when he said: "*Prove all things; hold fast that which is good*" (*1 Thessalonians* 5, 21) (15).

CONCLUSIONS

The tobacco smoking has an important etiologic role in cardiovascular diseases (myocardial infarct, sudden death, peripheral vasculopathies, and stroke) and pulmonary diseases (pulmonary emphysema, chronic bronchitis, lung cancer). The most dangerous factor is tobacco smoking in young age, between 20-25 years, with important medical and social consequences.

Alcoholism is defined as the alcohol dependence, characterized by a high desire to drink, the loss of control (the impossibility to stop the alcohol ingestion, despite the desire to do it), the physical dependence, with a specific clinical symptomatology and the alteration of alcohol tolerance.

The alcohol consumption, associated with familial or professional stress can represent a risk factor for cardiovascular diseases. The active association between tobacco smoking and alcohol consumption leads to the increase of cardiovascular diseases incidence, especially in a high consumption, comparatively with reasonable and medium intake.

The chronic excessive alcohol consumption is frequently associated with other cardiovascular risk factors: tobacco smoking, excessive salt and coffee consumption, hyperglycemic and hyperlipidic diet, sedentary, unhealthy lifestyle, in a familial or professional important stress.

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NEWS

THE INTERFERON-GAMMA RELEASE ASSAYS IN DIAGNOSIS OF TUBERCULOUS MENINGITIS

Tuberculous meningitis (TBM) occurs most often in children and is the most severe form of extra pulmonary tuberculosis with a high rate of mortality in developing countries. The diagnosis of this infection is difficult because the microscopic exam has a poor sensibility and isolation of mycobacteria in culture is time consuming. The interferon-gamma (IFN-γ) release assays are new rapid methods for the diagnosis of tuberculosis. This technic detects the level of IFN-γ produced in response to the *Mycobacterium tuberculosis* antigens using an immune enzymatic method. A recent study has evaluated usefulness of interferon-gamma combined detection in peripheral blood and cerebrospinal fluid in TBM diagnosis. The study included a group of 30 suspected cases of TBM and a control group of 39 cases of meningitis with another etiology. The detection of IFN-γ in peripheral blood and cerebrospinal fluid was realized by enzyme-linked immuno spot (ELISPOT) and enzyme-linked immunosorbent assay (ELISA), respectively. The results show that detection of IFN-γ in peripheral blood by ELISPOT had 70% sensitivity and 87% specificity in the diagnosis of TBM. ELISA for detection of IFN-γ in cerebrospinal fluid proved 83% sensitivity and 85% specificity. Also, after 4 weeks of treatment with anti-tuberculosis antibiotics, ELISA had detected a significant decrease of level of IFN-γ in patients with TBM. When the 2 methods showed positive results, the specificity for TBM diagnosis reached 100%. These findings support the usefulness of IFN-γ release assays in the diagnosis of TBM (Diya Lu, Cheng Chen, Shenglei Yu, Shu Chen. Diagnosis of Tuberculous Meningitis Using a Combination of Peripheral Blood T-SPOT.TB and Cerebrospinal Fluid Interferon-c Detection Methods. *Lab Med*. 2016;47(1):6-12).

Cătălina Luncă

MITOCHONDRIAL MYOPATHY: A NEW THERAPEUTIC APPROACH

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MITOCHONDRIAL MYOPATHY: A NEW THERAPEUTIC APPROACH (Abstract): Restoration of deoxyribonucleic acid in mitochondrial myopathies may occur after a mechanical or chemical injury of striated muscle or by endurance training. Therapies with enzymes, gene therapies, or treatments with substances that stimulate mitochondrial biogenesis are used at the moment. Genesis of mitochondria may also come from myonuclei by releasing the nuclear respiratory factor-1/2 during muscle contractions. Multiplying of myonuclei depends on muscle satellite cell activation. Since the electromyostimulation increase the number of circulating stem cells that may participate in the genesis of new muscle fibers (adding to the deposit of specific stem cells of the muscle), and intermittent hypoxia stimulates the proliferation of muscle satellite cells, we propose to combine the two processes for the treatment of mitochondrial myopathies. Respective combined therapy may be useful for restoring damaged mitochondria by drug side effects. **Keywords:** ELECTROMYOSTIMULATION, INTERMITTENT HYPOXIA, MITOCHONDRIAL MYOPATHIES

The syndrome comprising mitochondrial myopathy, encephalopathy and lactic acidosis have genetic causes, being inherited through maternal line. Restoring muscle mitochondrial deoxyribonucleic acid (DNA) may be the consequence of the healing process after producing a chemical or mechanical lesion of striated muscle (1, 2). In patients with mitochondrial myopathies, endurance training based on concentric exercises stimulate the transformation of stem cells into new muscle fibers with mitochondrial DNA restoration (3). A subsequent study found that 12 weeks of endurance exercises for the lower limbs in patients with the same condition resulted in an increase in aerobic capacity, mitochondrial activity and number of muscle cells satellites, but not in a significant restoration of mitochondrial DNA (4). One case study showed how the force exercises have reduced the rate of a sporadic transfer ribonucleic acid (tRNA) point mutations at an adult with mitochondrial myopathy, but that work has not been evaluated the muscle performance nor the satellite muscle cells characteristics (3). The current therapeutic strategies for mitochondrial myopathies are based on therapies with enzymes, gene therapies, nucleoside therapies, and also on the stimulation of mitochondrial biogenesis by the administration of resvera-

trol, 5-aminoimidazo-4-carboxamide ribonucleotide, nicotinamide riboside, or even antioxidants (5). In this paper we propose a new therapeutic strategy, based on the assessment of relations between the biogenesis of mitochondria and muscle stem cells.

ELECTROMYOSTIMULATION COMBINED WITH INTERMITTENT HYPOXIA - POTENTIAL METHOD TO RESTORE MUSCULAR MITOCHONDRIA HYPOTESIS

The decrease in intramuscular ratio of adenosine triphosphate/adenosine diphosphate (ATP/ADP) and of phosphocreatine concentration activates adenosine monophosphate-activated protein kinase (AMPK), which promotes mitochondrial biogenesis by peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and nuclear transcription factors (NRFs) (6, 7). Subsequently it was established that a factor arising from myonuclei (nuclear respiratory factor-1/2 - NRF-1/2) that stimulate mitochondrial biogenesis is released due to physical exercise (8).

Given that muscular stem cells can generate myonuclei or differentiate into new muscle cells (9} it follows that in both modes have an indirect contribution to mitochondria biogenesis. In vivo activation of satellite stem cells is made by nitric oxide (NO) released during exercise (10). In support of this mechanism it comes that at a young age, muscular stem cells activation is made by muscular attrition (11). Practically after only 4 weeks of endurance exercise the number of mitochondria in muscle fibers almost doubled (12). Electromyostimulation (EMS) made with frequency between 50 and 2,000 Hz performed on human subjects for 21 days has led to an increase in number of myonuclei in muscular fibers and possibly to hyperplasia of striated muscle, process assumed to be induced by proliferation of muscle cell satellites (13). Correlating this fact with the above observations strengthen the hypothesis that electromyostimulation activates satellite stem cells and implicit mitochondrial biogenesis. Intermittent hypoxia combined with surface electromyostimulation cause circulating stem cell proliferation (14), which can add to satellite cells during the formation of new muscle cells (13). Moreover, hypoxia activates Notch signals with role in muscular satellite cell proliferation, the same action having the stimulation of Wnt/B-catenin pathway (15).

It seems that mutant mitochondrial DNA in muscle satellite cells is lost if their replication to form new muscle fibers occurs rapidly, creating new prospects for treating mitochondrial myopathies (16). Given the supposed effects of intermittent hypoxia combined with electromyostimulation on muscle stem cell activation and implicitly on mitochondrial biogenesis, this therapy could be indicated for myopathy caused by mitochondrial defects.

DISCUSSION

An indirect argument for the fact that electromyostimulation induce biogenesis of mitochondria is that six weeks of low frequency EMS (10 Hz) had the effect of increasing muscle volume and degree of pinnate, lower blood pressure and increase aerobic capacity (i.e. the number of mitochondria of muscle) (17). In addition to the combination with intermittent hypoxia, EMS can be combined with modern therapies of mitochondrial myopathies, and in

particular with the use of riboside-nicotinamide (compound believed to increase the intracellular synthesis of nicotinamide adenine dinucleotide - NAD+) (18) and therefore the efficiency of communication between the nucleus and mitochondria (19).

The combination electromyostimulation - intermittent hypoxia could be used to control quality and/or quality defects of mitochondria appeared as unwanted side effects of some drug therapies: analgesics and anti-inflammatory (aspirin, acetaminophen, naproxen), anesthetics (bupivacaine lidocaine, propofol) antiarrhythmics (amiodarone), antianginal (perhexiline, Dethylaminoethoxyhexesterol), antibiotics (tetracycline), antiepileptics (valproic acid), antidepressants (amitriptyline, amoxapine, citalopram, fluoxetine), antipsychotic agents (chlorpromazine, fluphenazine, haloperidol, risperidone, quetiapine, clozapine, olanzapine), anxiolytics (alprazolam, diazepam), barbiturates (amobarbital, aprobarbital, butabarbital, butalbital, methylphenobarbital, pentobarbital, phenobarbital, primidone, propofol, secobarbital, thiobarbital), cholesterol medications (statins - atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, bile acids - cholestyramine, clofibrate, ciprofibrate, colestipol, colesevelam), diabetes medication (metformin, troglitazone, rosiglitazone, buformin), chemotherapy (mitomycin C, profiromycin, adriamycin), anti-parkinsonian (tolcapone), lithium, disulfiram, medication anti HIV (abacavir sulphates, Hivid (ddC, zalcitabine), zidovudine, ddI, didanosine, d4T, stavudine) (20).

CONCLUSIONS

In our study, based on proven functional relationships between mitochondrial biogenesis and muscle stem cells, we propose the combination electromyostimulation-intermittent hypoxia to be used as mitochondrial myopathies treatment, possibly in association with gene therapy or administration of substances that stimulate mitochondrial biogenesis.

Electromyostimulation combined with intermittent hypoxia may potentially be used for the repair of mitochondrial damage caused by various drugs.

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EVOLUTION OF SYNCHRONOUS BILATERAL BREAST CARCINOMA IN A YOUNG PATIENT

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EVOLUTION OF SYNCHRONOUS BILATERAL BREAST CARCINOMA IN A YOUNG PATIENT (Abstract) Bilateral breast cancer incidence is appreciated to be between 0.3 to 12% and is determined either by a hereditary load associated with chromosomal instability under the effect of environmental factors, or by the evolution in a particular hormonal context which gives biological aggressiveness. We present the case of a patient, aged 38 years, clinically, imagistic and bioptic diagnosed with left axillary lymph node metastases of breast carcinoma NST invasive G3, IHC - RE = 60%, RP = 30%, HER2neu = 2 +, Ki67 = 20% , in August 2013. Patient followed neoadjuvant chemotherapy treatment during September-October 2013. In December 2013 she was clinically and imaging diagnosed with bilateral breast cancer, for which surgical intervention was done which consisted of bilateral radical Madden mastectomy with bilateral axillary lymphadenectomy. BAP - invasive carcinoma NST: left breast - pT2mN3a G2, right breast - pT3mN3a G2, IHC - RE = 90%, RP = 70% HER2neu = 2 +, Ki67 = 50%. During the period of January-March 2014, the patient followed adjuvant chemotherapy and Herceptin. Bilateral breast ultrasound assessment in April 2014 revealed: left axilla - liquid blade 29 / 6mm; right axilla - oval ganglion 9 / 5mm. Abdominal and pelvic ultrasound: empty uterine cavity, bosselated contour; at left ovary level multiple cystic formations. During the period of May-June 2014, adjuvant radiation therapy and ovarian irradiationwas administered to the patient. Subsequently hormone therapy was initiated. Following CHT / ovarian irradiation patient continues to experience intermittent uterine bleeding, which is why a total hysterectomy with bilateral ovariectomy was done, and BAP: cervical, endometrialand left ovary with tumor multifocal infiltration with histopathological aspect of invasive breast carcinoma NST. Periodic imaging evaluations do not reveal local or distant recurrence. The particularity of this case is synchronous bilateral breast cancer diagnosis in a young patient complicated in its evolution by ovarian metastases. This form of metastasis is rare in young women and occurs in advanced stages of the disease. **Keywords**: SYNCHRONOUS BILATERAL BREAST CARCINOMA, MULTIMODAL TREATMENT, OVARIAN METASTASES.

Breast cancer is the most common type of cancer among women with increased incidence due to screening, resulting in improved prognosis and survival [1, 2]. In breast neoplasm, clinical forms and biological characteristics are very diverse. Of all breast cancers, BBC (bilateral breast cancer) is 2-6% [1]. Bilateral breast cancer incidence is appreciated to be between 0.3 to 12%. In breast cancer the most common

secondary dissemination are of the bone, liver, lung and brain, but may be metastatic sites other organs such as the peritoneum, ovaries and teguments.

MATERIAL AND METHOD:

In this paper we present the case of a patient, aged 38 years, clinically, imagistic and bioptic diagnosed with left axillary lymph node metastases of mammary gland carcinoma NST invasive G3, IHC (immunohistochemistry) ER (estrogen receptor) = 60%, PR (progesterone receptor) = 30%, Her 2neu = 2 +, Ki67 = 20%, in August 2013.Neoadjuvant chemotherapy followed during September-October 2013, 3 CAF protocol cycles (cyclophosphamide 800mg, doxorubicin80mg, 5-FU 800mg).

In December 2013, a bilateral breast ultrasound detected in the right breast a nodular formation of about 1.5 - 2cm, while on the left breast endured placard and bilateral axillary infracentrimetricaladenopathy.

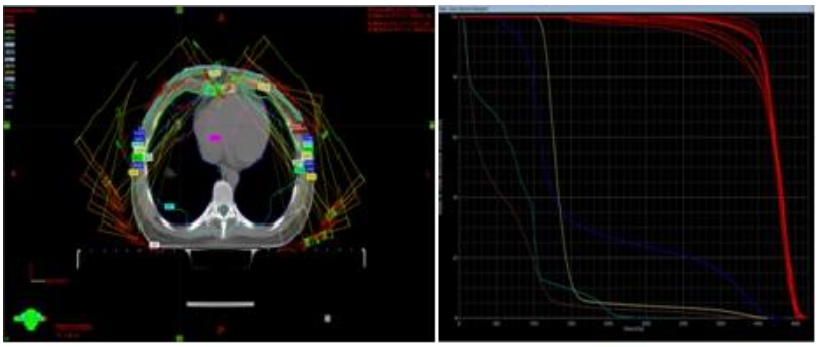
A pelvic ultrasound from December 2013 detects a heterogeneous consistence uterus, 45/36 / 72mm with two fibroid nodules 19 / 15.5mm and 12 / 15mm with 5.7mm endometrium, left ovary 40 / 24mm positioned retrouterin, normal consistency, right ovary 32 / 17mm normal echostructure and empty Douglas space.

A bilateral radical mastectomy type Madden with axillary bilateral lymphadenectomy with BAP (ID pathological) - NST invasive carcinoma: left breast - pT2mN3a G2, 11 excised lymph node metastasis; right breast - pT3mN3a G2, 15 lymph node metastasis in 19 excised IHC- RE = 90%, RP = 70%, Her 2neu = 2 +, Ki67 = 50% , FISH: HER2neu amplified.During the time period January-March 2014, the patient followed adjuvant chemotherapy: 1 CAF protocol cycle, 4 series of chemotherapy with Paclitaxel 300mg and initiated therapy with 300mg of Herceptin.

In April 2014, imaging evaluation through bilateral breast ultrasound revealed: left axillary - liquid blade 29 / 6mm; right axillary - oval ganglion 9 / 5mm. Abdominal and pelvic ultrasound: empty uterine cavity, bosselated contour; at left ovary level multiple cystic formations.

During May-June 2014 was administered to the patient adjuvant radiotherapy at ACC ClinaciX10, photons E = 10MV, DT = 42.56Gy/16fr/2.66Gy/fr/ chest wall bilaterally and axillary lymph nodes areas, bilateral supraclavicular region and right lateralcervical (Fig.1, Fig.2) and ovarian irradiationat Clinac accelerator 2100sc, with photons E = 10MV, DT = 12Gy/4fr/3Gy /fr.Subsequently adjuvant hormone therapy was initiated with Tamoxifen.

Fig. 1. Treatment plan: a- target volumes delineation; a, b – patient in simulation position



a b

Fig. 2 Treatment plan: a- radiation fields; b- dose-volume histogram evaluation

In the context of abdominal and pelvic ultrasound appearance and persistent uterine bleeding even after ovarian suppression by chemotherapy and radiation therapy, total hysterectomy with bilateral ovariectomy was performed. BAP: cervical, endometrial and left ovary with tumor multifocal infiltration with histopathological aspect of invasive breast carcinoma NST.

Thorax CT (computer tomography) in September 2015 - without signs of local or distant recurrence, persistence of microadenopathies of ~ 9 / 10mm right hilar. In upper abdominal sections a simple hepatic cyst was identified in the segment IVb of ~ 4.5mm.

Pelvis MRI (magnetic resonance imaging) in November 2015 – total hysterectomywith bilateralovariectomy without pathological contrast intake – stationery aspect, absence pelvic adenopathies.

DISCUSSION:

The risk of developing BBC is two to six times higher in women with history of unilateral breast cancer [2, 3].

BBC can be classified into subcategories, synchronous and metachronous. This classification is based on the time interval between diagnosis of primary tumor in the first breast and the second breast. Some previous studies have defined the synchronous bilateral breast cancer (SBBC) as the development of breast cancer in the time interval of 0- 12 months [4].

According to the WHO guidelines in 2012, BBC is defined as SBBC when contralateral breast carcinoma is diagnosed within three months [1].

SBBC is defined in some studies, from a epidemiological point of view, as the development of breast cancer within 12 months [5,6]. The SBBC incidence is relatively low, between 1 and 3% of all breast cancers. SBBC and MBBC have different biological characteristics, reflected in the histopathological characteristics, stage and prognosis [7].

The appearance of the second contralateral breast tumors can be synchronous (in less than 6 months after the first primary tumor in the first breast) or metachronous (after 6 months of diagnosis of the primary tumor in the first breast) [8].

In the presented patient case, the contralateral breast cancer was diagnosed 5 months after the left breast cancer diagnosis and was considered synchronous, ac-

cording to the interval of less than 6 months.

Some studies have indicated a worse prognosis and low survival rate for BBC. Conversely, other studies have claimed that there is no significant difference in the survival rate between UBC and BBC. Despite previous and ongoing studies, the prognosis and survival of patients with BBC remain uncertain [1].

Synchronicity and metachronicity are usually associated with local recurrence, hematologic and lymphatic spread to the lungs, bones and liver.

Early detection of contralateral breast cancer is of the most importance, emphasizing the importance of breast self-examination [9]. The standard for breast cancer screening is the mammography, some authors recommend breast MRI scan, which is more sensitive in comparison to mammography, but the high cost and lack of availability is limiting as a screening indication. There are no clear treatment guidelines for bilateral breast cancer. Patients are often treated with bilateral mastectomy, conservative surgery has an unclear importance [9].

In our case, multimodal treatment was administered according to the stage, histological and prognostic factors individually for each of the tumors.

Studies suggest that there is no significant difference in survival rate for patients with MBBC compared with those with SBBC. Synchronous tumors were associated with decreased survival in comparison to metachronous tumors [10].

Most often, patients with BBC are receiving radical surgery based on the idea that these tumors are biologically aggressive. However, scientific evidence to support this are split. Conservative treatment for BBC may be feasible also in the case of unilateral breast cancer [11].

BBC is more common in young aged women and most of these tumors are estrogen dependent. There is a concordance between the primary tumor and contralateral breast cancer from a histological point of view, of the receptors ER, PR and HER2. Some studies argue that there is no clear relationship between positive ER and PR and tumor bilateral localization. It seems that the BBC incidence is higher in cases with the overexpression HER-2 [2].

BC can metastasize to the bone, liver, lung and brain, but can affect other organs such as the peritoneum, ovaries and tegument.

Metastatic ovarian tumors, that are called Krukenberg tumors, occur frequently after the age of 40 years [12]. The appearance of these tumors at a young age is very rare and represents approximately 2% of all cases. The occurrence of ovarian metastases in breast cancer represents an advanced stage of the disease [13].

It seems that patients with Krukenberg tumors with BC as a starting point, unlike other tumor locations, have a good prognosis [13]. Surgery is the optimal treatment for these tumors, as it has been practiced in this case.

CONCLUSIONS:

The particularity of this case is synchronous bilateral breast cancer diagnosis in a young patient complicated in its evolution with ovarian metastases. This form of metastasize is rare in young women and occurs in the advanced stages of the disease. The prognosis for this patient is reserved considering the age under 40 years, synchronous neoplasia, nodal status (pN3a bilateral), the advanced stage (IIIC),Her

2neu positive status.

In the case of synchronous bilateral breast carcinoma there should be applied adequate multidisciplinary management and rigorous supervision of patient to improve survival and quality of life.

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DEVELOPMENT AND VALIDATION OF THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE QUANTITATIVE DETERMINATION OF ERYTHROMYCIN IN DERMO-PREPARATIONS

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DEVELOPMENT AND VALIDATION OF THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE QUANTITATIVE DETERMINATION OF ERYTHROMYCIN IN DERMO-PREPARATIONS (Abstract): **Purpose**: For the analysis of erythromycin (ER) in topical pharmaceutical forms we developed a high performance liquid chromatography dosage method (HPLC) greatly improved over the method formalized by the European Pharmacopoeia 5th ed. **Materials and methods:** The work conditions for using a chromatography column with C18 stationary phase were established as follows: optimal column temperature, 45 ° C; the mobile phase consisted of a mixture of 0.001 M disodium phosphate solution : acetonitrile in the ratio 20:80, volume of sample injected 20 µL, injection rate 1 mL/min and spectrum was recorded at λ = 200 nm. The method was validated by determining the following parameters: system precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and accuracy. **Results:** We determined linearity in the concentration range studied, 0.025 to 1.5 mg/mL. The equation of the regression line is: A = 53.7430 · C + 0.00203 with a regression coefficient of 0.9997. The method has LOQ = 0.0196 mg / mL and LOD = 0.005969. The accuracy of the method is appropriate, the ER recovery degree is in the range of 101.27 - 104.99%. **Conclusions:** The HPLC method with UV detection for the quantitative determination of ER is manageable, responsive, linear, precise and accurate and it can be used for the quantitative analysis of ER in topical pharmaceutical formulations. **Keywords:** ERYTHROMYCIN, LIQUID CHROMATOGRAPHY, UV DETECTION.

The European pharmacopoeia 5th ed. formalizes a method for the quantitative determination of erythromycin by high performance liquid chromatography (HPLC) which has two major drawbacks: the stationary phase is the styrene-divinylbenzene copolymer, and column temperature is 70 °C (1, 2). The objective of this study was to develop and validate a highly selective method for the identification and quantitative determination of erythromicin in dermo-preparations.

MATERIALS AND METHODS

Materials: Acetonitrile (ACN) and chromatographic grade disodium phosphate (Merck, Germany). ER reference substance, purity 99.70% (Sigma-Aldrich, USA). Erythromicin (ER) purity 99.85% (Zhejlang Pharmaceutical Co. Ltd. China Sanmen Hengkang); doubly distilled water, conductivity 0.01 μS/cm. *Solutions: K_2HPO_4 0,001M solution;* the *standard solution* was prepared by dissolving 50 mg ER (reference substance) in the mobile phase in a 5 mL volumetric flask, the *stock solution* is obtained by diluting a determined volume of the stock solution with the mobile phase in a volumetric flask in order to prepare solutions of concentrations of 0.025 - 1.5 mg/mL.

Methods: *Chromatographic conditions:* Thermo Fisher Scientific Ultimate 3000 Liquid Chromatograph, equipped with UV-VIS with Diode Array Detector. The method is based on the use of a chromatography column type Teknochroma Tracer Excel 120 OSDB C18, 150 mm x 4.6 mm, particle size 5 μm. Column temperature was maintained at 45 °C. The mobile phase was a mixture of 0.001 M disodium hydrogen phosphate : acetonitrile solution in the ratio 20:80. A volume of 20 μL was injected at a rate of 1 mL/min and the spectrum was registered at λ = 200 nm. *Optimization of the chromatographic conditions* - ER retention time optimization was achieved by changing the ratio of the two components of the mobile phase and of work temperature. *System accuracy (repeatability):* an ER solution with a concentration of 0.5 mg/mL was prepared. A volume of 20 μL of each solution was injected 10 times in succession in the chromatographic system and the peak areas and the retention time were registered for each solution. The mean value, standard deviation (SD) and relative standard deviation (RSD) were determined. *Linearity:* We investigated ER response linearity in a range of 5% of the reported ICH value and 300% of the concentration of interest of 0.5 mg/mL (3). In those circumstances, linearity was studied in the range of 0.025 - 1.5 mg/mL ER. We plotted the calibration curve and mathematical data were processed by linear regression ($R^2 \geq 0.990$). *The limit of detection (LOD) and the limit of quantification (LOQ):* we considered three points close to the reporting threshold and calculated the regression coefficient for the linear model in order to assess the considered trend. From the calculation of the intercept and of the residual standard deviation of the intercept, we estimated the limits of quantification and detection according to the formulas below

$$LOQ = \frac{10 \times \sigma}{b} \qquad (1)$$

$$LOD = \frac{3{,}3 \times \sigma}{b} \qquad (2)$$

Wherein σ - residual relative standard deviation, b - slope calculated for the range in question.

Accuracy was estimated by determining the recovery rate, using three solutions of three concentrations ranging between 0.1 - 1 mg/mL. Concentration was calculated using the calibration line and the values obtained were compared according to the experimental concentrations.

RESULTS AND DISCUSSION

Wavelength for ER was established on the basis of the maximum absorption in the recorded UV spectrum (fig. 1).

In order to optimize retention times, we used ACN with a ratio of up to 85% and found that introducing different ratios of ACN : 0,001M K_2HPO_4 solution produces a

different pattern of signals retrieved for ER. It was not possible to use a temperature over 55 °C, because the maximum column temperature is 60 °C. The use of a temperature of 45 °C efficasized separation, the number of theoretical trays and signal height having their highest values (tab. I, fig. 2).

ER peak area shows a RSD = 1.785 and retention time a RSD = 1.023. These values demonstrate that the system is compatible (tab. II, fig. 3).

We notice a linear variation of the peak area depending on sample concentration (fig. 4).

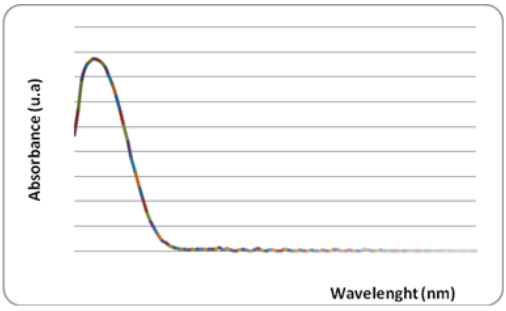

Fig. 1. UV adsorption spectrum of ER

TABLE I

Parameters of the development of the analytical method for determining ER

Mobile phase		Temperature	Retention time (min)	Theoretical trays/ signal height
% K_2HPO_4 (Sol. 0,001M)	%ACN	T(°C)	ER	ER
25	75	40	4.58	1888.699
20	80	45	4.1	3030
15	85	55	5.3	2710.372

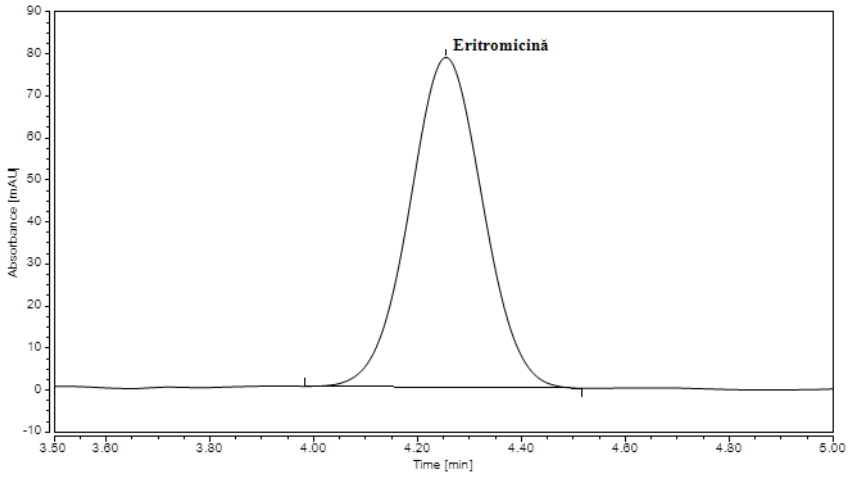

Fig. 2. ER chromatogram - λ = 200 nm

TABLE II

System repetability

Sample	Area	Individual deviations	Relative deviations	Retention time	Individual deviations	Relative deviations
SC1	26.4433	0.12781	0.48101	4,23	0.1555	2.91498
SC2	26.3015	0.26961	1.01467	4,31	0.1355	2.54006
SC3	26.4518	0.11931	0.44902	4,27	0.0725	1.35907
SC4	26.9181	0.34699	1.30589	4,29	0.0555	1.04039
SC5	27.302	0.73089	2.75069	4,33	0.0325	0.60924
SC6	26.724	0.15289	0.57539	4,36	0.1235	2.31511
SC7	25.5667	1.00441	3.78008	4,25	0.0175	0.32805
SC8	26.1853	0.38581	1.45199	4,33	0.0515	0.96541
SC9	26.7077	0.13659	0.51405	4,32	0.1015	1.90270
SC10	27.1107	0.53959	2.03073	4,24	0.1575	2.95247
Average	26.5711			4,293		
SD	0.47420			0.0439		
RSD	1.78467			1.0231		

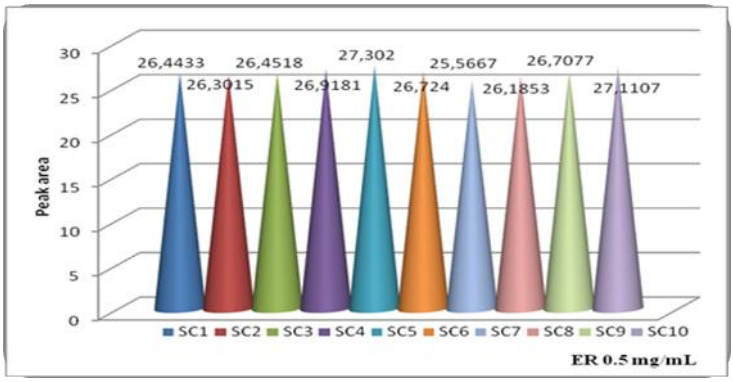

Fig. 3. Peak individual area

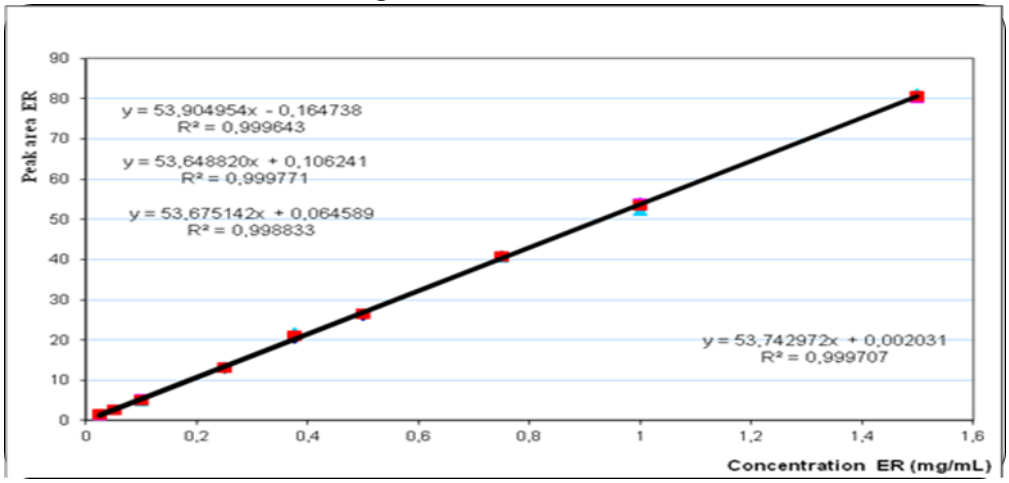

Fig. 4. Calibration line for ER determination under the chromatography conditions described

The results show that the method has LOQ = 0.0196 mg/mL and LOD = 0.005969 mg/mL (RSD = 0.0955). These values support the use of the method of ER determination at concentrations used in topical and even in internal medications. For each concentration level, we obtained a recovery rate in the range of 101-104% appropriate to validate the accuracy of the method (tab. III) (4 -8).

TABLE III

Assessment of the accuracy of the ER method of analysis

Concentration, mg/mL	Area, mAU* min.	Recovery, %	SD	RSD
0,1	6.1225	103.8846	1.081757	0.952337
	6.2357	104.9909		
	6.1561	104.5098		
0,5	28.8848	102.4849	0.989141	0.957882
	28.8391	103.3148		
	28.4968	104.0410		
1	54.8214	102.0029	0.967685	0.961843
	54.4285	101.2718		
	54.5880	101.5686		

CONCLUSIONS

In this paper we presented data obtained in the development of a reliable method for the quantitative determination of ER by HPLC with UV detection. The method was validated by the determination of several specific validation parameters. Thus, linearity was measured in the studied concentration range of 0.025 - 1.5 mg / mL. The regression line equation is:

$A = 53.7430 \cdot C + 0.00203$ with a regression coefficient of 0.9997. The method has LOQ = 0.0196 mg/mL and LOD = 0.005969. The accuracy of the method is appropriate and the recovery rate of ER is in the range of 101.27 - 104.99%. The new HPLC method with UV detection we developed and validated for the quantitative determination of ER is manageable, responsive, linear, precise and accurate and can be used for the quantitative analysis of ER in topical formulations.

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NEWS

NOI SUBSTANȚE MEDICAMENTOASE CU ACȚIUNE ANTIDIABETICĂ - EMPAGLIFLOZIN

Empagliflozin face parte din clasa medicamentelor antidiabetice noi, care determină glicozurie prin blocarea recaptării glucozei în tubul proximal. Pierderea a 50-100 g de glucoză / 24 de ore prin urină determină reducerea glicemiei *à jeun*, dar mai ales a glicemiei postprandiale, un consum crescut de energie cu 200-400 kcal / zi şi scăderea tensiunii arteriale. Eficacitatea tratamentului nu scade în timp, deoarece nu depinde de propria producție de insulină. S-au efectuat studii care evaluează siguranța tratamentului cu empagliflozin care va apărea în curând în portofoliul de antidiabetice în Republica Cehă. Aceste studii au demonstrat eficacitate mare, siguranță şi tolerabilitate bună pentru empagliflozin. Totodată, a fost descrisă o incidență mai mare a infecțiilor genitale non-severe, în special la femei. Medicamentul nu produce hipoglicemie. În combinație cu sulfoniluree, hipoglicemia poate să apară. Empagliflozin nu provoacă deshidratare semnificativă clinic sau hipotensiune arterială la pacienții cu vârsta de aproximativ 60 de ani, dar se recomandă precauție în tratamentul cu empagliflozin la pacienții vârstnici. Empagliflozinul prezintă avantajul interacțiunilor clinic nesemnificative cu alte medicamente şi posologia simplă de 1 comprimat / zi, administrat oral. În concluzie, empagliflozin este agent antidiabetic oral eficient cu un potențial de aplicare pe scară largă în toate etapele de diabet zaharat de tip 2, în monoterapie sau în combinație cu alte medicamente. Tratamentul este asociat cu pierderea in greutate şi cu scăderea tensiunii arteriale. Apariția efectelor secundare este rară, cu excepția incidenței crescute a infecțiilor genitale în special la femei şi risc crescut de hipoglicemie atunci când empagliflozin este combinat cu antidiabetice de tip sulfoniluree. Rusavy Z. New SGL T2 inhibitor empagliflozin: modern and seif treatment of diabetes. *Vnitr Lek* 2014; 60(11): 929-930

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INFLAMMATORY BOWEL DISEASES AND AIR POLLUTION

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INFLAMMATORY BOWEL DISEASES AND AIR POLLUTION (Abstract). Air pollution is responsible for the increase in mortality, morbidity and a high number of hospitalizations for respiratory and cardio-vascular diseases. Recently, several studies have shown that air pollution can also affect the digestive system. The major impact was observed on inflammatory bowel diseases (Crohn's disease and ulcerative colitis).Air pollutants that have the great impact on the digestive system health are: gases, especially ozone, particulate matter and heavy metals like lead, cadmium. **Material and Methods**: Forty-six articles and one book were evaluated for the purpose of this study. The articles are indexed in international databases, including PubMed, PMC, Scholar Google, and MEDLINE. **Results**: We present here the current understanding of the mechanisms by which air pollution can affect intestinal tissues. There are three mechanisms that can be taken into consideration: the direct effect of pollutants on epithelial cells; the alteration of the immune response and the influence of air pollution on the intestinal microbiota. The gene – environment relationship was also discussed in the case of the inflammatory bowel diseases. **Conclusions**: Up to this moment there are several indicators which show that pollution can have a negative impact on the intestinal tract, but the evidence is limited. The current studies have many limitations and confusion factors that must be measured and corrected so that the specific factors can be correctly evaluated for intestinal diseases. Studies on animals must verify the biologic plausibility of obtained data. **Keywords:** INFLAMMATORY BOWEL DISEASES, AIR POLLUTION, CYTOKINES, MUCOSAL INFLAMMATION, GUT MICROBIOTA, GENE-ENVIRONMENT RELATION.

The concept of air pollution refers to the presence of substances in air that can have a negative effect on human health.

The most famous incident related to the effect of acute air pollution on human health is the toxic fog accident in London in 1952. There were two previous episodes where air pollutants have determined increased mortality and morbidity: in 1928, in the Meuse Valley and in 1948 in Donora. The sources that have released pollutants in these areas were mostly stationary sources represented by industrial facilities. It was the meteorological conditions that have led to the increase in mortality and morbidity due to the phenomena of thermic inversion. Several studies have proved that long-term exposure to pollutants can reduce average lifespan by a few years and increase mortality. Thus, the APHEIS and APHEA study (1, 2), which investigated the impact of air pollution with particles and other air pollutants in Europe has demonstrated that, in Bucharest, the age standardized mortality rates (per 100,000 people) will be equal to 1127 if the level of air pollution with PM_{10} (particulate matter)

is in a range 14 –73 μg/m^3. If the air pollution level will drop by 5 μg/m^3, between 3,300 to 7,700 early deaths annually will be prevented, 500 to 1000 of which are associated with short term exposure (1). After the acute episodes of pollution mentioned above, legislative measures have been adopted, which have considerably lowered air pollution levels. A study conducted in U.S.A. assessed the changes registered in the life expectancy in relation with the levels of air pollution with fine particulate in the period between 1980 and 1990. The result of the study showed that, when the level of pollution with fine particle was lower than 10 μg/m ^{3}, there was an increase in the mean of life expectancy of 0.61±0.20 year (p=0.004) (3).

OBJECTIVE

This article tries to find connections between air pollution and the potential effects on human health especially between air pollution and intestinal diseases, especially inflammatory bowel diseases.

MATERIAL AND METHODS

Forty-seven articles and one book were evaluated. The articles are indexed in international databases, including PubMed, PMC, Scholar Google, and MEDLINE.

Effects of air pollution on human health

Studies have shown an increase in morbidity, number of hospitalizations and mortality during and immediately after an episode of acute air pollution.

a) Increased levels of air pollution can have impact on respiratory system with decreased ventilatory function in healthy individuals, modified ventilatory performance in exposed children, worsening of respiratory symptoms in pulmonary diseases like chronic obstructive pulmonary disease and acute attacks of asthma.

b) Also, recent studies have proven a consistent relation between air pollution with particles and cardio-vascular pathology. Exposure to an increased level of particles in ambient air can determine an increased rate of mortality by rhythm abnormalities and coagulation modifications, increasing the chance of myocardial infarction and stroke (4).

c) Another type of pathology that can be induced by air pollutants is significant intestinal pathology. There are very few studies in this regard.

The influence of air pollutants on intestines

In case of air pollutants exposure, the substances that enter the organism by inhalation are mostly eliminated. The main elimination path of particles and gases dissolved in mucus is done using the mucociliary system. By using the mucocilary escalator, the pollutants are taken towards the buccal cavity, where they are coughed or swallowed. By swallowing, the air pollutants can rapidly reach the intestines. Gaseous pollutants and particles can induce systemic inflammation that can have significant impact on intestines.

Pollutants that can induce intestinal pathology

Regarding the evaluation of the impact of environmental pollutants on digestive diseases, a relatively small number of studies have been done.

Pollutants that can induce intestinal pathology include:

- Gaseous pollutants like ozone, sulfur dioxide, nitrogen oxides and nitrogen diox-

ide in particular, carbon monoxide and carbon dioxide,

- Volatile organic substances like benzene,
- Particles. These represent a mix of biological elements like bacteria, fungi, pollen as well as particles with a high absorbent capacity for different gases like polycyclic aromatic hydrocarbons like benzo(a)pyrene. The particle composition can also have nitrates, sulfates, organic carbon or metals like lead, cadmium, etc. The particle structure depends on the source that releases the pollutants in the air.

The pollutants sources can be either fixed or mobile. The pollutants can result from the burning of fossil fuels in industry, for thermal energy, from the combustion of biomass or from traffic.

Of all air pollutants, it appears that ozone and particles have the greatest impact on intestinal pathology. It was determined that exposure to ozone can induce inflammation in both humans and animals, which will cause an increased cellular permeability and the destruction of mucosal barrier integrity (5). Important effects have, also, been identified for particles, as presented earlier. Regarding the exposure to nitrogen oxides, important intestinal effects have been identified, both in humans and animals, in case of exposure to very high concentrations.

The relation between air pollution, smoking and intestinal inflammatory diseases

Air pollution with particulates matter can appear due to several type of sources like burning fossil fuel for heating and smoking, especially in indoor spaces. Smoking can influence the group of intestinal bowel diseases. Smoking is associated with Crohn's disease, having a negative impact on the evolution of the disease (6, 7). Ulcerative colitis is more frequent in nonsmokers or previous smokers and the evolution of the disease is positively influenced by smoking (8).

Mechanisms by which air pollution can affect intestinal tissues

Multiple mechanisms can be taken into consideration, which could explain the impact of air pollution on bowels. Because there are few elements that evaluate the effect of air pollution on bowels, the proposed mechanisms for analysis are extrapolated starting from research on other organs and systems like the respiratory and cardiovascular systems.

a. The direct effect of air pollutants on epithelial cells

The direct effect of pollutants on epithelial cells could be considered. There is aggression, especially of particulate matter, against epithelial cells of the respiratory system. Modifications were found in exposed individuals that have pre-existing lesions as well as healthy individuals. These modifications have been seen on animal experiments as well as *in vitro* studies (9-13). A study done by Manzo *et al.* has demonstrated that in case of exposure of healthy cells, from the tracheal pathways or at the alveolar levels to diesel type air particles, have not registered a notable effect. However, if the pulmonary epithelial cells are already in a state of inflammation through the action of exogenous cytokines, like TNF-α (tumor necrosis factor α), IL-1β (interleukin 1β), and IFN-γ (interferon γ), due to previous exposure to diesel particles, they become incapable of responding to the increased level of oxidative stress, which will cause injury and dysfunc-

tion of the epithelial barrier and increased permeability (14). The action of environmental pollutants like diesel particles will, thus, have a greater effect on individuals with genetic predispositions and other conditions that induce inflammatory afflictions like Inflamatory Bowel Diseases (IBDs) (14).

Another study has evaluated the effect of administering a single oral dose of diesel particles on rats and has observed DNA injuries in colonic epithelial cells (15).

The effect on epithelial cells caused by exposure to air pollutants is the same, regardless of the exposed zone, respiratory or digestive. Epithelial cells generate radical oxygen species (ROS) as a response to exposure to particulate matter from air, inducing epithelial barrier disruption and increasing gut permeability (16).

b.The alteration of the immune response

In both Crohn's disease and ulcerative colitis, there is a modified and over reactive immune response (17). Air pollution has an immunomodulation effect, of inducing systemic inflammation, which causes an increased risk for autoimmune diseases and related diseases like type 1 diabetes, neuro inflammation or increased cardiovascular risk by systemic inflammation. This means it has a significant effect on inflammatory bowel diseases as well (18-21). Many of these effects are associated with urban development and industrialization, which would be favorable elements in the hygiene hypothesis of inflammatory bowel disease (22). This hypothesis suggests that persons with a decreased exposure to infections developed in childhood and at more precarious hygienic conditions in adulthood can have a deficit of „friendly" microorganisms or elements that stimulate the development of T regulatory cells, or they do not develop sufficient immune mechanisms due to the fact that they did not encounter harmful microorganisms. Individuals in this category have a higher incidence in chronic immune diseases, including inflammatory bowel diseases.

Zanobetti *et al.* (2009) and Ritz (2010) have shown that, at least in part, autoimmune diseases can be explained by the effect of air pollutants (23, 24).

The mechanisms used could be systemic oxidative stress, the stimulation of hematogenic medulla, the increase in the cytokine levels in blood and the increase in number of white blood cells The effect demonstrated *in vitro* (9, 25-33) of cellular immune activation could support this theory (34, 35). For example, IL-6 and TNF-α produced by peritoneal macrophages in mice are induced by the exposure to particles (both fine particles and large diameter particles). TLR2 (toll-like receptor 2) and TLT4 appear to mediate differently the response to air pollution. However, the exposure of alveolar macrophages in humans to particles in the urban air and diesel type particles induce a suppression of TNF-α and secretion of IL-6 and IL-8 (36).

Another study, performed in Seoul, has shown a dose dependence of the pro-inflamatory level of cytokines (IL-1, TNF-α, and IL-6), the type of cytokines Th0 (IL-2) and the type of cytokine Th1 (IL-12 and IFN-γ) in exposure to particles with the diameter of 2.5 μm. The activity of the oxidative stress related genes, like HSP 1a (heat shock protein 1a), HSP 8 (heat shock protein 8) and SOD (superoxide dismutase), and tissular lesions related genes, like MMP 15-19 (matrix metaloproteinases 15 and 19) and SLPI (secretory leukocyte peptidase inhibitor) were also influenced, dependent on exposure time

(37).

PM 2.5 can also induce an increase in the distribution of T cells in lymphocytes and a decrease of the CD4+/CD8+ ratio. Based on these result the authors of the Soul study suggest that the pollution of air with PM 2.5 can induce a Th1 type inflammatory response with oxidative stress, which can determine adverse effects on human health.

c. The effect on intestinal microflora

Another mechanism that can influence air pollutant action on intestinal mucosa is the effect on intestinal flora. The intestinal flora has a key role in inflammatory bowel diseases (38). It was proven in animal experiments that inflammatory bowel diseases do not appear if the mice are held in a sterile environment. The intestinal flora of patients with inflammatory bowel diseases is different than that of healthy subjects (39). The genetic loci associated with IBDs suggest the important role of the relation between the intestinal immune system and intestinal flora in the pathogenesis of inflammatory bowel diseases (40).

The structure of the intestinal flora can be affected by pollutants, especially heavy metals like lead and cadmium (39-42), which reduce taxonomic diversity.

Intestinal bowel diseases:
gene – environment relationship

The discovery of the genetic implication in inflammatory bowel diseases etiopathogenesis represented an important element. Still, only 20% of the cases can be thus explained. It is suggested that environmental factors contribute more than the genetic component in IBD pathogenesis (43). The importance of environmental factors is proven especially in children (44).

Up to this moment there are no studies to directly link and describe how the genetic and environmental factors act together in IBDs.

A study was performed on 310 patients with Crohn identified NOD2 gene as the most significant gene associated with Crohn's disease in smokers (45).

CONCLUSIONS

Up to this moment there are several indicators which show that pollution can have a negative impact on the intestinal tract. However, up to this moment the evidence is limited. The current studies have many limitations that must be eliminated and many confusion factors that must be measured and corrected so that the specific factors can be correctly evaluated for intestinal diseases.

This can include identifying and evaluating the interaction between genetic factors and environmental factors.

A careful interpretation must be done of the data that evaluates the relation between air pollution and inflammatory intestinal diseases and it must represent an incentive towards the continuation of research in this direction.

Studies on animals must verify the biologic plausibility of obtained data.

Air pollution with particles as well as gas and heavy metals can represent an element which cannot be ignored in determining the etiopathogenesis and evolution of inflammatory bowel diseases (46, 47).

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NEWS

BIOFILM-INHIBITORY COMPOUNDS AGAINST *PSEUDOMONAS AERUGINOSA* FROM BURDOCK LEAF

In a study by Lou Z *et al,* basic anti-biofilm profile data for the compounds in burdock leaf were analyzed and a convenient method for fast screening of anti-biofilm compounds from natural plants was provided. According to this study, 34% ethanol elution fraction of burdock leaf could completely inhibit biofilm formation of *Pseudomonas aeruginosa* at 1 mg·mL(-1). Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) was used to determine the chemical composition of burdock leaf fraction and to obtain the metabolic fingerprints of burdock leaf fractions before and after inhibiting *P. aeruginosa* biofilm. The analysis identified 11 active compounds: chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, ursolic acid, rutin, cynarin, luteolin, crocin, benzoic acid, and Tenacissoside I. Partial least squares discriminant analysis (PLS-DA) of the metabolic fingerprints showed that, among 81 compounds that were screened as potential anti-biofilm ingredients, rutin, ursolic acid, caffeic acid, p-coumaric acid and quercetin are the main anti-biofilm compounds in burdock leaf (Lou Z, Tang Y, Song X, Wang H. Metabolomics-Based Screening of Biofilm-Inhibitory Compounds against Pseudomonas aeruginosa from Burdock Leaf. Molecules. 2015;20(9):16266-77. doi: 10.3390/molecules200916266).

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DETECTION OF HUMAN *PAPILLOMAVIRUS* IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS: A LITERATURE REVIEW

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DETECTION OF HUMAN PAPILLOMA VIRUS IN HEAD AND NECK CANCERS: A LITERATURE REVIEW (Abstract): Human papilloma viruses (HPV) are the most common sexually transmitted viruses. There is mounting evidence that incriminates HPV as a risk factor for malignant transformation of oropharyngeal epithelium. In 2011 the International Research Agency of Cancer and National Cancer Institute (USA) declared HPV-16 as an independent risk factor for oropharyngeal squamous cell carcinoma (OPSCC). Leaders in the field of HPV research admit that this subtype of head and neck cancer is a sexually transmitted entity and its global incidence is on the rise. In the 1980s, clinicians observed a new group of patients with head and neck squamous cell carcinoma (HNSCC) independent of tobacco smoking or alcohol use. The new HNSCC patient is a middle-aged man, non-smoker, non-drinker with higher social status and the suspected risk factors for HNSCC being related to sexual practices (oral sex, multiple sexual partners, unprotected sex and drug use). Routine HPV testing of HNSCC patients is seriously considered as HPV-positive oropharyngeal cancers comprise a distinct molecular, clinical and pathologic entity that has a markedly better prognosis than HPV-negative oropharyngeal cancers. The current treatment protocols for OPSCC include radiation, chemo-therapy and surgery alone or in combination, involving high toxicity levels. Future therapeutic concepts for OPSCC may be personalized in relation to HPV-status to avoid unnecessary toxicity. The current review summarizes the contemporary trends in the diagnosis of HPV-related head and neck cancers, presenting the advantages and disadvantages of the main methods. **Keywords:** HEAD AND NECK CANCER, HPV TESTING, BIOMARKERS

Papillomaviridae (reclassified in 2004) are a family of small double-stranded DNA viruses that infect warm-blooded vertebrates (1). Papillomaviruses infect different species of mammals and also distantly related hosts such as marsupials and birds (2). Traditionally HPVs has been classified in "types" based on phylogenetic analyses of the L1 gene, which is the most conserved gene in PVs. Based on their tropism the HPVs have also been grouped into mucosal or cutaneous types. Mucosal HPV types designated as "high-risk" (HR) types, isolated preferentially from malignant tumors are: 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70. The "low-risk" (LR) types (HPV 6, 11, 42, 43 and 44) are founded in benign genital warts and other non-malignant lesions (1, 2).

The causative role of HPV in cervical

cancer is well established. Approximately 70% of cervical cancers worldwide occur due to HR-HPV types 16/18 infection (3). The exact molecular mechanism responsible for malignant transformation is still unknown. The HPV oncogenic proteins E6 and E7 are incriminated in this process. Persistent infection with HR-HPV is a necessary but not sufficient cause of this cancer, which develops over a long period through precursor lesions. It may pass as much as 20 years between the initial HPV infection and clinical occurrence of cancer (4). In women with normal cervical cytology findings the estimated global HPV prevalence is 11.7% and the most common types are HPV-16 (3.2%), HPV-18 (1.4%), HPV-52 (0.9%), HPV-31 (0.8%) and HPV-58 (0.7%) (5).

Romania has the highest incidence of cancer in Europe, 23.9/100,000. There are 4,343 new cervical cancer cases each year and 1,909 cervical cancer deaths. HPV prevalence among women with or without cervical lesions in the Northeastern region of Romania is 37.4% and the most frequent types are: 16 (10.5%), 53 (5.44%), 51 (5.05%), 52 (4.08%), 18 (2.91%) and 31 (2.73%) (6, 7).

HPV INVOLVEMENT IN HEAD AND NECK CANCER

The involvement of HPV in head and neck cancers was suggested for the first time in 1983 by *Syrjanenet al.* (8). The numerous studies dedicated to test this theory supported HPV involvement on the basis of: morphological similarities between genital and oropharyngeal epithelia, broad epithelial tropism of HPV, detection of HR-HPV genotypes in samples of oropharyngeal squamous cell carcinoma (OPSCC), and the HPV-induced immortalization of human oral keratinocytes *in vitro*. In 2011 the International Research Agency of Cancer and National Cancer Institute (USA) declared HPV-16 as an independent risk factor for oropharyngeal squamous cell carcinoma (OPSCC) (9). Experts in the field of HPV research admit that this subset of head and neck cancers is a sexually transmitted entity with an increasing global incidence. A growing body of evidence is supporting that HPV is responsible for approximately 20% of oral cancers and 60-80% of them are squamous cell carcinomas (10, 11).

In the 1980s, clinicians observed a new group of patients with HNSCC independent of tobacco smoking or alcohol use (12). The new HNSCC patient was a middle-age man, non-smoker, non-drinker with higher social status and the suspected risk factors were related to sexual practices (oral sex, multiple sexual partners, unprotected sex and drug use) (13-16).

HNSCC is the sixth most common malignant tumor worldwide with approximately 650,000 new cases diagnosed each year. While the reported incidence rate for all head and neck carcinomas is decreasing and the rates of coincident identification of oncogenic HPV as a new risk factor for OPSCC is increasing, it is obvious that a virus-related cancer epidemic is discussed (16-18). The involvement of oncogenic HPV types was clearly demonstrated only in cancers arising from the oropharynx (especially palatine and lingual tonsils). Romania is lacking HPV-related OPSCC studies. HR-HPV can be found in the oropharynx of healthy subjects and it is very important to differentiate between high-risk and low-risk HPV-types. Indeed, oral infection with HR-HPV types increases the odds ratio for developing oropharyngeal

cancer. In a recent systemic review of the literature the pooled prevalence of oral HPV in healthy individuals was found to be 4.5% with 3.5% of them having oncogenic HPV types (19). However, in the tonsils of healthy people the incidence of oncogenic HPV was only 1% (20).

Very often these tumors are diagnosed at an advanced stage, with an enormous impact on 5-year survival rate (80% for early-stage disease and only 20% for late-stage disease). In contrast to skin cancer screening where visual examination has high sensitivity (93%) and specificity (98%) the detection of oral cancers and premalignant lesions by conventional visual and tactile examination is problematic due to the complex anatomy of the oropharynx. Therefore, adjunctive screening tests are desperately needed (21).

MAJOR DIFFERENCES BETWEEN HNSCC BY HPV STATUS

HPV-positive oropharyngeal cancers comprise a distinct molecular, clinical and pathologic disease entity that has a markedly improved prognosis compared to HPV-negative oropharyngeal cancers**.** In HPV-negative OPSCC genetic alterations lead to mutations in p53 and retinoblastoma (pRb) tumor suppressor genes and also loss of heterozygosity. In contrast, HPV-positive cancers harbor the wild type of p53 gene, p16 is upregulated and pRb is down regulated (22, 23). Many researchers are looking for a predictive transcriptomic signature of oropharyngeal cancer according to HPV status in order to develop new reliable biomarkers for screening and early detection of OPSCC (24). Also, HPV-positive OPSCCs are strongly infiltrated by lymphocytes, are predominantly non-keratinizing squamous carcinomas and are described as poorly differentiated or basaloid carcinomas. The contrary facts are available for HPV-negative OPSCC (25). Also, the clinical response to therapy and overall survival are improved (a 28-58% reduction in the risk of death) for HPV-positive compared to HPV-negative cancers (26).

DIAGNOSIS OF HPV-POSITIVE HNSCC

At the moment, there is no consensus on the optimal algorithm to identify HPV-positive HNSCCs (Table I). Common practices include the detection of p16 protein through immunohistochemistry as well as HPV genetic material. Accurate HPV detection and typing is based on nucleic probe technology as HPVs cannot be cultured (27). At present HPV detection tests are included in three large categories: nucleic acid-hybridization, signal amplification and nucleic-acids amplification assays. In this review we will not address HPV serology tests (inconsistent seroconversion rates) and immunohistochemical detection of E6 and E7proteins (expressed at very different levels) since these methods are lacking sensitivity and therefore their routine implementation is of limited value (28, 29).

Nucleic acid-hybridization assays include techniques such as Southern blotting, *in situ* hybridization (ISH) and dot-blot hybridization. The advantage of ISH is its high specificity, the detection of HPV DNA is site-specific and has the ability to differentiate between episomal and integrated DNA. ISH is time-consuming, requires large amount of purified DNA and the sensitivity is low (83%) with an estimated 13-14% false-negative rate. All these makes ISH improper for routine clinical use (29).

TABLE I

Sensitivity and specificity of main diagnostic methods used in HPV infection detection, related with OPSCC

Authors Year (reference)	Sample size	Detection method	Sensitivity	Specificity
Schache et all (41) 2013	79 FFPE	RNA Scope (RNA ISH) and DNA qRT-PCR	97% 91%	93% 87%
Chaudhary et all (30) 2010	222 OSCC	Hybrid capture 2 Ts-PCR E6 primers	DR=32,4% DR=31,4%	-
Thavaraj et all (42) 2011	142 tonsil SCC	P16+HPV DNA ISH P16+PCR GP5+/6+ P16+ISH+PCR	Dr=53% Dr=61% Dr=63%	-
Ahn et all (44) 2014	81OPSCC saliva + plasma	Q-RT-PCR saliva Q-RT-PCR plasma	S+P=76 S or	S+P=100% +p.v=100%

Abbreviations: FFPE=formalin fixed paraffin embedded, ISH=in situ hybridization, qPCR= quantitative polymerase chain reaction, Ts=type-specific, OSCC=oral squamous cell carcinoma, SCC=squamous cell carcinoma, S=saliva and P=plasma, Dr=detection rate

Signal amplification. At the moment two signal amplification assays are commercially available and are approved by the Food and Drug Administration (FDA). These kits are: *Digene HPV test* using *Hybrid Capture 2* (hc2) technology and the *Cervista HR HPV* assay (29).

Hc2 detects 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 low-risk types (6, 11, 42, 43 and 44) based on the hybridization of the target HPV-DNA to labeled RNA probes in solution. This assay was not designed for genotyping single HPV type and distinguishes only between high-risk and low-risk groups. Also this test uses full-genome RNA probes complementary to HPV DNA, specific antibodies, signal amplification and chemiluminescent detection (28, 29). *Chaudhary et al*. found a slight difference between the positivity rates of HR-HPV infection detected by hc2 and PCR method in OSCC, with a better sensitivity attributed to hc2 assay (30).

The *Cervista HPV signal amplification* assay (FDA approval in 2009) detects 14 HR-HPV types as a group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) in addition to simultaneous HPV-16/18 detection. Also, this method demonstrates a lower false-positive rate, high sensitivity and specificity to genotyping HPV-16/18 (28, 29, 31).

PCR-based techniques can be *consensus* or type specific detection methods. Although it is very important to discriminate between HR and LR HPV types, genotyping of a single HPV type has a greater significance not only for research purposes but also for clinical findings since in persistent infections with HPV-16/18 the risk of a precancerous lesion is estimated at 10-15% and 3% for all other HPV strains combined (29, 32).

At the moment there are different primer sets for HPV detection in consideration to HPV biology. The most commonly used primer pair sets are MY09/MY11, GP5/GP6 and SPF10 LiPA sets which due to the high variability of HPVs (189 types)

target the L1 open reading frame (ORF) which is highly conserved among HPV strains. Upon viral integration into the host genome, the L1 ORF of the HPV genome may be deleted, consequently resulting in decreased sensitivity of HPV detection. PCR strategies targeting sequences retained upon viral host genome integration can offer more sensitivity for HPV detection. Such sequences are E6 and E7 HPV oncogenes. This was proved by a study on HPV detection in anal carcinoma where the HPV detection rate with L1 consensus primers was found to be 16%, whereas with strain-specific E6 primers the HPV positivity was 46% (28, 33-35). Also, in the integration process of HPV genome into the host genome, very often occur breaking points at E1 and/or E2 ORF (regulators of E6 and E7 protein expression) resulting in gene disruptions. When E2 gene is disrupted, the designed primers targeting E2 gene and aiming the entire E2 amplification will fail. Therefore primer sets targeting shorter regions will enhance the sensitivity of the method (35-37).

The drawbacks of conventional PCR methods are: low specificity, not site- specific like *in situ* hybridization assay (the detected HPV-DNA can originate from both malignant cells and surrounding non-transformed tissue), does not differentiate episomal from integrated HPV-DNA and also implies technical difficulties in performing. At the moment, there are no standardized PCR-based techniques leading to discordances in analytical sensitivities and specificities between laboratories. Furthermore, the genetic variation of HPV types and other unknown yet genomic features of HPVs are responsible for false-negative results (27, 29, 35, 38).

Equally important is the read-out system used to detect and visualize the PCR products. Novel PCR-based methods and commercially available kits improved their performance *via* more powerful read-out systems and implying hybridization technology. Most read-systems use hybridization techniques of PCR products to labeled oligonucleotide probes. The reverse line hybridization technique uses a solid support with a biotin-labeled PCR product hybridized to an array of immobilized oligonucleotide probes. Ultimately the visualization is performed through colorimetric or fluorescent staining procedures. Another read-out system incriminates an enzyme immunoassay method which cannot detect single genotype. This technique imply capturing of biotinylated PCR products to streptavidin-coated microplate wells and then hybridization with labeled oligonucleotide probes specific for the HPV types of interest (28, 30, 35, 38).

The real-time PCR (RT-PCR) has more analytical power this is higher sensitivity, and also provides a quantitative analysis of viral load. The quantification of targeted DNA is done *via* colorimetric markers during PCR amplification. High analytical sensitivity (a few copies of HPV DNA) does not translate into more clinical relevance because of presence of latent, non-productive and transient (not uncommon) HPV infections. On the other hand, the quantification of viral load and adjusting the cut-off viral load results in increased specificity and clinical relevance of the results of RT-PCR investigation. For a cut-off viral load >0.5 copies per cell, Smeets *et al* estimated the sensitivity at 92% and the specificity at 97%. Therefore false-negatives still exist (28, 35, 37, 39).

Detection of HPV mRNA in tumor samples is currently considered “the gold

standard". The clinical significance of HPV E6/E7 mRNA detection is greater than that of HPV-DNA detection since this provides evidence of active gene transcription, the HPV infection is a productive one and the virus is an etiological factor. Several authors reported that 14-50% of oropharyngeal cancers found to be HPV-DNA-positive mainly through PCR-based techniques were negative for E6/E7 mRNA expression, meaning that the false-positive rate was 14-50% and the specificity 50-86% (35, 40). "Why is specificity important?" Even though the HPV-status does not influence the treatment plan for OPSCC, the impact of a HPV false-positive test of a cervical specimen in female patients is much bigger often leading to over treatment and psychological discomfort. A drawback of HPV E6/E7 mRNA detection is that it is not site specific, but performing RNA *in situ* hybridization proves that HPV E6/E7 mRNA originates from HPV-induced malignant cells. These techniques are time consuming, expensive, and too laborious for use in routine screening (35, 41).

CONCLUSIONS

Even though the relation between HPV infection and HNSCC was formulated in 1983, over the years experts in HPV field have not yet reached a clear conclusion regarding the best techniques for the detection of premalignant lesions in order to implement effective methods of treatment. RT-PCR, detection of HPV mRNA and *in situ* hybridization of mRNA seems to be the most useful methods.

Future large-scale anti-HPV vaccination program worldwide will be able to decrease the incidence of HR HPV infection and the risk of OPSCC and HNSCC as well.

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CONFIDENTIALITY PRINCIPLES IN PSYCHIATRY

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CONFIDENTIALITY PRINCIPLES IN PSYCHIATRY (Abstract): Confidentiality stands out in psychiatry through its multiple connotations as an intrinsic necessity in the ethics of professional relationships. Thus it represents an important characteristic of this profession and at the same time a stringent request which, through its specificity, implies a direct contact with persons in need for help. Despite being inserted in professional codes and legislative systems, confidentiality in psychiatry is far from being considered a clarified matter and does not stand aside from ethical controversy. Keeping the professional secret is often a hard task due to the pressure of the law or of other professional groups who can bring multiple justifications, including that of action for the benefit of society. The therapist is often submitted to a tension caused on the one hand by the promise of keeping the professional secret and on the other hand by multiple requests of breaking the confidentiality. So the problem of confidentiality in Psychiatry deserves special attention because in this profession, more than in other branches of medicine, the gain of the patient's trust is essential in the psychotherapeutic relationship. **Keywords:** ETHICAL CONTROVERSY, PROFESSIONAL GROUPS, GAIN OF TRUST, PSYCHOTHERAPEUTICAL RELATIONSHIP

The idea of protecting the confidential character of communication between physician and patient was first expressed in the Hippocratic Oath, while the guarantee of confidentiality through law appeared in the English legislation as early as the 17th century. Moreover, the ethical principle of confidentiality was at the core of Percival's Code of Medical Ethics, representing the starting point for both the American Medical Society and the British Medical Society. From a legislative viewpoint there are, however, significant differences regarding the limitation of medical confidentiality within the legislative systems of different countries. Consequently, despite being inserted in professional codes and legislative systems, confidentiality could hardly be regarded as a clarified matter and often makes the subject of controversy. Keeping the professional secret is often a difficult challenge due to the pressure coming from either the legislative system or from certain professional groups that could provide a whole range of justifications, including that of action for the benefit of society (1, 2). The psychiatrist is thus challenged by an ethical tension caused, on the one hand by his engagement to keep the professional secret and on the other hand by the multiple requests of breach of confidentiality that she/he constantly receives.

THE DOCTOR - PATIENT RELATIONSHIP

Confidentiality essentially defines, beyond any legislative aspects, the professional relationship between doctor and patient. More specifically than in any other branch of medicine, confidentiality is essential in psychiatry firstly because it helps the patient engages in the psychotherapeutic relationship. Only that patient who fully trusts his/her therapist will reveal his/her most intimate thoughts, intentions, doubts or states of mind. In the Hippocratic times, although doctors had very few therapeutic means, they were able to offer the patients trust, hope and inspiration that helped them recover. These less tangible approaches can be hardly found in the over-technologies medical practice of the present day. The therapist's role is limited, according to the reductionist model strictly based on biological dimensions, to correcting certain organic and functional deficiencies by means of pharmacological and surgical methods. Being guided by these aspects, some voices even claim that Hippocratic medicine would be out-of-date and unable to overpass its symbolic condition. However, from an ethical point of view, the Hippocratic tradition is a key element in medicine and defines the human context necessary for the application of all these new technologies.

The relationship between the psychiatrist and the patient, on the one hand, as well as between the psychiatrist and the patient's family is crucial in achieving efficient psychotherapy, in increasing the patient's trust in the therapist's competence and his/her belief that the condition can be either improved or cured. Nowadays, due to the new technologies and paraclinical investigations possibilities, patients show an increased tendency to believe that the diagnosis and cure of a certain condition depend mainly on complicated tests and investigations and the miraculous effect of sophisticated treatment schemes. The therapist's intervention and contribution seem to be regarded as secondary and less important (3). This results in a state of tension and concern, as the patient searches for and requires more and more tests and treatments, the psychotherapeutic contact between doctor and patient being thus negatively impacted. In this context, the relationship between the therapist and the patient should be reconsidered, by trying to re-establish an affective contact and increase the role of psychotherapy, which should be mediated by means of other investigations. As well as this, hospital conditions should be improved, the trauma produced by hospital commitment and by the implications of psychiatric diagnosis should be reduced to a minimum and the best possible social and family integration should be constantly attempted. In this context, the psychiatrist's duty is to protect the patient, to keep the professional secret and treat the diagnosed condition with competence, resoluteness and optimism. Such an attitude is meant to increase the patient's confidence in the therapist's competence and expertise, the moral support offered to the patient motivating the latter to cooperate in regaining his/her hope of recovery and reintegration in his/her social, family and professional life.

Confidentiality is philosophically founded and supported by arguments and justifications belonging to various theoretical concepts. Regarded from the medical ethics perspective these concepts are related to ethical and philosophical dimensions faithfully mirrored by social and community elements.

THE UTILITARIAN THEORY

As far as the utilitarian theory is concerned, breach of confidentiality is only permitted when the respective breach results in producing a better effect or a less harmful effect upon the society as compared to keeping the professional secret. Any society is interested in ensuring health benefits for all its members, and guaranteeing the confidentiality of the medical act is meant to encourage the population in requiring health assistance. This particular aspect is more obvious in the case of a psychiatric condition, as the nature of the condition itself can label the patient in a socially stigmatizing manner. This is why psychiatric conditions are regarded with more conspicuous caution and prejudice by the rest of the population.

It was repeatedly noticed that the society's attitude towards the psychiatric patient and his/her condition directly impacts upon the psychiatric assistance (4). Depending on the hesitations and the prejudices influencing the community or the patient himself/ herself, the patient postpones his visit to the psychiatrist, psychiatric hospital or even the ambulatory medical unit. Unconventional treatments are often preferred, which leads to wrong diagnosis or inaccurate evaluation of the physical suffering, delays the administration of adequate therapy and hides the sometimes very severe evolution of the condition. This situation is mainly due to the reticence of the social environment towards the patient who was committed to a psychiatric hospital and received a psychiatric condition diagnosis (5). This is why the patient should feel safe under the shelter of confidentiality so that he is able to reveal certain information and help the therapist decipher his/her suffering. On the other hand, the therapist must maintain confidentiality of all information and data received from the patient and not only of that information that is relevant for diagnosis or therapy.

THE THEORY OF AUTONOMY

The theory of autonomy focuses on the mandatory dimension of confidentiality supporting the notion of individual autonomy that include every person's right to privacy and secrecy as far as personal data and information are concerned. The obligation to respect confidentiality can be reinforced by the doctor's promise to the patient to treat all information as confidential. In this case, as opposed to the utilitarian theory, there is no motivation whatsoever for breaking the promise of confidentiality. Some modern approaches of this theory state that confidentiality may be broken in situations in which keeping the information secret would result in causing harm to other people or the patient himself/herself. The justification of such an action is based on the principle that no right is absolute as long as the one who benefits from that specific right would infringe other norms or regulations that would cause harm to other people or to that individual himself/herself.

However, other approaches argument and plead in favor of the absolute right to confidentiality motivating that this would bring infinitely more benefits than any breach of confidentiality required by particular cases and situations. Immediate benefits can be followed by negative long-term consequences and especially by a decrease in medical assistance consumption that would in turn have a negative impact upon the general health of the population (6). Another argument would be the improper situation a therapist would put him-

self/herself into when revealing confidential information for political and legal reasons rather than medical ones.

THE SOCIAL CONTRACT THEORY

Another theory to be considered is the social contract theory that states that any traditional generally accepted practice can be formally protected by the law. This can also be applied to confidentiality, which represents an ancient medical tradition. The obligation to keep the professional secret and confidentiality is part of the social contract concluded between the doctor and his/her patient.

PARTICULAR SITUATIONS

As far as dilemmas raised by particular situations are concerned, the therapist finds himself/herself in a very delicate position: on the one hand s/he must value and respect confidentiality in his/her relationship with the patient and on the other hand s/he must act in compliance with the provisions of the law respect his/her duty as a citizen. There are cases in which breach of confidentiality as required by the law is only performed after the person involved formally consents. This category includes situations of legal obligation to reveal information on an individual's state of mental and physical health, such as medical reports or examinations required for hiring in a new workplace, obtaining a driver's licence or a handgun carry permit.

As opposed to the situations mentioned above, there are countless cases where evaluation is controversial. The necessity to break confidentiality is often motivated by the need of social protection (7). First of all there are frequent cases of degradation of family relations in which usually the wife, although physically and/or emotionally abused does not want to press charges on her violent husband, although her suffering is evidently perpetuated. Equally controversial are children abuse cases. Although it is generally accepted that these cases be reported once they are discovered, it is yet unclear if reporting the case actually solves the problem. Experience sadly shows that limited resources within the family is the most common cause for which children are required participate in certain activities while their school attendance drops or their access to education is limited. In such cases, simply reporting the situation to relevant authorities does not solve the issue. As well as this, children might consequently become victims of physical and psychic violence or even sexual abuse, especially when parents are alcoholics or drug addicts. In these cases besides reporting the situation to authorities, parents should be taken into social care and receive specialized assistance

SPECIAL CASES

Drug users are one of the special cases. As far as they are concerned, in many countries the law stipulates that the therapists should report them, for their own protection. That is why the drug users give up medical help altogether, so their addiction is not revealed. Drug or alcohol addiction should consequently be entitled to strict confidentiality, mainly due to the stigmatizing character of the addiction, of which the patient is fully aware. The patient's desire to remain anonymous often prevails over his/her desire to get help. This is the very reason for which various associations dealing with the protection and rehabilitation of alcoholics are called *Alcoholics Anonymous Associations* (8).

Another special case would be that of a psychiatrist who finds out from a patient that the latter has committed a crime. Any supporter of the absolute right to confidentiality would plead in favor of keeping the professional secret in this specific case. This is actually one of those instances in which the relationship between therapist and patient resembles the one between priest and parishioner under the confession oath (9). Supporters of the utilitarian theory would most probably argue against this position, as, according to their belief, doctors' duty is to contribute to the wellbeing and protection of society, as any other citizen.

On the other hand, breaking confidentiality every time a therapist senses a potential danger would only estrange and keep at a distance the patients who, feeling their trust betrayed, would no longer require the psychiatrist's help. One should not disregard the strictly therapeutic aspect of working off one's tensions by means of free and uncompromised expression of all thoughts, feelings and imagination that a patient frees himself/herself of during therapy. More than that, the absolute trust and reliance a patient has towards his/her therapist determines that patient to respect all therapeutic indications; when confidentiality is uncertain the patient would not be motivated to follow therapeutic indications properly. Thus, the only chance the psychiatrist has for intervention in particularly difficult and potentially dangerous cases, namely hospital commitment and treatment acceptance, would be totally compromised (10, 11, 12).

CONCLUSIONS

Many psychiatrists are revolted against this pressure to become defendants of public order rather than therapists, especially since violence prediction is not a very exact coordinate. Potential dangerousness may or may not turn into violent behaviour, and it can be manifested in so many various ways. Most legal systems do not state clearly the situations in which the psychiatrist shall warn the authorities. This attitude would probably be justified only in obvious and extreme cases where a person's or a private property's integrity would be endangered. If we also take into account the possibility of a relative identity of the potential victim or a vague identification of that victim, the next question arises: How should the therapist act in order to prevent an event? The rightest attitude from a medical and ethical viewpoint would probably be to simplify the compulsory commitment procedure.

In psychiatry, confidentiality arises thus, due to its multiple connotations, as an intrinsic necessity within professional relations ethics. This principle represents an important characteristic that governs many other professions and an undeniably essential demand in professions that imply a direct contact with people who seek some kind of professional help. In psychiatry, confidentiality and keeping the information provided by the patient secret become thus an implicit condition of professionalism, representing at the same time a guarantee for the confidence of the patient in the respective professional service.

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NEWS

EZETIMIBE/STATIN COMBINATION THERAPY TO TREAT PATIENTS WITH TYPE 2 DIABETES

Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world. Patients with diabetes present a cardiovascular risk two to four times higher than non/diabetes population. An important factor in cardiovascular complications is hyperlipidemia. Diabetic dyslipidemia is characterized by the so-called atherogenic lipid triad. The aim of this study was to investigate the efficacy of add-on ezetimibe therapy in cholesterol lowering for patients with type 2 diabetes and not controlled by statin therapy. The results of this study showed that adding ezetimibe to statin in add-on trials provided a relative decrease in LDL-cholesterol from baseline of 23.4% compared to statin monotherapy. This fact suggests that patients who poorly respond to statins may be more responsive to cholesterol absorption inhibitors such as ezetimibe. The study supports the hypothesis that an high effect of cholesterol-lowering can be achieved by administration of a minimum dose of statin with ezetimibe, a higher response to that obtained by administration of statin monotherapy maximum doses. Ezetimibe decrease the postprandial elevations of chylomicron cholesterol and triglycerides; after combination therapy with atorvastatin it induced a greater decrease in post-prandial TG levels. These favorable effects have been observed also in association with simvastatin, a good influence on fasting and postprandial triglyceride-rich lipoproteins in patients with type 2 diabetes, by favoring the production of less atherogenic cholesterol-poor chylomicrons and VLDL particles. Ezetimibe alone or in association with simvastatin had favorable effects on the removal of radio labeled chylomicron-like emulsions from plasma in patients with coronary heart disease. It can be concluded that ezetimibe therapy, in combination with statins, provide greater biological beneficial effects, compared to statin monotherapy, in patients with type 2 diabetes (Farnier M, Ezetimibe/statin combination therapy to treat patients with type 2 diabetes. *Atherosclerosis Supp*, 2015; 17: 2-8)

Florentina Lupaşcu

DEVELOPMENT OF A NEW RP-HPLC METHOD FOR ONDANSETRON DETERMINATION IN INJECTABLE FORMULATIONS

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DEVELOPMENT OF A NEW RP-HPLC METHOD FOR ONDANSETRON DETERMINATION IN INJECTABLE FORMULATIONS (Abstract): The aim of this study was to develop a simple, rapid and efficient reversed phase HPLC-UV method for ondansetron assay in injectable dosage forms. **Material and methods**: Analyses were performed with the aid of an 1100 series HPLC system (Agilent Technologies USA) by using an Inertsil ODS 150x4.6 mm column with 3 µm particle size (GL Sciences, Japan), operated at 45°C temperature. The mobile phase consisted of 70% KH_2PO_4 20 mM with triethylammonium phosphate and 30% acetonitrile and it was delivered at a flow rate of 1.5 mL/min. The detection was done at 216 nm. **Results**: Different chromatographic conditions were tested to reduce the time of analysis and to improve the chromatographic efficiency, with symmetry factor between 0.8-1.2. After establishing the optimal analytical conditions, analytical performance studies have been carried out. Linearity study reveals a good correlation in the concentration range 30 µg/mL – 70 µg/mL. Method accuracy and precision were less than 2%. Under the HPLC-UV specified conditions, ondansetron hydrochloride was separated with a retention time less than 2 minutes, with peak symmetry of 0.87 and adequate results at the validation tests. **Conclusions**: The proposed method allows a rapid analysis of ondansetron from injections by using a simple reversed stationary phase. **Keywords***:* ONDANSETRON, HPLC-UV, INJECTIONS.

Ondansetron hydrochloride is a carbazole derivative with a imidazolilmethyl group on position 3 with the chemical name 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one hydrochloride. It is used to prevent nausea and vomiting caused by surgery, cancer chemotherapy or radiation treatment. The racemic form of ondansetron is a selective, competitive serotonin 5-HT3 receptor antagonist in the gastrointestinal tract as well as centrally. It is available as tablets, oral solutions, injections (1).

Ondansetron is a chiral molecule with one asymmetric carbon and it is used as racemate. It is a white or almost white powder, sparingly soluble in water and in alcohol, soluble in methanol, slightly soluble in methylene chloride (2-3). Ondansetron is less sensitive to temperature and moisture, it must be protected from light and neutral and alkaline pH influence

its stability (2).

Different methods regarding the assay of ondansetron hydrochloride are reported, many of them being reversed phase HPLC methods with octylsilyl, phenylsilica or TiO_2 as stationary phases and mixtures of polar solvents as mobile phases (4). Other studies are focused on simultaneous determination of ondansetron in combinations with the aid of HPLC technique, combinations such as ondansetron-famotidine, ondansetron-rabeprazol, idarubicin ondansetron in 0,9% natrium chloride, ondansetron-morphine (4-7). RP-HPLC with ions pairs was applied for the determination of ondansetron hydrochloride from pharmaceutical formulations, particularly the effect of sodium heptane sulfonate (8-10).

In the monography of Ondansetron hydrochloride from European Pharmacopoeia 8-th edition a HPLC method for separation and identification of ondansetron and his main impurities (Impurity A-H) is described by using a nitrile silica gel stationary phase with a mobile phase consisting of acetonitrile and sodium dihydrogen phosphate monohydrate adjusted to pH 5.4 with sodium hydroxide (3). Cyano phases has a strong dipole character that can interact with other dipoles on solutes and have moderate hydrophobic character from alkyl ligands. Their stability in comparison with C8 and C18 is moderate and it depends from one producer to another. For cyano phases the chemical bond to the silica surface is more accessible because of the small size of the ligand and it is unstable under hydrolytic conditions or can cause hydrolysis.

To develop a new HPLC method without using the official nitrile stationary phase and a complicate composition of mobile phase, a rapid and improved RP-HPLC method for ondansetron determination in injections is proposed by using a simple octadecyl column.

MATERIAL AND METHODS

Instruments and chromatographic conditions

Agilent Technologies 1100 series HPLC system, equipped with quaternary pump and UV-VIS detector. Column: Inertsil ODS 150x4.6 mm column (C18) with 3µm particle size (GL Sciences, Japan). Mobile phase was consisted of a solution of KH_2PO_4 (Merck) with triethylammonium phosphate TEAP (Sigma Aldrich) and acetonitrile ACN (LC Grade, Fluka). Final injection volume was 5 µl.

During method development, temperature of the column was varied between 35°C and 45°C, the flow rate between 1 mL/minute and 1.5 mL/minute. The tested wavelengths were 254 nm and 216 nm. The concentrations of phosphate and TEAP were varied between 10 and 20 mM and between 2 and 20 mM, respectively. The final chromatographic conditions were: 70% KH_2PO_4 20 mM with 20 mM TEAP and 30% ACN, flow rate 1.5 mL/min, temperature 45 °C, detection at 216 nm.

Reference substance and preparation of standard solutions

Ondansetron hydrochloride working standard (Glaxo SmithKline, UK) was used. Stoc solution preparation: an appropriate quantity of ondansetron hydrochloride dihydrate standard was dissolved with methanol (LC Grade, Sigma Aldrich) at the final concentration 100 µg/mL ondansetron.

The stock solution was further diluted to give standard solutions with concentrations between 30 and 70 µg/mL.

Sample preparation

The ondansetron injection, with 2

mg/mL ondansetron, was diluted with methanol at 50 µg/mL concentration before analysis.

RESULTS AND DISCUSSION

Different chromatographic conditions were tested to reduce the time of analysis, to improve the efficiency parameters for active pharmaceutical ingredient by taking into consideration a peak symmetry factor between 0.8-1.2 according to pharmacopoeia provisions (tab. I).

TABLE I

Tested chromatographic conditions during method development

No	Injection volume, µL	Mobile phase composition*		Flow rate, mL/min	t, °C	λ, nm	Symmetry
		70% aqueous phase					
		KH_2PO_4	TEAP				
1	10	20 mM	-	1	35	254	0.519
2	10	40 mM	-	1	35	254	0.52
3	10	10 mM	-	1	35	254	0,56
4	10	0	-	1	35	254	0.575
5	10	20 mM	2 mM	1	35	254	0.685
6	5	20 mM	2 mM	1	35	254	0.738
7	10	20 mM	6 mM	1	35	254	0.731
8	5	20 mM	6 mM	1	35	254	0.75
9	5	20 mM	6 mM	1	45	254	0.764
10	5	20 mM	10 mM	1	45	254	0.789
11	5	20 mM	10 mM	1.5	45	254	0.845
12	5	20 mM	20 mM	1	45	254	0.806
13	5	20 mM	20 mM	1	45	254	0.871
14	5	20 mM	20 mM	1.5	45	216	0.87

*the organic phase consisted of 30% ACN

In the first place the concentration of KH_2PO_4 was modified from 10 mM to 40 mM without TEAP proportions of the mobile phase components were 70% KH_2PO_4 at different concentration and 30% ACN, flow rate 1 mL/minute, detection at 254 nm, column temperature 35°C. In case of using KH_2PO_4 40 mM the ondansetron hydrochloride peak symmetry was 0.52 (fig. 1), for KH_2PO_4 10 mM the symmetry increases at 0.56, but still unsuitable for our study.

The presence of phosphate is important for higher, tighter and efficient peaks, a concentration of 20 mM KH_2PO_4 seems to be optimal, but also the mobile phase pH influence was studied, in this case we adjusted the pH with TEAP at 3.5, thus combining the pH influence and the presence of ammonium quaternary salt for the inhibition of residual interactions.

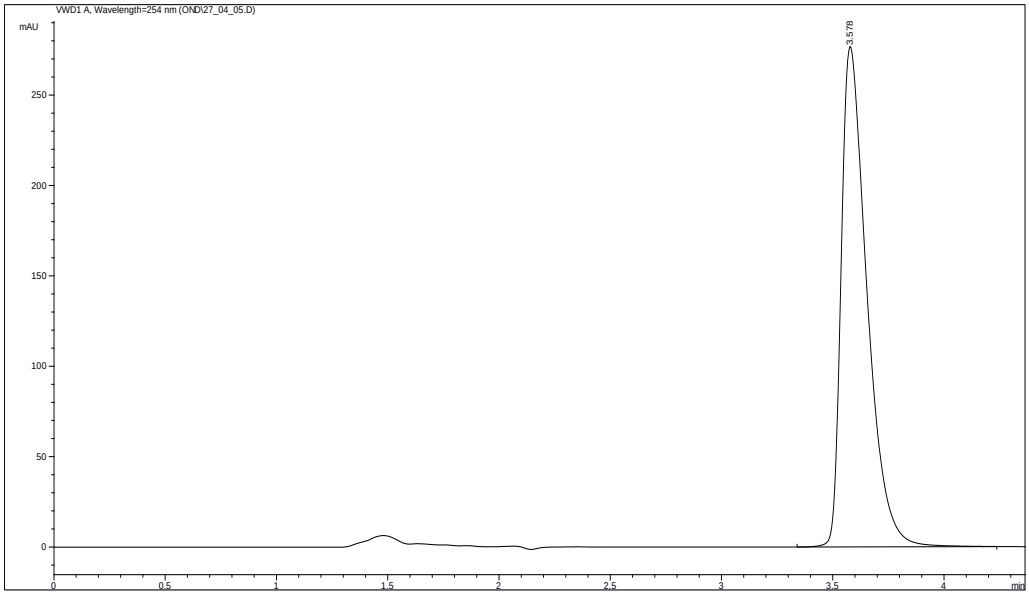

Fig. 1. Chromatogram of ondansetron hydrochloride standard solution 100 µg/mL, with mobile phase consisting of KH_2PO_4 40 mM 70%: ACN 30%, flow rate 1 mL/min, detection at 254 nm, separation temperature 35°C, peak symmetry 0.519.

The injection volume was also modified from 10 µl to 5µl, and the flow from 1 mL/minute to 1.5 mL/minute to improve peak symmetry. The separation temperature has a small influence on ondansentron hydrochloride peak symmetry, an increasing from 35°C at 45°C lead to better symmetry value 0,87. The detection was finally established at 216 nm, according to pharmacopoeia provision, and to detect any interference by using an unspecific detection wavelength (fig. 2).

To test the analytical performances of the developed method, linearity, accuracy and precision were investigated. For the linearity test, five levels of concentrations in five different series were analyzed by applying the final chromatographic conditions. The results are presented in table II.

There is a linear positive correlation between peaks areas and concentrations of ondansetron solutions, with the mean calibration curve Area = 18.49(±0.41)-3.40(±16.00), the correlation coefficient being greater than 0.998. A randomly distribution of residuals was obtained in ±2% range.

For precision test, the concordance of results from a range of 7 replicate quality control samples with the concentration 50 µg/mL of ondansetron hydrochloride was established, the RSD being 1.13%.

For the quantitative analysis of ondansetron from injectable dosage form, 2 mg/mL, the pooled sample was diluted with methanol at 50 µg/mL concentration, the analysis being performed in triplicate. No interference of excipients is expected being non-retainable substances under the proposed chromatographic conditions. A content of 104.5% was determined, in conclusion the sample is in accordance with Pharmacopoeia requirements regarding labelled content.

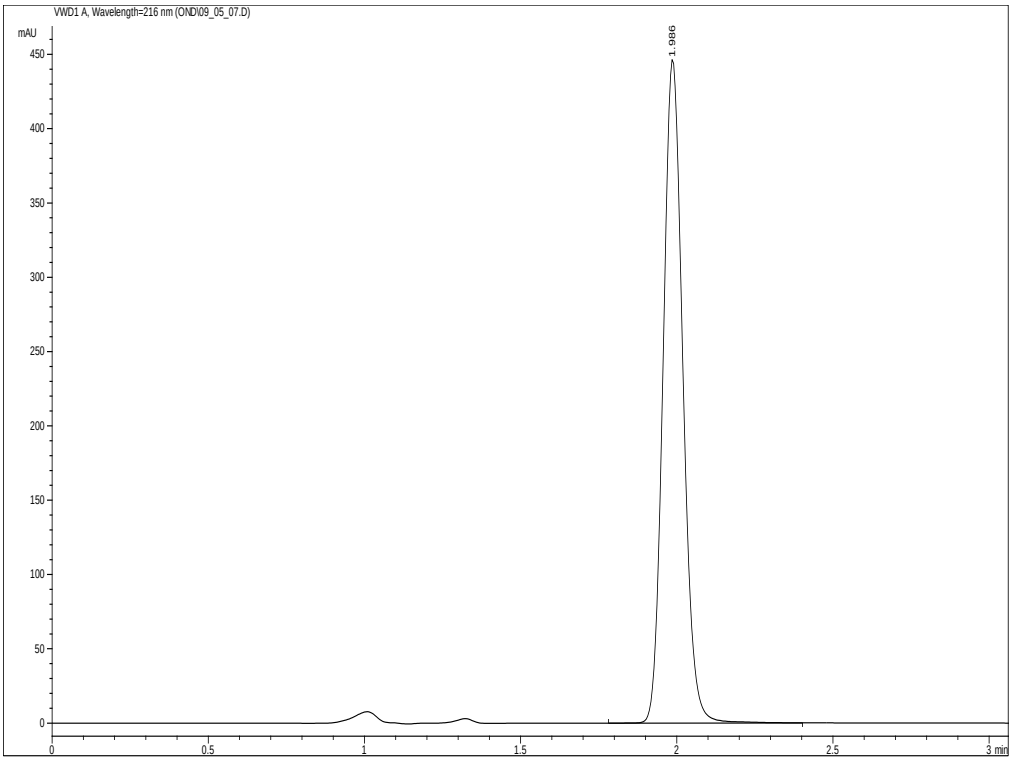

Fig. 2. Chromatogram of ondansetron hydrochloride standard solution 100 µg/mL, with mobile phase consisting of KH_2PO_4 20mM with TEAP 20mM 70%: ACN 30%, flow rate 1.5 mL/min, detection at 216 nm, separation temperature 45°C, injection volume 5 µL, peak symmetry 0.87**.**

TABLE II

Linearity test results

Theoretical concentration, g/mL	Found concentration, µg/mL					Average, g/mL	DSR%	Er%
	Series 1	Series 2	Series 3	Series 4	Series 5			
30.00	30.31	30.68	29.35	29.95	29.86	30.03	1.66	0.10
40.00	39.78	39.67	40.24	40.39	40.03	40.02	0.76	0.05
50.00	50.23	49.46	51.38	50.28	50.35	50.34	1.36	0.68
60.00	58.95	59.35	59.11	58.46	58.96	58.97	0.55	-1.72
70.00	70.73	70.84	69.92	70.92	70.6	70.60	0.57	0.86

CONCLUSIONS

The study proposed a rapid reversed phase HPLC-UV method for ondansetron assay with a good peak symmetry, by using

a C18 column, more available and less expensive than *Pharmacopoeia official* nitrile column, and a simple mobile phase. The developed method it was proven to be linear for the chosen concentration range and the quantitative analysis from injectable dosage forms was carried out with optimal results.

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IS IT ENOUGH SCINTIGRAPHY FOR EVERYONE? A CROSS-SECTIONAL ANALYSIS REGARDING THE IMPACT OF JUSTICE IN THE DISTRIBUTION OF HEALTH CARE RESOURCES

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IS IT ENOUGH SCINTIGRAPHY FOR EVERYONE? A CROSS-SECTIONAL ANALYSIS REGARDING THE IMPACT OF JUSTICE IN THE DISTRIBUTION OF HEALTH CARE RESOURCES (Abstract): **Aims**: The aim of this study was to evaluate the influence of three demographic criteria: gender, age and education level on the patients that perform a scintigraphy. **Materials and Methods:** The cross-sectional study was applied to 220 patients to whom it was prescribed a scintigraphy by their treating physician. Of these, 74 were men and 145 women, 10 children and 210 adults. According to their education, 88 people did not graduate a high school, a total of 56 people graduated a high school, 27 persons graduated a college, 22 people had a university diploma and 26 people a PhD title. The chi-square test was used to analyze the frequencies for the measurable variables on a nominal scale. The significance threshold is considered to be 0.05, so, only the values which are smaller were taken into consideration. We presented and analyzed only the data that fulfill this condition. **Results and Discussion:** From our study it has been found that gender criterion played an important role in performing a scintigraphy for the first time. 71.6% of male patients were undergoing a scintigraphy for the first time. The frequency of exploration by scintigraphy is increasing if you are a female patient than if you are a male patient: chi-square calculated value is χ^2 (3)=12.398, p=0.006. From our study it has been found that age item plays an important role in the scintigraphy investigations for all the patients. The first scintigraphy was significantly performed in the 40-50 years old gap, whilst for the patients being aged over 60; they were probably not performing a first time scintigraphy. We did not find significant statistical differences in respect of the education level. **Conclusions**: So, we can conclude that access to that medical investigation is not depending on the socio-economic status of the patient, but in some occasions, the number and the frequency distribution of performing a scintigraphy depend on gender and age. We can also conclude that the principle of egalitarianism is fulfilled and so the justice principle in the distribution of health care resources must be re-evaluated. **Keywords:** SCINTIGRAPHY, CROSS-SECTIONAL STUDY, HEALTH CARE, JUSTICE PRINCIPLE.

As Oprea has showed (1), based on a Deaton's study (2), health care services are unevenly distributed. This debate must submit appropriate distribution of health services in the case of medical imaging techniques. But, does a barrier really exist

for patients to get access to those methods of investigation? From this point of view, a barrier could result from the fact that in the clinics of the public medical system, time resource (staff's programme at work) and material resource (the actual cost of an investigation) are finite. So, in many laboratories and imaging services, scheduling of patients is carried out with delays, many patients have to wait up to one week or more to have access to those investigations (3). This, in reality, led to a limitation of access to medical services apart for what European general rules state (4).

Not only the antagonism between egalitarianism - suficientism is involved in an imaging investigation. Sufficient or equal access to medical services as presented by Huzum (5) does not make a difference in the way the patient is properly investigated, even though patient access is limited by the time factor.

The patient access to complex investigation and/or with a high degree of novelty (6) stressed that health care distribution is influenced by SES (socio-economic status) (7). Despite the prevailing circumstances given by their level of education, from our study we have found, that a high percentage of those who turn to complex medical services (par excellence, CT, MRI, scintigraphy techniques), have had access to them. Generally, as it is known, a lower level of instruction places the subject in the category of those with precarious material level and with a weak access to medical resources (8).

MATERIAL AND METHODS

The present study was based on a questionnaire survey. The questionnaire contains 20 questions (items), including a series of questions regarding the person's demographics, such as gender, age, and level of education (item 20). The application of questionnaires was performed starting with March till the end of July 2014. Questionnaires were completed by patients in the Laboratory of Nuclear Medicine of St. Spiridon University Hospital of Iasi. Their consent was obtained in advance. The subjects were assured for the confidentiality of the responses, and they have been informed that they can refuse to complete the questionnaire at any time.

For the questionnaire used in this study the approval of the Ethics Committees of the Grigore T. Popa University of Medicine and Pharmacy of Iaşi and St. Spiridon University Emergency Hospital of Iaşi was requested and obtained.

The subjects of this research were 220 patients, to whom it was prescribed a scintigraphy by their treating physician. Of these, 74 were men and 145 women, 10 children and 210 adults. According to their education, 88 people performed less than high school education, a total of 56 people graduated a high school, 27 persons graduated a college, 22 people owned a university diploma and 26 people a PhD title. Depending on the age, the variable was represented by 6 persons aged 1-10 years old, 4 people with the age between 10 to 20 years old, 2 persons aged 20-30 years old, 12 people aged between 30 to 40 years old, 24 people aged between 40 to 50 years old, 61 persons aged 50 to 60 years old and 111 people aged over 60.

All the data acquired were analyzed using *SPSS* version 22.0 for Mac. We used a statistical technique called *SPSS* (Statistical Package for the Social Sciences) which is widely used in different fields, including the health sciences as well. The chi-square test was used to analyze the frequencies for the measurable variables on a nominal scale. In this method, the significance

threshold is considered to be 0.05, so, only the values which are smaller are statistically significant. We presented and interpreted only the data that fulfill that condition.

RESULTS AND DISCUSSION

For a more analytical presentation, the results to be interpreted were splited in three different criteria: gender, age and level of education (within item 20).

Gender criterion

Answering to the first item: *„Are you for the first time in the Laboratory of Nuclear Medicine?”,* 58.2 % of the subjects responded that they were performing a scintigraphy for the first time. If we undergo an analysis of the distribution of measurable variables frequencies of gender criterion (within item 20) with the first time performing a scintigraphy criterion (item 1) we obtained the following data (tab. I).

TABLE I

Analysis of gender of the patients with item 1

		Gender		
		Male	**Female**	**Total**
Item 1	**Yes**	53	75	128
	No	21	71	92
Total		74	146	220

Chi square analysis attests: $\chi 2$ (1) =8.524, p=0.004. Analyzing the results, we can observe that irrespective of the answer to item 1, there were more women than men who came to the Laboratory of Nuclear Medicine. On the other hand, if in the case of female patients, there were no significant differences between those who were for the first time and those who were not, for male patients we recorded significant differences. The figures show that 71.6 % of the male patients were for the first time in the laboratory, while 28.4 % of the male patients were, in fact, repeating the investigation.

The second item was: *„If your answer to item 1 was No, how many times have you performed a scintigraphy?”* Analyzing through *SPSS* the item 2 with the patient's gender-related item 20 we arrived to the following interpretation: there were no significant differences based on gender with regard to responses to item 2 and chi square test shows: $\chi 2$ (3)=2.927, p=0.403. However, by chi square adjustment we recorded a significant difference between female patients and male patients that answered they had performed once or those that answered they had performed twice a scintigraphy (tab. II).

TABLE II

Analysis of gender and the number of performed scintigraphies

		Gender		Total
		Male	**Female**	
Item 2	**Once**	14	40	54
	Twice	8	20	28
	More than twice	17	29	46
	It is not the case	35	57	92
Total		74	146	220

The third item was: *„If you have performed multiple scintigraphy investigations, which was their frequency?”* Analysis of frequencies for the variables item 3 with the patient's gender in the subitem 20, showed that there are significant differences in scintigraphy investigations, χ^2 (3) =12.398, p=0.006. The meaning of these calculated values is that for the patients who were investigated by scintigraphy only once, a higher frequency was found for female than for male patients (tab. III).

TABLE III

Analysis of gender and the frequency of performed scintigraphy criteria

		Gender		
		Male	**Female**	**Total**
Item 3	**Once a year**	17	64	81
	Twice a year	2	6	8
	More than twice a year	3	1	4
	It is not the case	52	75	127
Total		74	146	220

Combining the results of item 1 with item 5 ("At your arrival in the Laboratory of Nuclear Medicine, your diagnosis was already established by your treating doctor?"), as well as with patient's gender-related criterion (within item 20), it was found that only for female patients there are significant differences, $\chi^2(2)=10.953$, p=0.004. In this case, we noticed an increased likelihood for the female patients with previously established diagnosis and that were not for the first time in the Laboratory of Nuclear Medicine with all the other categories of patients (tab. IV).

TABLE IV

Analysis of first time presence (item 1) with previously established diagnosis (item 5) for females (within item 20)

		Item 5			Total
		Yes	**No**	**I don't know this**	
Item 1	**Yes**	54	9	12	75
	No	66	2	3	71
Total		120	11	15	146

Gender demographic analysis of item 20, applied to the resulting analysis of frequencies for variables in item 5 and item 3, pointed significant differences in female patients, $\chi^2(6)=24.913$, p=0.000, which attests that female patients perform with a much higher probability at least one scintigraphy per year, if their diagnosis is already indicated (tab. V).

TABLE V

Analysis of previously established diagnosis (item 5) with the annual frequency of scintigraphies (item 3) for female patients

		Item 5			Total
		Yes	**No**	**I don't know this**	
Item 3	**Once a year**	60	1	3	64
	Twice a year	6	0	0	6
	More than twice a year	0	1	0	1
	It is not the case	54	9	12	75
Total		120	11	15	146

Age criterion

From our study, age seems to play an important role in the scintigraphy investigations. If we perform an analysis of item 1 (first time in the laboratory) and with age gap (within item 20), chi square is: $\chi^2(6)=19.689$, p=0.003. The significance of this value shows that there were statistically significant differences by age criterion with regard to the fact of being or not for the first time in the Laboratory of Nuclear Medicine. Differences were found for category 40 to 50 years old and also for those over 60 years old. Thus, for the category 40-50 years of age, patients who were for the first time (75 %) were much more numerous than those who did not come for

the first time (25 %). For the patients over 60 years old, there were several cases of those that did not come for the first time (67.6 %) compared to those who came for their first time (32.4 %) (tab. VI).

Analyzing in terms of age, item 1 (first time performing a scintigraphy) and item 5 (diagnosis previously established), we have found only for the patients over 60 years old a statistically significant difference. We have obtained a calculated value: $\chi^2(2) = 8.115$, $p=0.017$. This value signifies that patients over the age of 60 years old have more chances to be not for the first time in the laboratory, and, also, more chances that when arriving to the laboratory, patients' diagnoses were previously established (tab. VII).

TABLE VI

Analysis of age criterion (within item 20) and the first time in the laboratory (item 1)

		Age (years)							Total
		1-10	10-20	20-30	30-40	40-50	50-60	Over 60	
Item 1	Yes	3	2	1	10	6	31	75	128
	No	3	2	1	2	18	30	36	92
Total		6	4	2	12	24	61	111	220

TABLE VII

Analysis of the first time presence (item 1) with previously established diagnosis (item 5) for patients over 60 years old (within item 20)

		Item 5			Total
		Yes	No	I don't know this	
Item 1	Yes	53	11	11	75
	No	34	1	1	36
Total		87	12	12	111

We analyzed the measured variables from item 5 (previously established diagnosis) and item 3 (frequency of performing a scintigraphy) with age criterion. We have found that for patients belonging to age category 50-60 years old, there were significant differences, $\chi 2\ (2)=17.322$, $p=0.008$. The meaning of this value is that regardless of patients' gender those aged between 50 and 60 years old and previously diagnosed, have a higher probability to perform a scintigraphy at least once a year (tab. VIII).

TABLE VIII

Analysis of previously established diagnosis criterion (item 5) and frequency of scintigraphies (item 3) for patients aged 50-60 years old

		Item 5			Total
		Yes	No	I don't know this	
Item 3	Once a year	21	1	2	24
	Twice a year	5	0	0	5
	More than twice a year	0	1	0	1
	It is not the case	23	2	6	31
Total		49	4	8	61

Education level criterion

Analogue analysis for square chi criteria related to patients education level applied to item 1, showed no evidence of significant statistical differences, obtaining a value $\chi^2(4)=5.471$, $p=0.242$. Due to the fact that we did not obtain statistically

significant scores, these are evidences that access to this imaging exploration opportunity involves no social rank categories. On the contrary, educational and economical factors are not barriers to access at the latest medical investigation methods from public health system. From the education level point of view in relation with all the other items, the data we have acquired were not statistically significant. That is why we consider the principle of egalitarianism from this perspective can be reconsidered and there is a justice in the distribution of health care resources.

CONCLUSIONS

This study shows that the gender criterion played an important role in the performing scintigraphy for the first time. Unlike female patients, for the male patients, it is highly probable that they are for the first time in a Laboratory of Nuclear Medicine. The frequency of performing scintigraphy is increasing if you are a female patient than if you are a male patient.

Analyzing the frequencies for the measurable variables it results that, for female patients to whom the diagnosis were previously established, there is a higher probability that they are not in the laboratory for the first time, so, they have performed such a test before. If the diagnosis is already known, female patients, with a much more significant probability, are explored by scintigraphy at least once a year.

It has also been found that age criterion plays an important role in the scintigraphy investigations for all the patients. The first visit (we mean the first scintigraphy) being significantly represented in the category 40-50 years old, whilst for the patients aged over 60, probably they are not for the first time in the Laboratory of Nuclear Medicine.

For the patients with a previously established diagnosis with age over 60, there is a statistical significance that they are not experiencing a scintigraphy for the first time.

No matter the gender criterion, for the patients aged between 50 to 60 years old, with the diagnosis previously established before their arrival in the laboratory, there is a higher probability, with statistical significance, that they were explored at least once by scintigraphy.

Due to the fact that for the persons explored by scintigraphy there are not significant differences in connection with the education degree, we can conclude that access to that medical investigation is not depending on the socio-economic status of the patient and there are enough proofs that there is justice in the distribution of health care resources.

ACKNOWLEDGMENTS

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NEWS

COMPARATIVE EVALUATION OF SERUM C-REACTIVE PROTEIN LEVELS IN CHRONIC AND AGGRESSIVE PERIODONTITIS PATIENTS AND ASSOCIATION WITH PERIODONTAL DISEASE SEVERITY

C-reactive protein (CRP) is an acute-phase reactant and has been proved to be a significant predictor of future cardiovascular events. Recent studies have demonstrated a correlation between periodontitis and elevated C-reactive protein levels. Most of the studies have focused on chronic periodontitis and very few studies are done in patients with aggressive periodontitis. The aim of a study realized by a group of indian researchers was to determine and to compare the relative levels of serum CRP in aggressive and chronic periodontitis patients. A total of 75 systemically healthy subjects were divided into three groups: group I (non-periodontitis subjects); group II (chronic generalized periodontitis patients) and group III (generalized aggressive periodontitis patients). All participants were subjected to quantitative C-reactive protein analysis using enzyme-linked immunosorbent assay. Mean CRP levels were significantly greater in both group II and III as compared to group I and group III having greater level than group II. CRP levels positively correlated with the amount of periodontal destruction as measured by probing depth and clinical attachment loss. The study indicates a positive correlation between C-reactive protein and periodontal disease severity with particular concern in younger individuals that could be a possible underlying pathway in the association between periodontal disease and the observed higher risk for cardiovascular disease in periodontitis patients (Goyal L, Bey A, Gupta ND, Sharma VK. Comparative evaluation of serum C-reactive protein levels in chronic and aggressive periodontitis patients and association with periodontal disease severity. *Contemp Clin Dent*, 2014; 5 (4): 484-488).

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EPIDEMIOLOGICAL STUDY OF METABOLIC SYNDROME AND RISK OF DIABETES MELLITUS IN A RURAL FAMILY MEDICINE PRACTICE IN BACAU COUNTY

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EPIDEMIOLOGICAL STUDY OF METABOLIC SYNDROME AND RISK OF DIABETES MELLITUS IN A RURAL FAMILY MEDICINE PRACTICE IN BACAU COUNTY (Abstract) **Aim:** This descriptive epidemiological study included 615 subjects investigated by their family doctor in the interval October 2011- August 2012. **Material and Methods:** Patients were selected according to 2012 IDF diagnostic criteria resulting 304 patients. **Results:** There was a Gaussian distribution of waist circumference (WC) with a maximum in age-group 70-80 years (46.3%), followed by age- groups 50-60 years (37.8%) and 60-70 years (41.6%). Waist circumference measurement showed an average of 100.76+/-11.59 cm, ranges 72 cm - 134 cm, without statistical significance. Mean body mass index (BMI) was 28.25+/-4.72 kg/m^2 *vs.* 27.79+/-4.48 kg/m^2. The difference is statistically significant (p=0.05). Considering categorical the patients with hypertension (AHT) according to the definition, we found that only 7 cases did not have AHT (4.4%) as compared to a prevalence of AHT of 13.7% in the MS (metabolic syndrome) group. Mean serum triglyceride level was 123.11+/-68.55mg/dl *versus* 113.75+/-65.62mg/dl in the MS group, significantly higher (p<0.003). Mean HDL cholesterol was 57.58+/-17.11mg/dl *versus* 58.54+/-15.02mg/dl, statistically significant difference (p<0.05). **Conclusions:** The prevalence of glucoregulation disorders is higher in the MS group. Proportional relationships were found between BMI, WC, and SBP and high blood glucose. Comparison of blood glucose levels at baseline and at 1 year follow up showed significant differences. **Keywords**: METABOLIC SYNDROME, DIABETES MELLITUS, EPIDEMIOLOGY, SCREENING.

Diabetes mellitus type 2 is associated with metabolic and biochemical abnormalities, including hyperglycemia, hypertension, inflammation, atherogenic dyslipidemia, endothelial dysfunction and inhibition of fibrinolysis, each of these anomalies promoting atherosclerosis and cardiovascular disease (1, 2, 3).

MATERIALS AND METHODS

This epidemiological study was conducted in Pancesti village, Bacau County, with a population of 1463 persons aged 0-89 years, having or not having health insurance, regis-

tered with the family doctors. The group of interest to our study, the adult population aged 35-89 years, accounted for 969 individuals (66.23% of the population), of which 542 (55.93%) women and 427 (44.07%) men. Of these, 615 subjects had high waist circumference (WC) according to IDF criteria (≥ 80 cm in women and ≥ 94 cm in men) (tab.I).

TABLE I
Distribution of subjects by study groups

<table>
<tr><td rowspan="4">Total population: 1463</td><td rowspan="4">35-90 years: 969</td><td rowspan="4">IDF, criteria, WC:615</td><td rowspan="2">Women: 422</td><td rowspan="2">68.62%</td><td>MS + 222</td><td>36.10%</td></tr>
<tr><td>MS – 200</td><td>32.52%</td></tr>
<tr><td rowspan="2">Men:193</td><td rowspan="2">31.38%</td><td>MS + 108</td><td>17.56%</td></tr>
<tr><td>MS – 85</td><td>13.82%</td></tr>
</table>

This epidemiological study was descriptive, transversal and included 615 subjects investigated by their family doctor in the interval October 2011-August 2012. All subjects underwent a complete history and physical examination, with measurement of waist circumference (soft tape) and arterial blood pressure (ABP). A second visit was scheduled for fasting night laboratory tests: blood glucose level, triglycerides, HDL-cholesterol, liver and kidney function tests. Then, more test were performed, including total serum cholesterol and LDL-cholesterol, to assess overall cardiovascular risk. To detect another co-existing cardiovascular risk factor the subjects were questioned directly about: alcohol use (yes or no and estimated amount), physical exercise (yes or no), and education level (less than 8 grades, or high school/higher education), family history of diabetes and hypertension (yes or no) in at least one first-degree relative, smoking (yes or no), economic or social stress. According to 2012 IDF criteria 615 subjects had a waist circumference ≥ 80 cm in women and ≥ 94 cm in men (63.47% of the total of 969 adult population). The patients were divided into two groups according to the presence/absence of the metabolic syndrome (MS): group I, consisting of 330 patients with overt metabolic syndrome (increased waist circumference and at least two other components of the metabolic syndrome) and group II consisting of 285 subjects without overt metabolic syndrome, but having one or two components of the metabolic syndrome (tab. I). Of all analyzed subjects we selected the 330 patients with metabolic syndrome. Of these, some died or left the village; thus, the study group included 304 patients (tab. II).

Of the 304 patients we selected a group of patients with fasting glucose > 100 mg/dl, the threshold at which it is a cardiovascular risk factor. So we further studied a group consisting of 204 cases. Of these, 132 were women (64.7%) and 72 men (35.5%) (tab.III). When these data were reported to the studied population and to those with overt metabolic syndrome the following *incidence data* were obtained: total 1462 subjects => incidence 13.9%, and 615 MS cases - incidence 33.1%. For further clinical-biological investigations of the patients with glucoregulation disorders a total of 159 cases were selected, the other patients refusing to continue this study or

were already known with diabetes and were on treatment. This patient group consisted of 101 women (63.5%) and 48 men (36.5%). The mean age of the study group was 68.23 + / -9.18 years, ranges 37 – 87 years. In this group, 11 cases (6.9%) had blood glucose levels above 126 mg/dl, which is they had diabetes mellitus.

TABLE II
Structure of the study group

		Frequency	%	Valid Percent	Cumulative Percent
Valid	f	200	61.8	61.8	61.8
	f d	6	1.8	1.8	63.5
	f p	13	3.8	3.8	67.4
	m	101	29.7	29.7	97.1
	m d	3	.9	.9	97.9
	m p	7	2.1	2.1	100.0
	Total	330	100.0	100.0	

TABLE III
Sex distribution

		Frequency	%	Valid Percent	Cumulative Percent
Valid	f	132	64.7	64.7	64.7
	m	72	35.3	35.3	100.0
	Total	204	100.0	100.0	

f- female, m – male, fd/md – deceased females/deceased males, fp/mp – females/males lost to study

RESULTS

Age distribution of glucoregulation disorders. Age distribution of impaired fasting glucose patients showed a Gaussian distribution with a maximum among age group 70-80 years (46.3%), followed by age groups 50-60 years (37.8%) and 60-70 years (41.6%). In this group the average waist circumference was 100.76+/-11.59cm, ranges 72 cm -134 cm, compared to an average of 99.36 +/-10.27 cm in the MS group (615 cases), not statistically significant difference. Categorical comparison of waist circumference-related data (WC=abdominal obesity, F> 80 and B> 94 cm) showed that most patients (98.7%) presented abdominal obesity. Basically, only two cases did not meet these criteria. Obesity measured by body mass index (BMI) gave an average of 28.25 +/-4.72 kg/m², with high variance data, 22.31 kg/m^2, which proves the dispersion of data. In the MS group (615 cases), the average was 27.79 +/-4.48 kg/m². The difference is statistically significant (p = 0.05). The obtained percentages reveal a significantly higher (p=0.087) proportion of diabetes mellitus (DM) among the obese group compared to the group with normal BMI. When considering categorical the patients with AHT according to the definition, we found that only 7 cases did not have AHT (4.4%) compared to a 13.7% prevalence of hypertension in the MS group. Gender distribution was balanced: 95%

females and 96.6% males, with no significant differences. In the MS group AHT was more common in males, 92.2%. Mean triglyceride level was 123.11 +/- 68.55 mg/dl *versus* 113.75+/- 65.62 mg/dl in the MS group, being significantly higher (p<0003). Mean HDL cholesterol was 57.58+/-17.11 mg/dl *versus* 58.54+/-15.02 mg/dl, with statistical significance (p<0.05). Descriptively, in the group of 159 patients the measurement of serum cholesterol levels showed a moderately increased mean value, 214.06 =+/- 49.92 mg/dl, the data being very scattered (104 to 347 mg/dl). The same was found for LDL cholesterol. The mean was 115.58+/- 36.33 mg/dl. Thirty-five (22%) patients were smokers, and 67 (42.1%) were alcohol users. One hundred and forty patients (88.1%) had an education higher than middle school. Ischemic heart disease and stroke were present in 33.3% and 3.8% of cases, respectively.

Association of metabolic syndrome elements revealed a high prevalence of the association between abdominal obesity (increased WC), hypertension and BMI over 25 kg/m (tab. IV).

When comparing the 2 groups: with or without blood glucose ≥ 100mg/dl, 204 cases, *vs.* the group of 107 cases we found no statistically significant differences in cholesterol level. In the group with blood glucose above or equal to 100 mg/dl WC was statistically significantly higher than in the group with blood glucose below 100 mg/d. The mean was over 3 cm and the median 2 cm higher, unlike BMI where differences were insignificant (fig. 1, 2).

No statistical difference was found between SBP and DBP (fig. 3, 4).

No statistical differences in cholesterol and LDL cholesterol levels were found between the two groups (fig. 5, 6).

Triglycerides were statistically significantly higher in the group with blood glucose <100 mg/dl, and HDL cholesterol was significantly lower in the group with blood glucose <100 mg/dl (fig. 7, 8).

TABLE IV

Association of metabolic syndrome elements

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		1	.6	.6	.6
	AHT/BMI≥25	1	.6	.6	1.3
	WC/ low HDL / BMI≥25	1	.6	.6	1.9
	WC / AHT	30	18.9	18.9	20.8
	WC / AHT / low HDL	4	2.5	2.5	23.3
	WC / AHT / low HDL / BMI≥25	21	13.2	13.2	36.5
	WC / AHT / BMI≥25	58	36.5	36.5	73.0
	WC / AHT / Triglycerides >=150	7	4.4	4.4	77.4
	WC / AHT / Triglycerides>=150 / low HDL / BMI≥25	12	7.5	7.5	84.9
	WC / AHT / Triglycerides>=150 / BMI≥25	19	11.9	11.9	96.9
	WC / BMI≥25	4	2.5	2.5	99.4
	CA / Triglycerides>=150	1	.6	.6	100.0
	Total	159	100.0	100.0	

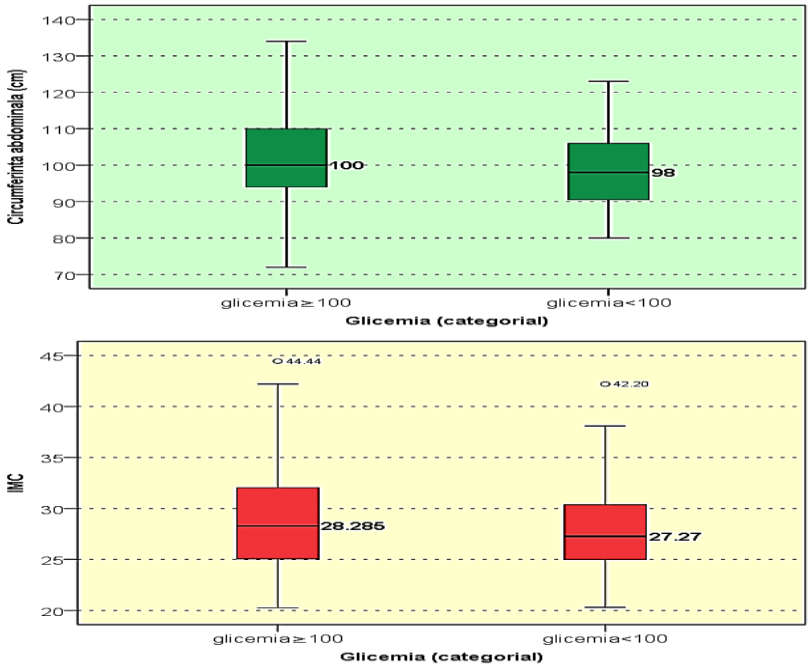
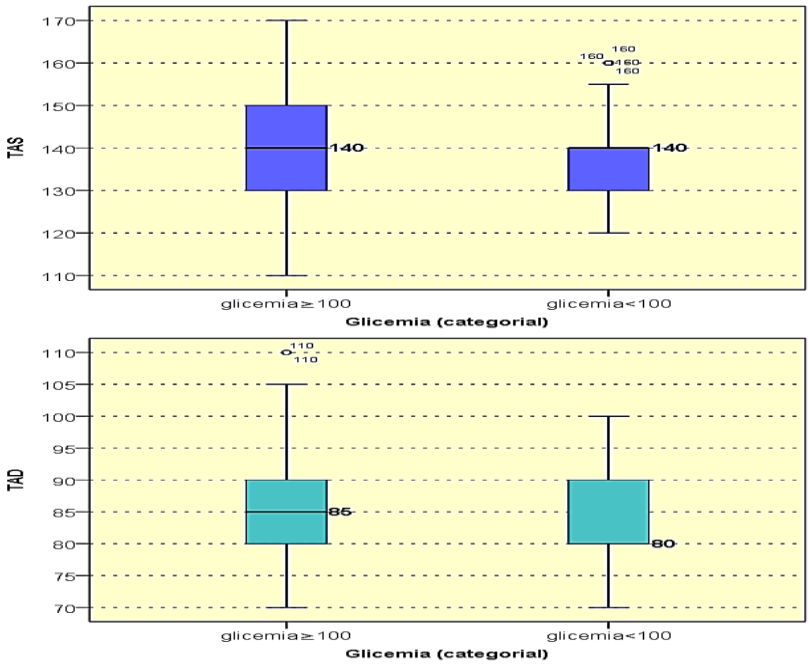
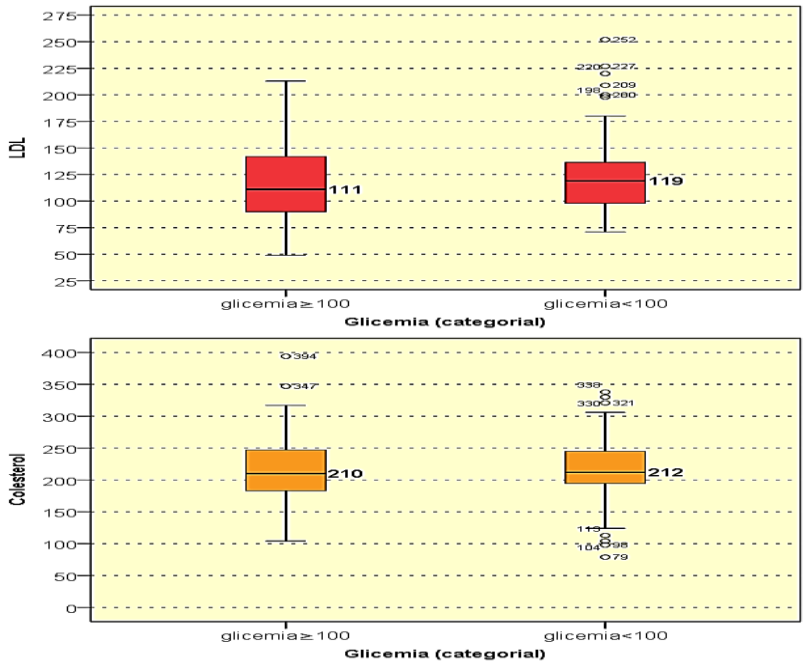


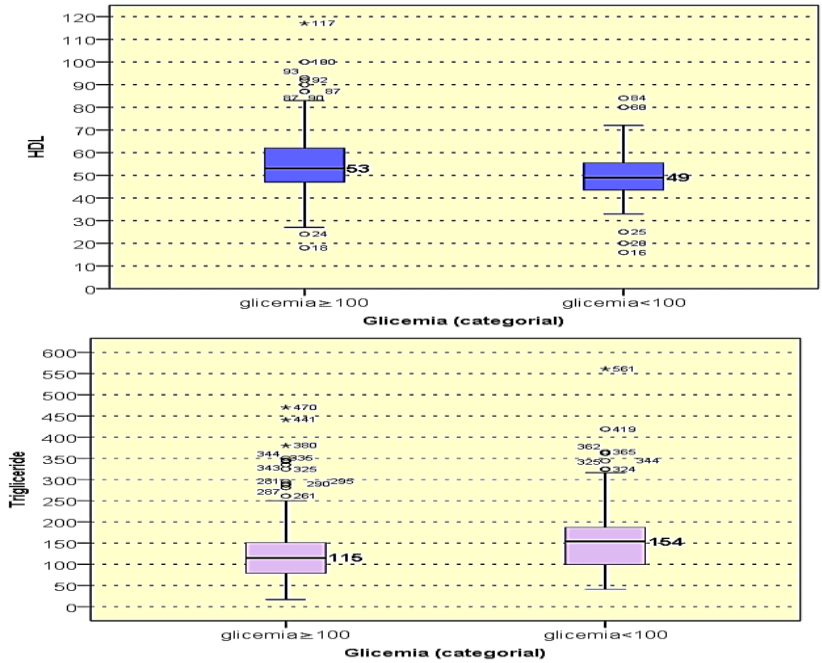
Fig. 1, 2. Comparisson of WC and BMI with blood glucose leves



Figs. 3,4. Comparison of SBP and DBP with blood glucose levels



Figs. 5,6. Comparison of LDL cholesterol and total cholesterol with blood glucose levels



Figs. 7,8. Comparison of HDL cholesterol and triglycerides with blood glucose levels

1) *Regression analysis*. In the group of 311 patients we determined the relationship between the variable *blood glucose* and the possible predictors: BMI, WC, age, SBP, DBP, cholesterol, triglycerides, HDL cholesterol. Using multiple linear regressions we obtained significance for WC, SBP and DBP. The coefficient of determination was 0.062 showing that only 6.2% of the variation in glucose levels can be explained by the model. Such a small percentage is unacceptable.

2) *Analysis of associations by the correlation method*. Our data do not show a normal distribution of blood glucose levels. The correlation coefficient can be calculated by using Pearson (parametric) or Spearman (nonparametric) methods (tab. V).

TABLE V

Analysis of associations by correlation method

Blood glucose	**BMI**	**Waist circumference**	**Age**	**SBP**	**DBP**	**Cholesterol**	**Triglycerides**	**HDL**
Pearson Correlation	.159**	.162**	.014	.156**	.000	-.052	-.058	.005
Sig. (2-tailed)	.005	.004	.801	.006	.993	.365	.307	.933
N	311	311	311	311	311	311	311	311
Blood glucose	BMI	Waist circumference	Age	SBP	DBP	Cholesterol	Triglycerides	HDL
Spearman's Correlation	.160**	.172**	.014	.147**	-.002	-.057	-.127*	.109
Sig. (2-tailed)	.005	.002	.804	.009	.972	.317	.025	.056
N	311	311	311	311	311	311	311	311

** Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed)

Using the correlation method we obtained directly proportional relationships for BMI, WC and SBP. The other variables showed no statistical significance, meaning a lack of influence on blood glucose according to our data.

Comparison of fasting glucose at baseline and after one year of clinical and biological follow-up showed significant differences: the mean baseline blood glucose level was statistically significantly higher than that measured after 1 year of clinical and biological follow-up: 110.38+/-12.67 mg/dl *versus* 104.2+/-12.58 mg/dl ($p < 0.001$). We can say that there was an average drop by approximately 6 units between baseline and 1-year blood glucose levels.

DISCUSSION

The prevalence of diabetes mellitus (DM) varies significantly depending on the population studied, age, sex, socioeconomic status and lifestyle. According to IDF/WHO, the prevalence of DM will reach 9% in 2025. Type 2 DM accounts for 80-90% of all DM cases and is more common in overweight or obese persons. More than half of the patients have one or more chronic complications of DM at the time of diagnosis (5). Patients with type 2 DM have a 2-4 times higher risk for cardiovas-

cular disease (CVD) than nondiabetic population (6). CVD is a common complication of DM being responsible for 80% of deaths in people with diabetes (7). Selecting cases with glucoregulation disorders aimed at emphasizing the importance of active screening of impaired fasting glucose for the early diagnosis of DM and implicitly for initiating preventive measures and appropriate therapy. We found statistically significant differences in terms of both anthropometric parameters and biological elements compared to extended group presenting only increased WC. Another important finding was the statistically significant improvement of fasting blood glucose control after 1 year.

CONCLUSIONS

The difference is statistically significant of BMI ($p = 0.05$). In the MS group AHT was prevalent in males, 92.2%. Mean serum triglyceride level was 123.11+/-68.55 mg/dl *versus* 113.75+/-65.62 mg/dl in the MS group, significantly higher ($p < 0003$). Mean HDL cholesterol was 57.58+/-17.11 mg/dl *versus* 58.54+/-15.02 mg/dl, statistically significant difference ($p < 0.05$). The proportion of glucoregulation disorders was higher in the MS group. According to correlation method we obtained directly proportional relationship for BMI, WC, and high blood glucose. Comparison of fasting glucose levels at baseline and at 1 year follow-up revealed significant differences.

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USING ARCGIS SOFTWARE IN THE PRE-HOSPITAL EMERGENCY MEDICAL SYSTEM

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USING ARCGIS SOFTWARE IN THE PRE-HOSPITAL EMERGENCY MEDICAL SYSTEM (Abstract): **Aim**. To measure the accessibility to healtcare services in order to reveal their quality and to improve the overall coverage, continuity and other features. **Material and methods**. We used the software ESRI Arc GIS 9.3, the Network Analyst function and data provided by Ambulance Service of Iasi (A.S.I.) with emergencies statistics for the first four months of 2012, processed by Microsoft Office Excel 2010. As examples, we chose "St. Maria" Children's Emergency Hospital and "St. Spiridon" Emergency Hospital. **Results**. ArcGIS Network Analyst finds the best route to get from one location to another or a route that includes multiple locations. Each route is characterized by three stops. The starting point is always the office of Ambulance Service of Iasi (A.S.I.), a second stop at the case address and the third to the hospital unit chosen according to the patient's diagnosis and age. Spatial distribution of emergency cases for the first four months of 2012 in these two examples is one unequable, with higher concentrations in districts located in two areas of the city. **Conclusions**. The presented examples highlight the poor coverage of healthcare services for the population of Iasi, Romania, especially the South-West area and its vulnerability in situations of emergency. Implementing such a broad project would lead to more complex analyses that would improve the situation of pre-hospital emergency medical services, with final goal to deserve the population, improve the quality of healthcare and develop the interdisciplinary relationships. **Keywords**: HEALTCARE SERVICES, EUROBAROMETER, ROUTING, SERVICE AREA

Accessibility to health care services is conditioned by the real availability of the facilities offered by the healthcare system and real demand for health. Within the European Union, the access to healthcare services is officially decided at the legislative level, representing a fundamental right of the individual to receive medical treatment and prevention.

Access to healthcare systems is a criterion that reveals that system is well organized and optimally conducted, among other criteria: overall coverage, continuity, the possibility to choose the healthcare provider, rehabilitation, a.s.o. (1).

Measuring access to healthcare services represents an element of equity and a right of the customer who pay taxes to the provider, but the quality of services as well. In order to measure access to healthcare, the

Eurobarometer uses indicators such as: the distance to the nearest hospital (under 20 minutes, between 20 and 50 minutes, an hour or more); accessibility by foot to the nearest hospital (walking).

In the European Union, rapid access (under 20 minutes) to the hospital is not universal. Between 38 and 50% of the population has access to the hospital so quickly. It must be taken into account that small countries have an advantage, because the distance rural-urban is more reduced, such as The Netherlands or Estonia. Meanwhile, large countries such as France or Poland, recorded better access than Romania (2).

MATERIAL AND METHODS

In order to initiate the study, we used the software ESRI Arc GIS 9.3, the Network Analyst function and data provided by Ambulance Service of Iasi (A.S.I.) with emergencies statistics for the first four months of 2012, processed by Microsoft Office Excel 2010.

GIS is a software environment for spatial analysis that has, as main feature, the representation of the real world or geographical space as layers or thematic maps (3). ArcGIS Network Analyst provides network-based spatial analysis such as routing, travel directions, the nearest emergency unit (closest facility) and service or coverage area (service area). It is built as a own subsystem and uses an advanced network model (Network Data Model) that allows the use of data sets with multiple attributes (4).

RESULTS AND DISCUSSION

Route

ArcGIS Network Analyst finds the best route to get from one location to another or a route that includes multiple locations. If we have more inside route stops, displaying the best route will be based on the order of locations specified by the user. ArcGIS Network Analyst will display the best routing for that path. Whether it is about finding a simple path between two points or a path with a sequence of locations, people usually try to find the best route. But "the best route" can assume different things to different situations.

The best route may be the fastest, shortest or most scenic path, depending on the chosen impedance. *Impedance* is the barrier or the limit set by the user. If the chosen barrier is time, then the best route will be one of the fastest routes. Therefore, the best route may be defined as the path with the lowest impedance. Any valid attribute can be used as impedance to determine the best route (5).

Accessibility refers to the facility with which you can get to a location. In ArcGIS Network Analyst, accessibility can be measured in terms of travel time, distance or any other impedance network (6). Each route is characterized by three stops. The starting point is always the office of Ambulance Service of Iasi (A.S.I.), a second stop at the case address and the third to the hospital unit chosen according to the patient's diagnosis and age. The route display is performed in a few seconds, after careful selection of these three points.

Description of routes

- First route (yellow route), departing from the A.S.I. to 64 Pacurari Street, for a major emergency - diagnosis: seizures in a 26-year female patient, transported by a type B ambulance to Clinical Hospital of Neurology.
- Route two (blue route), starting from the A.S.I. to 5 Minerva Street, for a major emergency – diagnosis: broken membranes of a 35-year female patient, transported by a type A ambulance to "Cuza Voda" Hospi-

tal of Obstetrics and Gynecology.

- First route (purple route), departing from A.S.I to 6 Jelea Str., for a major emergency – diagnosis: attempted suicide of a 52-year male patient, transported by a type B ambulance to "St. Spiridon" University Emergency Hospital.
- First route (green route), departing from the A.S.I. to 10 Chimiei Blvd., for a major emergency – diagnosis: seizures in a 19-year male patient transported by a type C ambulance to Clinical Hospital of Neurosurgery (fig. 1).

Service areas

The Network Analyst may identify service areas around any location on a network. A service area is a region that encloses all accessible streets, representing the streets that are in the specified impedance area (7).

As examples, we chose “St. Maria” Children's Emergency Hospital and “St. Spiridon” Hospital. Spatial distribution of emergency cases for the first four months of 2012 in these two examples is one unequable, with higher concentrations in the districts of “Dacia”, “Alexandru cel Bun”, “CUG”. For “St. Maria” Hospital, the service area was performed compared to the emergencies cases in population age group under 18. It notes that it serves a small area of the surface of the city, which leads to solve the cases in a longer period of time, especially a small number of emergency cases. Districts that do not enclose in the service area (0-2000 meters) were located in the North-West and South-West of the city, such as districts of “Ticau”, “Copou” “Pacurari”, “Dacia”, “Galata”, “Nicolina”, and “CUG” (fig. 2).

Instead, for “St. Spiridon” Hospital, the service area covers the central area of the city, facilitating more rapid resolution of emergencies cases, due to a high accessibility to other districts. Districts that do not enclose in this service area is those of South-West and South-East (fig. 3). This representation was applied to emergency patients over 18 years old.

Nowadays, Public Health problems are much larger than the field of their application. Specialists depend on modern technologies such as GIS, and mapping of applications useful in their analyses. GIS leads to understanding and improving public health problems and improve quality of healthcare and increase accessibility.

Mapping and analysis solution consign to dispatchers and field workers vital information. It allows “911” centers use real data of incident to predict expected emergency calls. Mapping intelligence allows an ambulance to be put to a better location to reach an incident in the fastest time.

CONCLUSIONS

In this research, we have considered accurate exemplify of relationships between the access to healthcare services, the health of the population, and pre-hospital emergency medical services. The presented examples revealed the poor coverage of healthcare services for the population of Iasi, especially the South-West area and its vulnerability in situations of emergency.

Initially, we calculated the population accessibility to emergency care units from the City of Iasi, highlighting the fact that at this scale of analysis, we can correlate the centrality with the accessibility. Implementing such a broad project would lead to more complex analyses that would improve the situation of pre-hospital emergency medical services, with final goal to deserve the population, improve the quality of healthcare and develop the interdisciplinary relationships.

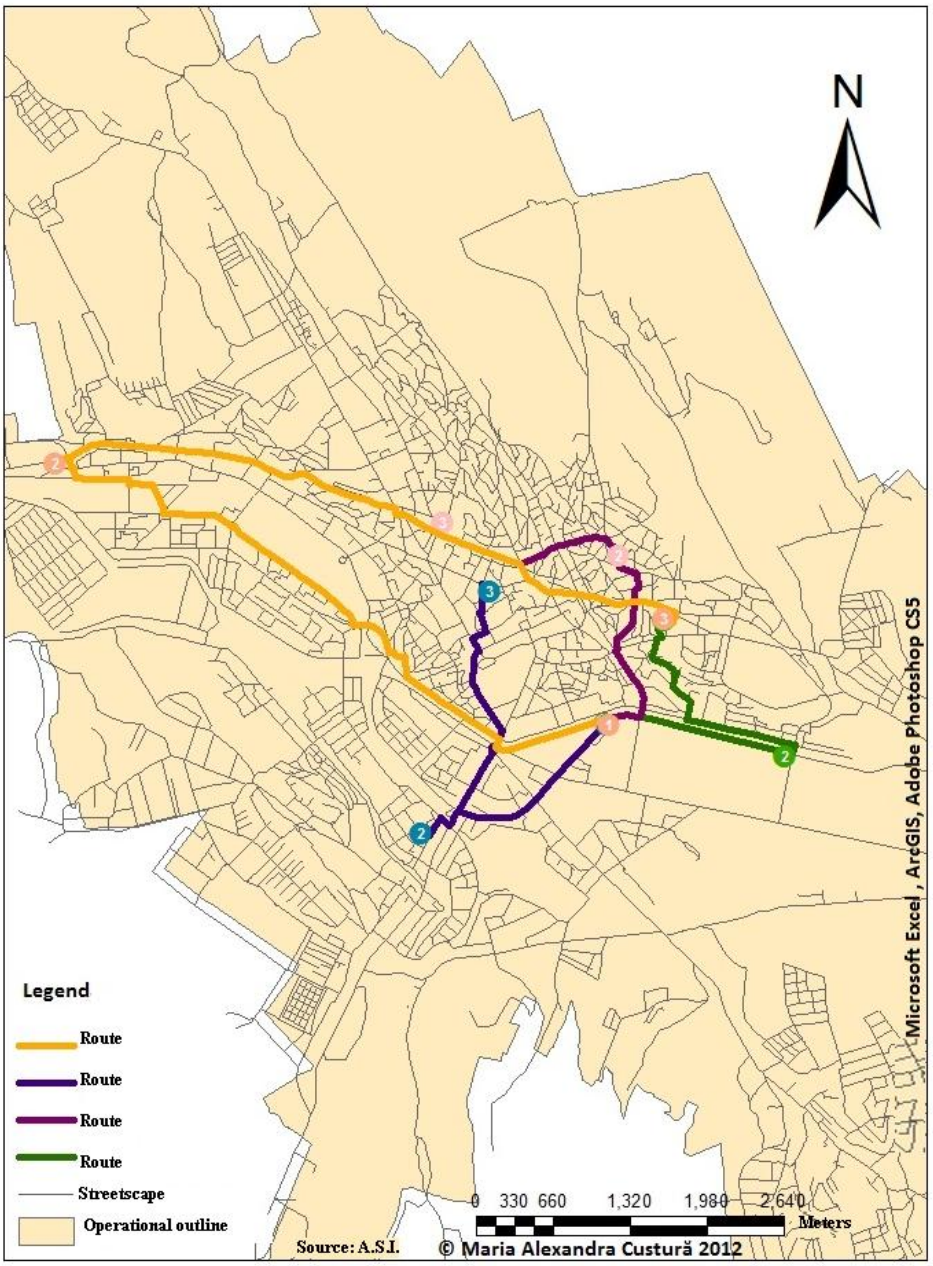

Fig. 1. Optimal routes for various medical emergencies, aleatory chosen from the database

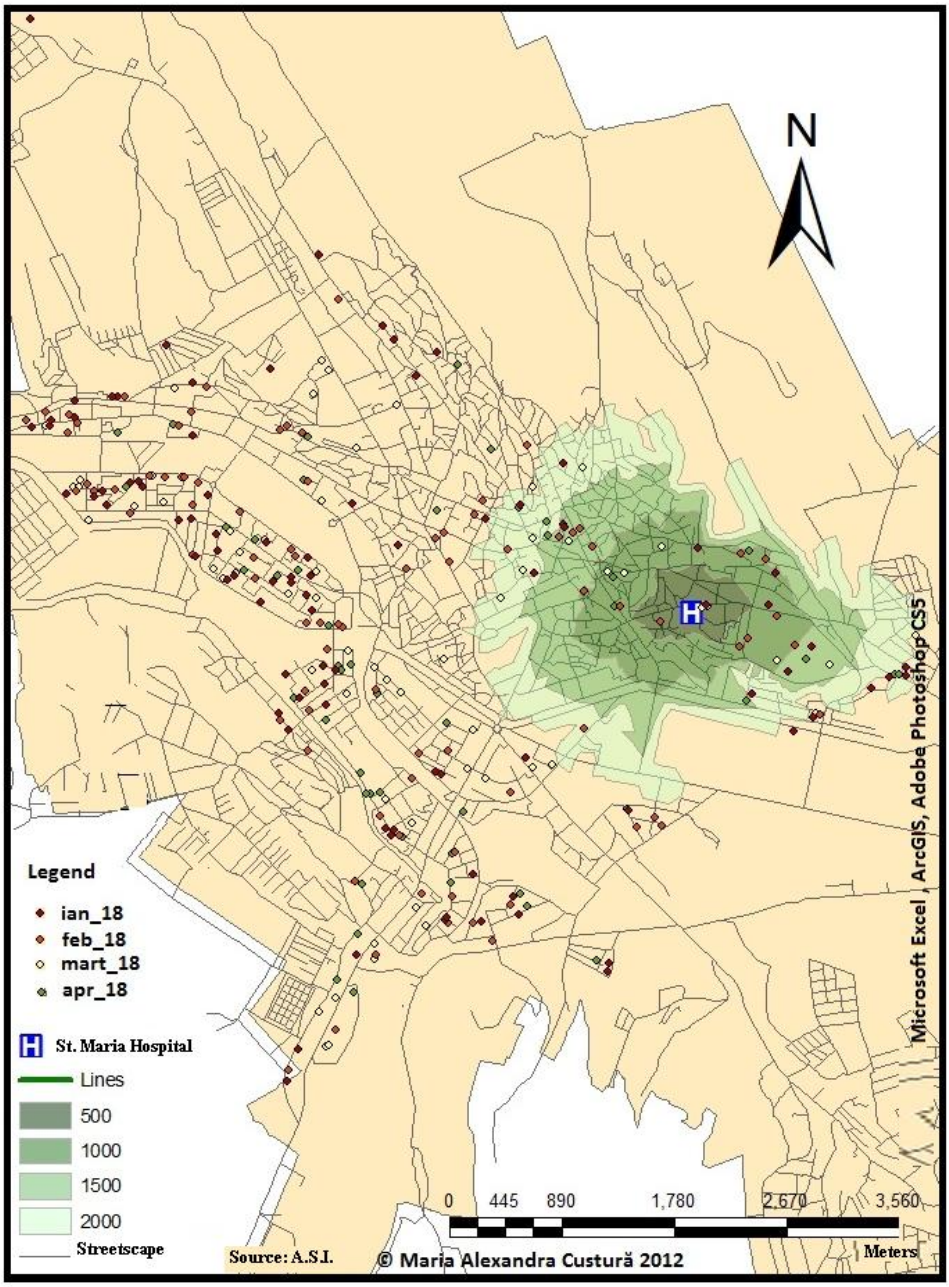

Fig. 2. Service area for "St. Maria" Children's Emergency Hospital

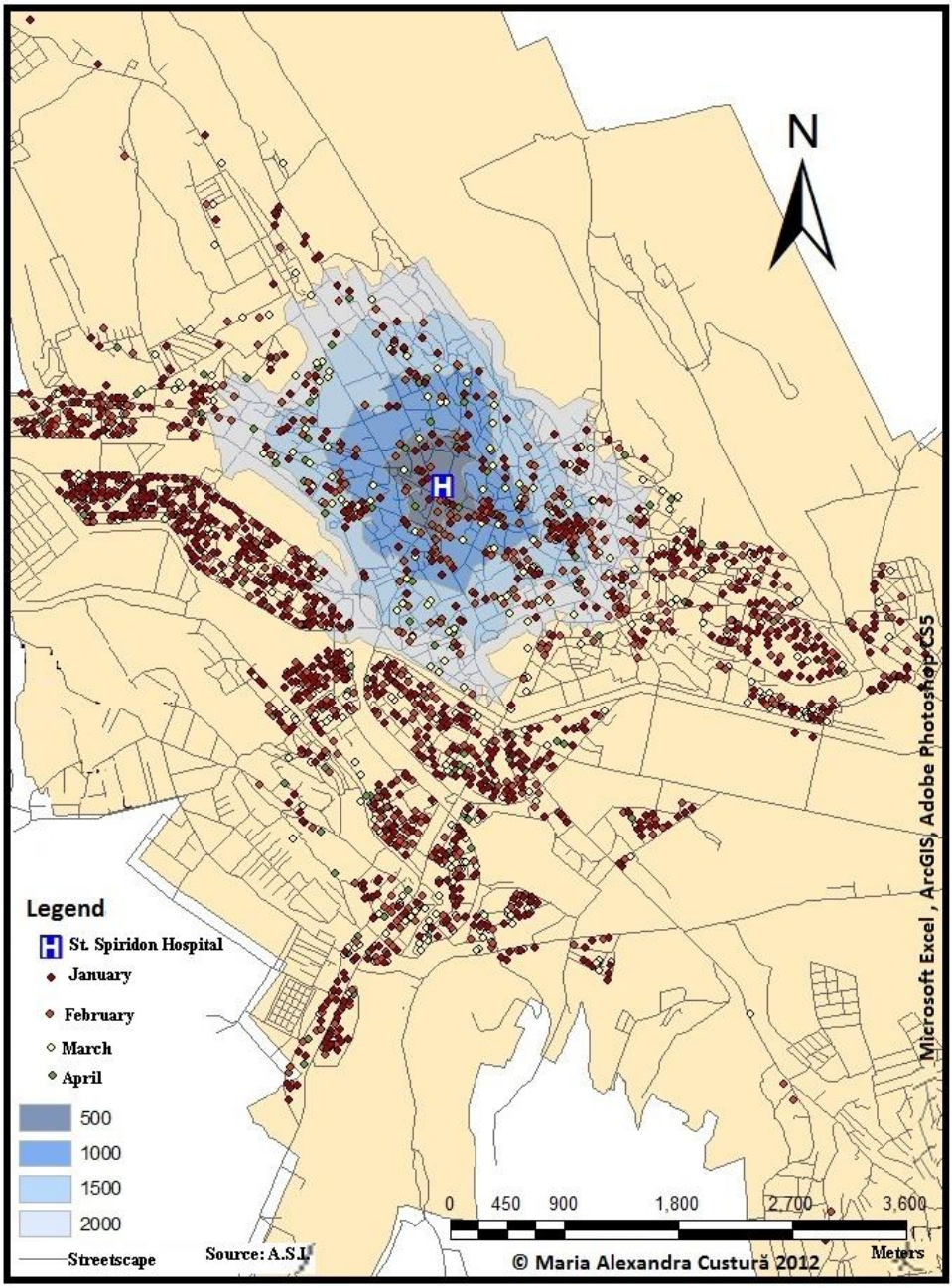

Fig. 3. Service area for "St. Spiridon" Emergency Hospital

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NEWS

CALCIUM AND VITAMIN D SUPPLEMENTS AND CHOLESTEROL PROFILE

Vitamin D has multiples roles in the calcium metabolism, modulation of cell growth, immune function and reduction of inflammation. Many studies have linked low levels of the vitamin D with an increased risk of type 1 diabetes, various types of cancers (breast, colon, prostate, and ovaries), heart attacks, rheumatoid arthritis or multiple sclerosis. In a recent study, Schnatz and colleagues assessed influence of calcium and vitamin D supplements on cholesterol profile in postmenopausal women. The researchers administered a daily dose of 400 units of vitamin D and 1,000 milligrams of calcium and respectively placebo to a group of 576 postmenopausal women. They followed serum 25-hydroxyvitamin D3 (25OHD3) concentrations and lipid levels before and after calcium and vitamin D administration. The results showed a significantly increase of serum 25OHD3 concentrations for women who took the supplement (24.3 ng/mL) compared with placebo (18.2 ng/mL) and a 4.46-mg/dL mean decrease in low-density lipoprotein cholesterol. In addition, the supplement users had elevated levels of high-density lipoprotein cholesterol and lower levels of triglycerides. These results support the importance of an increased serum level of 25OHD3 for improving cholesterol profiles (Schnatz PF, Jiang X, Vila-Wright S et al. Calcium/vitamin D supplementation, serum 25-hydroxyvitamin D concentrations, and cholesterol profiles in the Women's Health Initiative calcium/vitamin D randomized trial. *Menopause*, 2014; 1 DOI: 10.1097/GME.0000000000000188).

Cătălina Luncă

STUDY OF DENTAL AND SKELETAL DISORDERS IN MONO- AND DIZYGOTIC TWINS

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STUDY OF DENTAL AND SKELETAL DISORDERS IN MONO- AND DIZYGOTIC TWINS (Abstract): The aim of this study was to analyze and to compare the dental and skeletal morphology of mono- and dizygotic twins (MZ and BZ). **Material and methods:** 10 sets of monozygotic (mean age: 18.5 years) and 10 sets of dizygotic (mean age: 19.7 years) twins were examined, cast analysis, ortopantomography and lateral cephalometric films were analyzed. **Results:** The upper frontal teeth dimension and shape is the same in 99.69% of MZ and 93.91% of the DZ twins. In MZ twins we found different values for transversal arch width and upper intercanine distance. The dental malpositions were different in the DZ sets, especially in tranversal and sagital plan. The same differences were seen in the arch forms. The skeletal pattern was also different between the components of DZ twins. The cephalometric values showed slight differences of the interincisal angle both in MZ and DZ twins. **Conclusions:** Genetically influenced patterns can be explained by the results of our study.
Keywords: MOMOZYGOTIC TWINS, DIZYGOTIC TWINS, MALOCCLUSIONS

A twin is one of two offspring produced in the same pregnancy. Twins can either be monozygotic ("identical"), meaning that they develop from one zygote that splits and forms two embryos, or dizygotic ("fraternal") meaning that they develop from two eggs, each fertilized by separate sperm cells.

The twin method is one of the most effective methods available for investigating genetically determined variables in orthodontics, as well as in other medical fields, depending on the variance in the shape and the size of skull and teeth, both on genetic and environmental influences. In the meantime, the results of these studies cannot be always transposed to the singleton population, unless extensive analysis of the parents and nutritional, functional habits is made (1).

The purpose of this study was to analyze the dental and skeletal pattern, the type of malocclusion in the components of

MZ and DZ sets.10 MZ (5 boys and 5 girls, mean age: 18.5 years) and 10 DZ (same sex, 7 boys and 3 girls, mean age: 19.7 years) consisted the analyzed sample. In each case dental casts, panoramic X-ray and lateral cefalograms were studied.

At the dental cast analysis we examined every tooth's position in all of the three planes, the position of the frontal and lateral dental groups, the shape of the arches, the symmetry of the arches (symmetroscopy), the transversal, sagital and vertical development of the dental arches (Pont and Korkhaus index, intercanine width), we studied the static occlusion in all of the three planes (2, 3, 4).

On the panoramic X-rays we followed the presence of all teeth, the shape and the development stage of the roots, agenesis or supranumerary teeth (fig. 1).

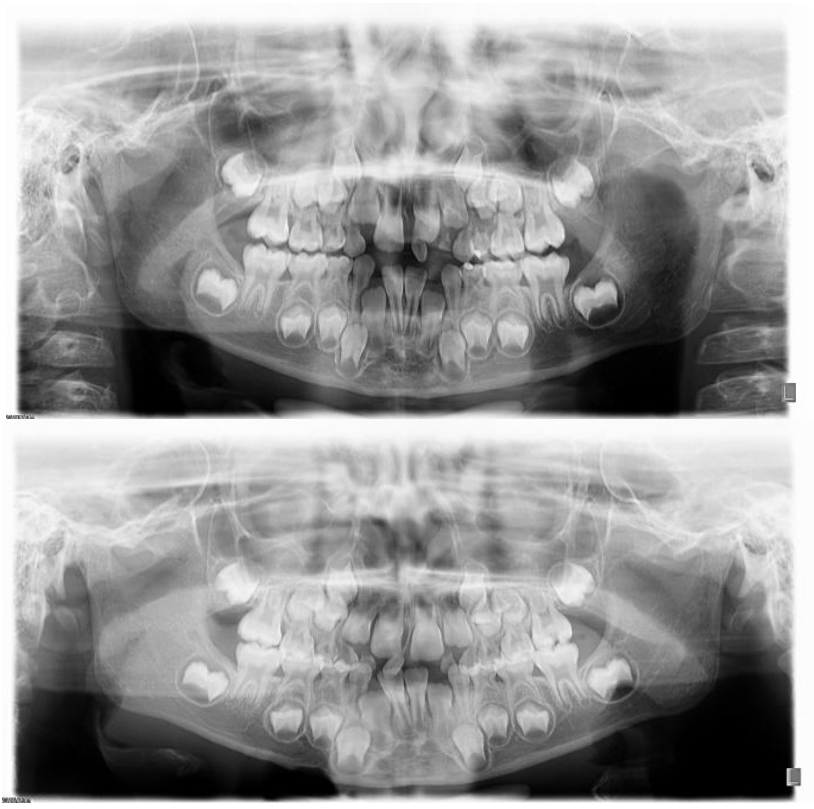

Fig. 1. H.K. and H.L., MZ twins (8 years) – simetrical supranumerary teeth

The lateral cefalogram were interpretated using the Tweed analysis technique. Cefalograms were traced according the mentioned technique with different colored pencils in case of a twin set. Frankfort-mandibular incisal angle (FMIA), Frankfort-mandibular plane angle (FMA), incisor-mandibular plane angle (IMPA), sella-nasion-point A (SNA), sella-nasion-point B (SNB), point A-nasion-point B (ANB), sella-gnation-Frankfort (SGn-F) and interincisal angles were measured. We superimposed each pair of cefalogram on sella-nasion plane in S (6,7) (fig. 2).

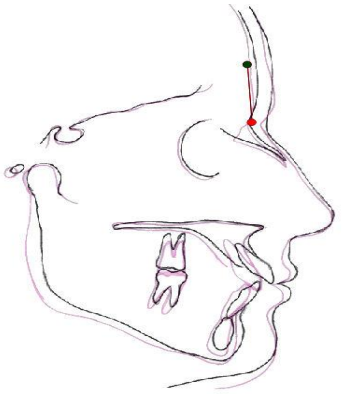

Fig. 2. Superimposition of lateral cefalograms of a set of MZ twins

RESULTS

The upper anterior teeth dimension and shape were similar in 99.69% of MZ and 93.91% of DZ sets. Upper arch transversal width was found different in MZ sets, especially in intermolar region. Intercanine width was slightly dissimilar in the upper arch in the MZ sets (fig. 3, 4). We found difference in the static occlusion analysis, 4 sets of DZ twins presented different sagital relationship in molar and canine region.

As far as dental malpositions are concerned, only 50% of both MZ and DZ sets presented symmetrical dental positions, in 5 MZ twins we found a so called "mirror image": left ectopic or retained canine in one, right ectopic or retained canine in the other component of the twin set or different way of mandibular shift at the two components (fig. 5).

In cefalometric analysis we found slight difference in the IMPA and interincisal angle both in MZ and DZ sets.

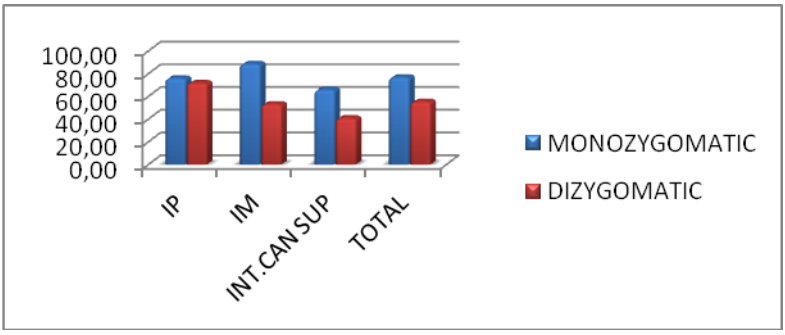

Fig. 3. Comparison of transversal arch width measurements in the upper arch

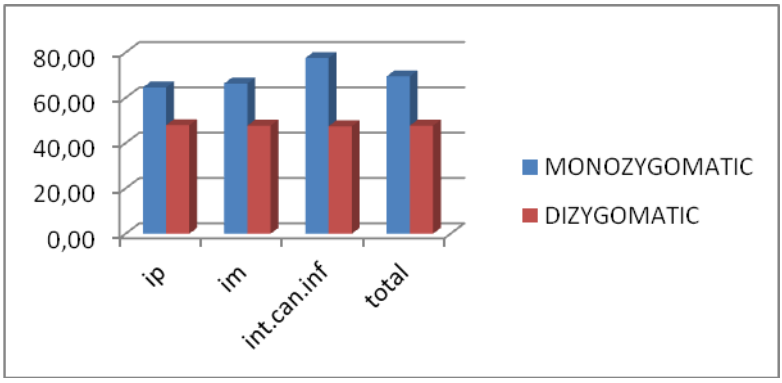

Fig. 4. Comparison of transversal arch width measurements in the lower arch

Fig. 5. Ectopic canines in mirror image in a MZ twin set

DISCUSSION

Since Francis Galton's classic paper (8) of 1875, twin studies have been viewed as an ideal way to evaluate the interaction between genetic (nature) and environmental (nurture) influences on a particular phenotype, including dentofacial variations (9). Biologically, twinning results from a single fertilized ovum; so MZ twins are identical genetically. The chance that both MZ twins would express the same uncommon abnormality or condition as a random happening is highly unlikely (10).

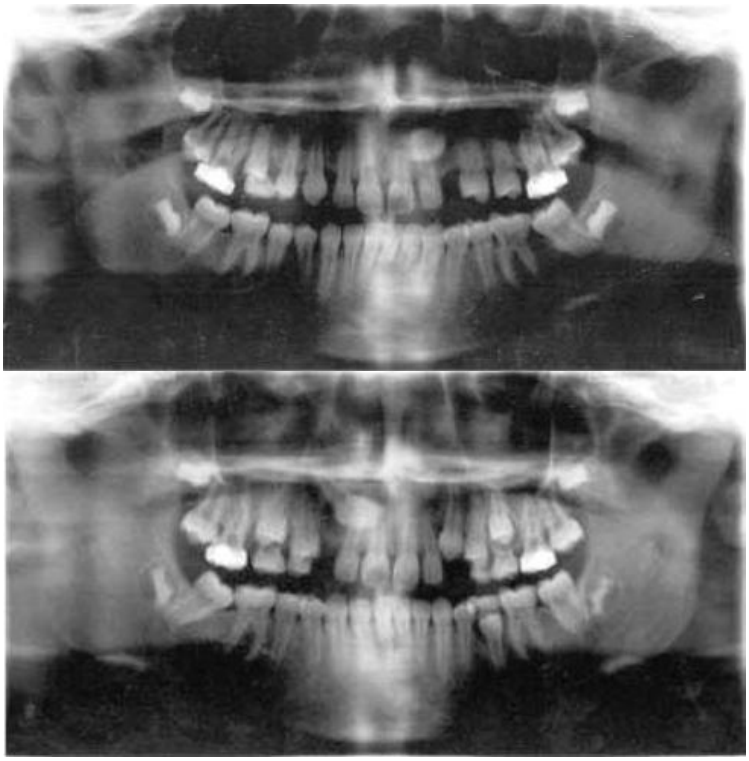

Fig. 6. Retained canines in mirror image in a MZ twin pair (Cs.B., Cs.N - 10 years)

The study of craniofacial relationships in twins has provided much useful information concerning the role of heredity in malocclusion. The procedure is based on the underlying principle that observed differences within a pair of monozygotic twins (whose genotype is identical) are due to environment and that differences within a pair of dizygotic twins (who share 50 per cent of their total gene complement) are due to both environment and genotype. A comparison of the observed within-pair differences for twins in the two categories should provide a measure of the degree to which monozygotic twins are more alike than dizygotic twins. The larger this differences between the two twin categories, the greater the genetic effect on variability of the trait. This model implies that zygosity is accurately determined and that environmental effects are equal in the two twin categories. At the present time, accurate zygosity classification is seldom a problem due to the ability to identify the large number of available polymorphic blood group and enzyme markers. (11).

The dentitions and faces of twins provide a good opportunity to study the fascinating phenomenon of mirror-imaging, in which one twin mirrors the other for one or more features (fig. 6). As Mosey (11) has pointed out, 'the key to the determination of the etiology of malocclusion, and its treatability, lies in the ability to differentiate the effect of genes and environment on the craniofacial skeleton in a particular individual'. Twin studies have confirmed that genetic influences contribute to variation in occlusal traits to varying degrees, with tooth size and arch shape displaying relatively high heritability, whereas heritability estimates for inter-arch variables such as overbite and over jet are considerably lower (12)

However, these findings have not led to major changes in the clinical management of malocclusions (13).

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NEWS

OSTEOPOROSIS AND PERIODONTAL DISEASE

Osteoporosis (OP) is a systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in fragility and susceptibility to fracture of bones. In the past, OP was considered a physiological process associated with ageing, but today it is recognized as a multifactorial chronic systemic disease. OP may affect also the jawbones, whose structure may be impaired by other conditions resulting in bone loss. One of these, is periodontitis (PD), a chronic infection-mediated condition modulated by different genetic and environmental factors, characterized, in advanced forms, by loss of the soft tissue attachment to teeth and resorption of alveolar bone . PD is the prototype of a low grade local infection (bacteria of the oral plaque) associated with local (within the periodontal tissues) immune-inflammatory response causing periodontal tissue damages/destruction and a mild individual systemic inflammatory response contributing to the global inflammatory burden and to its related dangerous effects. It might be expected that the alveolar bone destruction seen in periodontitis could be magnified in the presence of generalized skeletal disturbances such as OP. Thus, a relationship between OP and PD might be probable, but further prospective and sensitive studies are required in order to provide definitive evidence. By now, available data underline the primary importance of dentists in the early diagnosis of OP, because of the opportunity to assess the health of the entire skeleton of the patient through dental radiography. This is of considerable clinical interest, considering that such dental radiological investigations are routinely performed for diagnosis and treatment of dental and periodontal diseases, which are particularly frequent in the same population affected by OP. This may also provide clues for new preventive strategies and/or early therapeutic approach resulting in a potential reduction of bone resorption and contributing to maintain bone biomechanical characteristics (e.g. architecture, remodelling, quality of matrix collagen and its mineralization). In fact, the prevention of OP is the most rational and modern approach to defeat the disease, and early diagnosis is one of the foundations of modern medicine; the dentist seems to have an important role not only in monitoring/maintaining the oral and periodontal health and its relationships with systemic health, including OP, but also in drafting diagnostic/therapeutic paths and participating in counselling for OP in collaboration with general practioners and other specialists (Rosario Guiglia, Olga Di-Fede, Lucio Lo-Russo. Osteoporosis, jawbones and periodontal disease. *Med Oral Patol Oral Cir Bucal*. 2013 Jan 1;18 (1):e93-9).

Doina Butcovan

IN VIVO BIOCOMPATIBILITY EVALUATION OF SOME *BIDENS TRIPARTITA* EXTRACTS IN MICE

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IN VIVO BIOCOMPATIBILITY EVALUATION OF SOME *BIDENS TRIPARTITA* EXTRACTS IN MICE (Abstract): Adaptogens represent a class of herbs frequently used as a unique and natural alternative medicine and herbal remedy for treating the many forms of stress and different other pathological conditions. *Bidens tripartite,* a flowering plant from the genus Bidens, family *Compositae*, subfamily *Asteroideae* was widely used in traditional medicine for its antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic and sedative effects. Phytochemical analysis of this plant has revealed the presence of flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins. **Aim:** The *in vivo* biocompatibility evaluation of two extracts from *Bidens tripartita* plant in rats. **Material and methods:** The vegetable product used for the study was obtained after maceration and extraction in alcohol. Flower powder was dissolved in absolute chloroform, re-extracted and filtered. After a complete dryness the product was extracted by the addition of ethanol then evaporated. The chemical composition of the extracts was determined. The administered dose of *Bidens tripartita* retained was 1/20 of lethal dose 50 (LD50). The experiment was carried out on white male Wistar rats (200-250g) divided into 3 groups of 7 animals each treated intraperitoneally as follows: Group I (Control): distilled water 0.1ml/10g weight; Group II (coded BT-alcoholic): 200mg/kbw alcoholic *Bidens tripartita* extract; Group III (coded BT-aqueous): 250mg/kbw aqueous *Bidens tripartita* extract. The biocompatibility properties of alcoholic and aqueous extracts from *Bidens tritartita* were studied by assessing their effects on blood count and serum biochemical tests. The following immune parameters: phagocytic capacity of peripheral neutrophils (NBT test) and serum complement activity were also evaluated. The data were presented as +/- SD and significance was tested by SPSS for Windows version 13.0 and ANOVA method. Experimental protocol was implemented according to the recommendations of the University Committee for Research and Ethical Issues and guidelines of IASP Committee for Research and Ethical Issue. **Results**: Laboratory analysis did not show significant differences on leucocyte formula (GOT, GPT and LDH) or immune parameters (phagocytic capacity of peripheral neutrophils and serum complement activity) between alcoholic and aqueous *B. tripartita* extracts and distilled water, elements suggesting a good *in vivo* biocompatibility. **Conclusions**: In our experimental conditions, the alcoholic extract and aqueous extract from *B. tripartita* determined similar immune responses as distilled water following intraperitoneal administration in rats, indicative of good in vivo biocompatibility. **Keywords**: BIDENS TRIPARTITA, IN VIVO BIOCOMPATIBILITY, RATS.

Medicinal plants and herbs contain substances known to modern and ancient civilizations for their properties.

The word and concept of an adaptogen is a relatively new way of describing a type of remedy commonly found in traditional medicine (1, 2).

Adaptogens represent a class of herbs frequently used as a unique and natural alternative medicine and herbal remedy for treating the many forms of stress and different other pathological conditions (3, 4).

An adaptogen has non-specific activity and acts by increasing resistance of the body to a various adverse biological, chemical, and physical factors (5, 6).

The active substances present in these plants have the ability to balance endocrine hormones and the immune system, and they help the body to maintain optimal homeostasis and tend to help regulate or normalize organ and system function within the organism (7). They modulate the body response to stress (physical, environmental, or emotional) and help regulate the interrelations between endocrine, immune, and nervous systems (8).

Literature data show that around 80 percent of the world population largely depends on traditional natural medicine. Most of these natural remedies are based on medicinal plants and herbs, with some 35,000 plant species being used (9). Plants have evolved many protective defenses against pests and infection in the form of phytonutrients. These defenses are better developed in organic foods because of selective adaptive pressures necessary to resist pests, infections, and variable climate conditions (10, 11).

During time studies on plants were very limited. It can be estimated that only about a third of the million or so species of higher plants have been identified and named by scientists, and of these only a small fraction has been studied (12).

Bidens tripartita, a flowering plant from the genus Bidens, family *Compositae*, subfamily *Asteroideae* was widely used in traditional medicine for its antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic and sedative effects (13, 14).

Bidens tripartita, an annual plant, 30-100 cm in height with yellow flowers is commonly known as Three-lobe Beggarticks, Three-part Beggarticks, Trifid Burmarigold.

Different phytochemical researches revealed that *B. tripartita* contains a significant amount of active substances, such as: flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins proved to be involved in the pharmacodynamic effects of the plant (15, 16, 17, 18).

This medicinal plant is usually used in the treatment of acute respiratory infection, catarrhal rhinitis, angina, colitis, gout, or chronic dysentery (19, 20).

The present study is an *in vivo* biocompatibility evaluation of extracts from *Bidens tripartita*. The influence of this vegetable product on blood count, immune parameters level, some serum enzymes activity and other biochemical constants were assessed. Changes in different blood cells counts and levels of biochemical parameters were estimated up to 24 hours following *B. tripartita* extract intraperitoneal administration in mice.

MATERIAL AND METHODS

Animals. White male Wistar rats (200-250 g) were used. Lighting was on a 12 hours light/dark cycle (lights on at 6:00

a.m.), with standard laboratory food and tap water freely available, except during the time of the experiments. Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

Procedure. The vegetable product used for the study was obtained after maceration and extraction in alcohol. Flower powder was dissolved in absolute chloroform, re-extracted and filtered. After a complete dryness the product was extracted by the addition of ethanol then evaporated. The chemical composition of extracts was determined (21). The administered dose of *Bidens tripartita* retained was 1/20 of lethal dose 50 (LD50). The experiment was carried out on white male Wistar rats (200-250 g) divided into 3 groups of 7 animals each treated intraperitoneally as follows:

Group I (Control): distilled water 0.1 ml/10 g weight;

Group II (coded BT-alcoholic): 200 mg/kbw alcoholic *Bidens tripartita* extract;

Group III (coded BT-aqueous): 250 mg/kbw aqueous *Bidens tripartita* extract.

All experimental procedures involving the use of animals were conducted according to the standards of the Ethical Committee on Animal Experiments at the "Grigore T. Popa" University, which are in compliance with the ethical standards of the European Community. Each animal was used only once and the duration of the experiments was kept as short as possible. For ethical reasons, all animals were sacrificed at the end of the experiment (22).

We evaluated the acute toxicity of *Bidens tripartita* extracts by assessing their effects on blood count, serum biochemical tests, and some immune system parameters.

Twenty-four hours and 14 days after substances administration, blood samples (0.5 ml) were taken from retro-orbital plexus to assess blood count, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) activity.

We also investigated the following immune parameters: phagocytic capacity of peripheral neutrophils (Nitro Blue Tetrazolium test-NBT test), and serum complement activity.

The NBT test highlights the metabolic modifications that occurred during ingesting, quantitatively related to phagocytosis and identified through the reduction of the Nitro Blue Tetrazolium (NBT). The hemolytic activity of complement in serum was determined by its functional ability to lyse red blood cells in vitro.

Statistical analysis of data. The data were presented as +/- standard deviation and significance was tested by SPSS Statistics for Windows version 13.0 and ANOVA method. P-values less than 0.05 were considered statistically significant compared to controls.

RESULTS AND DISCUSSION

There were no statistically significant differences between the percentage values of leucocyte formula elements in the peripheral blood between the laboratory animals which systemically received alcoholic *B. tripartita* extract or aqueous *B. tripartita* extract and controls (tab. I).

Laboratory analysis did not show significant differences in GOT, GPT and LDH levels between alcoholic *B. tripartita* extractm, aqueous *B. tripartita* extract and distilled water, elements suggestive for a good *in vivo* biocompatibility (tab. II).

There were no statistically significant differences in the phagocytic capacity of peripheral neutrophils and serum comple-

ment activity levels between rats treated intraperitoneally with alcoholic or aqueous *Bidens tripartita* extract and those treated systemically with distilled water (tab. III).

TABLE I

Differential white cell count (leukocyte formula) determination in the control group and in groups treated with alcoholic *Bidens tripartita* extract and aqueous *Bidens tripartite* extract, respectively. Values are expressed as mean ± S.E.M. *p<0.05, **p<0.01 *vs* control

Groups/Time		Leucocyte formula				
		% values				
		PMN	Ly	E	M	B
Distilled water	24 hrs	29.8±0.2	65.2±0.2	0.7±0.1	3.7±0.1	0.2±0.05
	14 days	29.7±0.4	65.3±0.2	0.8±0.05	3.8±0.2	0.2±0.1
BT alcoholic	24 hrs	29.5±0.1	65.6±0.1	0.6±0.1	3.8±0.2	0.2±0.1
	14 days	29.6±0.1	65.7±0.2	0.8±0.05	3.6±0.1	0.2±0.05
BT aqueous	24 hrs	29.8±0.2	65.3±0.2	0.9±0.1	3.7±0.1	0.2±0.1
	14 days	29.7±0.3	65.5±0.3	0.7±0.05	3.6±0.1	0.2±0.1

TABLE II

Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase activity determination in control group and in groups treated with alcoholic *Bidens tripartita* extract and aqueous *Bidens tripartita* extract, respectively Values are expressed as mean ± S.E.M. *p<0.05, **p<0.01 *vs* control

Groups/Time		GPT (U/ml)	GOT (U/ml)	LDH (U/l)
Distilled water	24 hrs	42.5±2,4	98.2±3,6	343.26±58.17
	14 days	43.4±1.5	97.2±3.8	346.84±43.22
BT alcoholic	24 hrs	43.7±1,8	97.4±4,2	345.36±52.29
	14 days	44.1±3.2	97.6±2.3	349.41±37.52
BT aqueous	24 hrs	43.6±1,8	98.2±1,7	347.17±54.33
	14 days	42.9±1.8	98.7±2.2	348.43±37.65

TABLE III

Phagocytic capacity of peripheral neutrophils (NBT%) and serum complement activity evaluation in control group and in groups treated with alcoholic *Bidens tripartita* extract and aqueous *Bidens tripartita* extract, respectively. Values are expressed as mean ± S.E.M. *p<0.05, **p<0.01 *vs* control

Groups/Time		NBT (%)	Serum complement activity (UCH_{50})
Distilled water	14 days	15.17	53.62
BT alcoholic	14 days	15.67	54.18
BT aqueous	14 days	15.33	53.22

CONCLUSIONS

It has been demonstrated that the alcoholic *B. tripartita* extract and aqueous *B. tripartita* extract intraperitoneally administered in rats determined modifications of blood parameters and biochemical responses similar to those of distilled water, indicative of good in vivo biocompatibility.

Systemic administration of alcoholic and aqueous extracts from *B. tripartita* in rats did not alter their immune reactivity as compared with controls.

These studies suggest that alcoholic and aqueous extract from *B. tripartita* may be suitable for *in vivo* use as a drug delivery system.

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NEWS

N-3 FATTY ACIDS IN PATIENTS WITH MULTIPLE CARDIOVASCULAR RISK FACTORS

In previous trials, administration of omega 3 fatty acids have proven beneficial in patients who have been diagnosed with myocardial infarction or heart failure. The present trial was assessed to verify a possible benefit in patients who have a combination of cardiovascular risk factors and subclinical atherosclerotic disease, but had no history of myocardial infarction. Patients were randomized to receive omega-3 fatty acids (1 g / day) or placebo (olive oil). After 5 years of follow-up, in which 6244 patients received omega-3 fatty acids and 6269 patients received placebo, the number of deaths, heart attacks and brain were 733 in patients who received omega-3 fatty acids and 745 in that receiving placebo ($p = 0.58$). In conclusion, prophylactic administration of omega 3 fatty acids in a dose of 1 g / day has not been shown effective in reducing cardiovascular morbidity and mortality in patients accumulating cardiovascular risk factors. (The Risk and Prevention Study Collaborative Group, n-3 Fatty Acids in patients with multiple cardiovascular risk factors, *N Engl J Med* 2013; 368: 1800-1808).

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VALUE OF ICDAS INDEX IN A PRESCHOOL COMMUNITY FROM IASI

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VALUE OF ICDAS INDEX IN A PRESCHOOL COMMUNITY FROM IASI (Abstract): The International Caries Detection and Assessment System (ICDAS) is a clinical scoring system for use in dental education, clinical practice, research, and epidemiology. The ICDAS is designed to: lead to better quality information to inform decisions about appropriate diagnosis, prognosis, and clinical management at both the individual and public health levels. It provides a framework to support and facilitate personalized management of the caries process and dental decay for improved long term health outcomes. **Aim:** This baseline study aims to find out the distribution of the caries lesions in primary teeth and their associations between the socio-economic statuses of families, in an Iasi town preschool community (Romania). **Material and methods:** The study series included 500 children (276 girls, 224 boys), aged 3-7 years old (43 subjects aged 3-3, 9 years, 126 subjects aged 4-4, 9 years, 186 subjects aged 5-5, 9 years, 136 subjects aged 6-6, 9 years and 9 subjects aged 7 years), attending a Iasi town kindergarten, in which the ICDAS index, was applied. **Results:** The visits to the dentist are statistically correlated only with the average number of healthy teeth from the frontal and the lateral area. In other areas of the dental arch, there is no statistically significant data, but in the frontal area, the children that had visited the dentist present an average number of 3.5 healthy teeth, and the children that had not been to the dentist present an average number of 3.717 healthy teeth. The number of teeth with enamel destruction without dentin visibility is much reduced in children also, statistically significances being present only in lateral mandibular and maxillary areas. Though in lateral mandible area, this value varies from 0 (for children between 6-7 years old), to 0.1176 (for children between 5-6 years old). The frequency of tooth brushing, has a significant influence on the (ICDAS = 4) value in lateral mandibular and maxillary areas, where these values decrease, as the tooth brushing is more often. Though in lateral maxillary area, the value is 0.066 for the children that brush their teeth twice a day, for the children that brush their teeth once a day, the value is 0.0353 and for the children that brush their teeth occasionally the value is 0.018. **Conclusions:** This study confirmed the high caries level in this region. The total caries experience indicators are normally higher, in the mixed dentition because the primary teeth have been exposed longer for the risk factors of dental caries for example frequent sugar snacks and drinks. It can be concluded that ICDAS method, gives much more relevant information about caries process than other methods. The distributions of the ICDAS codes are correlated between the primary and the permanent molars of the mixed dentition. **Keywords:** ICDAS INDEX, CHILDREN, DENTAL CARIES.

The International Caries Detection and Assessment System (ICDAS) is a clinical scoring system for use in dental education, clinical practice, research, and epidemiology (1, 2, 3).

The ICDAS is designed to: lead to better quality information to inform decisions about appropriate diagnosis, prognosis, and clinical management at both the individual and public health levels. It provides a framework to support and facilitate personalized management of the caries process and dental decay for improved long term health outcomes (4, 5).

It was found that, in the USA, dental caries has been synonymous with presence of cavitation, or decay. In Europe, in the research community, the understanding of dental caries appeared to be more advanced than in America. The standards for measuring caries include clinical stages of the disease process which precede cavitation, not simply the decay in the tooth. This is beginning to change, but at the time of the review, this information was accurate (6, 7).

The ICDAS group met in 2009 and developed Coronal Primary Caries Detection Criteria (8). The ICDAS detection codes for coronal caries range from 0 to 6 depending on the severity of the lesion (tab. I).

TABLE I

ICDAS - description of codes (8)

Codes	Description
0	Sound
1	First Visual Change in Enamel
2	Distinct Visual Change in Enamel
3	Localized Enamel Breakdown
4	Underlying Dark Shadow from Dentin
5	Distinct Cavity with Visible Dentin
6	Extensive Distinct Cavity with Visible Dentin

This baseline study aims to find out the distribution of the caries lesions in primary teeth and their associations between the socio-economic statuses of families, in an Iasi town preschool community (Romania).

MATERIAL AND METHODS

The study series included 500 children (276 girls, 224 boys), aged 3-7 years old (43 subjects aged 3-3,9 years, 126 subjects aged 4-4,9 years, 186 subjects aged 5-5,9 years, 136 subjects aged 6-6,9 years and 9 subjects aged 7 years), attending a Iasi town kindergarten, in which the ICDAS index, was applied.

Clinical examination was performed by four dentists in daylight. Tooth surfaces were visually inspected and the suspect surfaces were explored with the dental probe. The children were given a toothbrush and toothpaste and requested to brush their teeth before the examination. The written informed consent form was signed by the parent/caretaker. Very few par-

ents/caretakers did not want their child to participate, and some children were absent from school in the day of the examination. Data about the socio-economic status of children families were obtained through a questionnaire filled out by the parent. Statistical processing of the obtained data was performed using *SPSS 17.0*®.

RESULTS AND DISCUSSION

The visits to the dentist are statistically correlated only with the average number of healthy teeth from the frontal and the lateral area. In other areas of the dental arch there is no statistically significant data, but in the frontal area (fig. 2), the children that had visited the dentist present an average number of 3.5 healthy teeth, and the children that had not been to the dentist present an average number of 3.717 healthy teeth (U = 28494.000, p = 0.023). In the lateral area, the children that had visited the dentist present an average number of 4.978 healthy teeth compared children that had not been to the dentist have an average number of 5,438 healthy teeth.

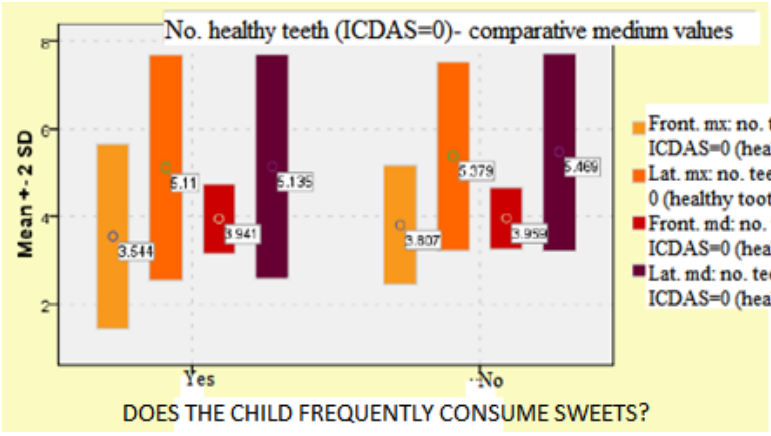

Fig. 1. Correlation between sweets consume and dental integrity

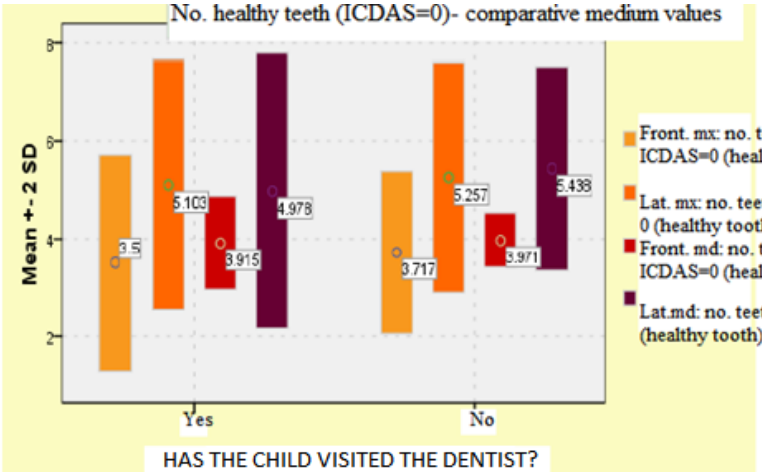

Fig. 2. Correlation between frequency of dentist visits and dental integrity

The frequency of sweets consumption affects the average number of teeth from the frontal and lateral maxillary area, lateral mandible area, but the frontal mandible area is not affected (fig. 1). The average number of teeth from this area is lower for the children that consume sweets compared to the children that do not consume sweets:

- in mandibular frontal area 3.54 (YES), compared to 3.807 (NO) – U=2307.000, p=0.006 statistically significant.
- in lateral maxillary area 5.11 (YES), compared to 5.379 (NO) – U=2319.500, p=0.045 statistically significant.
- in lateral mandibular area 5.135 (YES), compared to 5.649 (NO) – U=21613.500, p=0.001, statistically significant.

The same phenomenon happens with hard food consumption that influences the average number of healthy teeth from frontal maxillary area and lateral maxillary area, whiles the lateral mandibular area, is not influenced (fig. 3). The average number of healthy teeth is much higher in these areas, to the children that consume hard food compared to the children that do not have this eating habit.

- in frontal maxillary area a value of 3.708 (YES), compared to a value of 3.461 (NO) – U=25562.000, p=0.002 statistically significant.
- in lateral maxillary area a value of 5.286 (YES), compared to a value of 5.011 (NO) – U=25986.000, p=0.046 statistically significant.

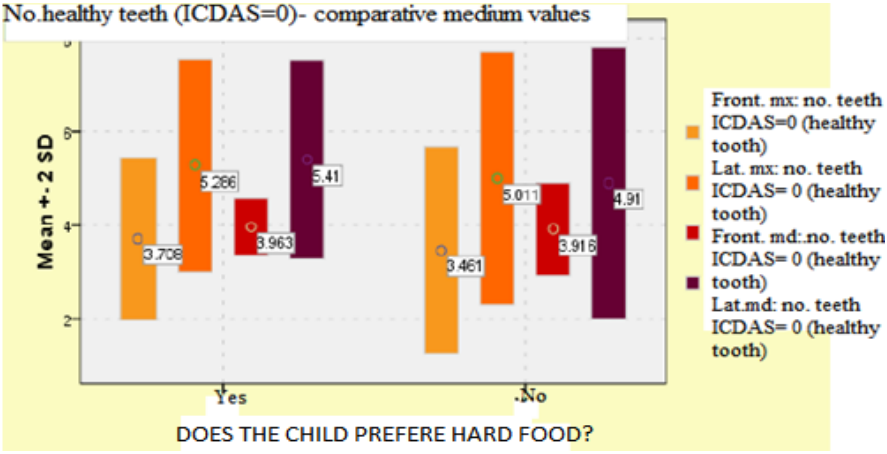

Fig. 3. Correlation between hard food consume and dental integrity

The number of teeth with enamel destruction without dentin visibility is much reduced in children also, statistically significances being present only in lateral mandibular and maxillary areas (fig. 4). Though in lateral mandible area, this value varies from 0 (for children between 6-7 years old), to 0.1176 (for children between 5-6 years old) p=0.017, which is statistically significant.

The frequency of tooth brushing, has a significant influence on the (ICDAS=4) value in lateral mandibular and maxillary areas, where these values decrease, as the tooth brushing is more often (fig. 5).

Though in lateral maxillary area, the

value is 0.066 for the children that brush their teeth twice a day, for the children that brush their teeth once a day, the value is 0.0353 and for the children that brush their teeth occasionally the value is 0.018, that is statistically significant (p=0,001).

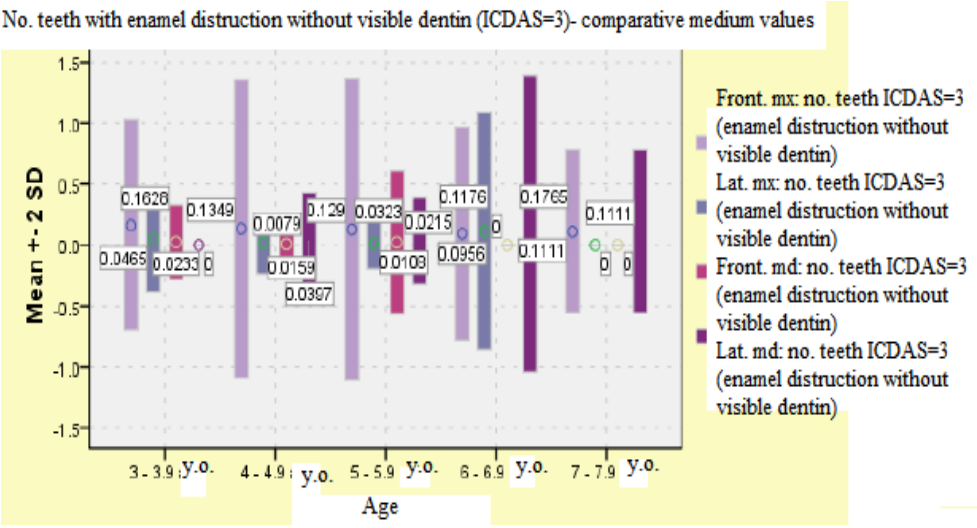

Fig.4. Distribution of teeth with enamel destruction without visible dentin

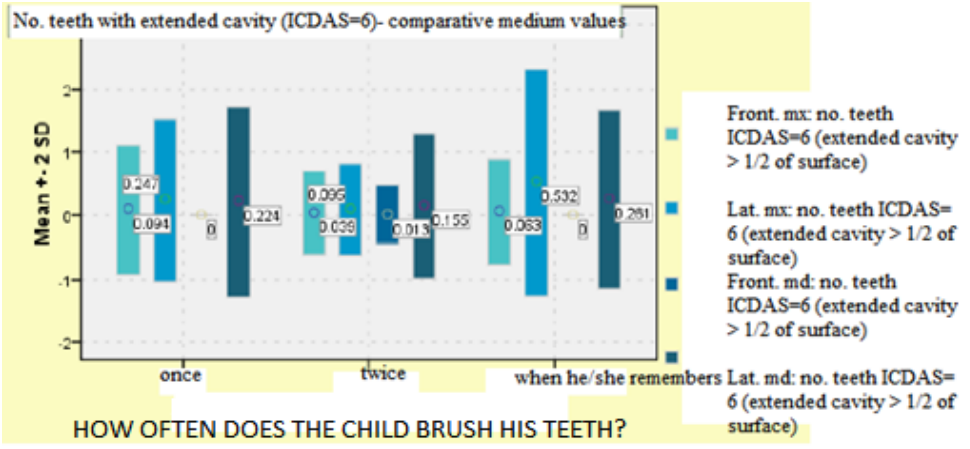

Fig.5. Correlation between teeth brushing frequency and caries cavity extension

The distribution of the ICDAS codes is difficult to explain. The code 2 (enamel caries detected on a wet tooth surface), might be easier to detect than the code 1 (enamel caries visible only when tooth surface is dry) and code 3 (minor cavitation on enamel only). This has been confirmed also in other ICDAS studies (9, 10).

CONCLUSIONS

This study confirmed the high caries level in this region. The total caries experi-

ence indicators are normally higher, in the mixed dentition because the primary teeth have been exposed longer for the risk factors of dental caries for example frequent sugar snacks and drinks.

High number of caries on the primary molars could be expected to predict caries in the permanent molars. The association of the number of caries lesions between the first permanent molars and the second primary molars was analyzed by Spearman correlation coefficient.

There were no statistical differences in the main number of dentine caries lesions according to the age. The older children could have been expected to have higher caries experience than the younger ones. However the older children had less dentine caries than the younger ones. This could be a worrying sign of the rapidly changing diet.

ICDAS is a especially valuable method for detecting the enamel caries lesions for planning the individual remineralization therapy or for monitoring the caries pattern at the population level.

It can be concluded that ICDAS method, gives much more relevant information about caries process than other methods. The distributions of the ICDAS codes are correlated between the primary and the permanent molars of the mixed dentition.

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THE ESSENTIAL ROLE OF CONE BEAM COMPUTED TOMOGRAPHY TO DIAGNOSIS THE LOCALIZATION OF IMPACTED MAXILLARY CANINE AND TO DETECT THE AUSTERITY OF THE ADJACENT ROOT RESORPTION IN THE ROMANIAN POPULATION

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THE ESSENTIAL ROLE OF CONE BEAM COMPUTED TOMOGRAPHY TO DIAGNOSIS THE LOCALIZATION OF IMPACTED MAXILLARY CANINE AND TO DETECT THE AUSTERITY OF THE ADJACENT ROOT RESORPTION IN THE ROMANIAN POPULATION (Abstract): The impaction phenomenon is a pathological condition which is defined as the lack of eruption of a permanent tooth in the oral cavity. After third molar, the first permanent tooth impacted in the alveolar bone is the permanent maxillary canine, with varies from 1% to 3%. The **aim** of this study was to use the most recently imaging technique- CBCT, to evaluate the position of the impacted maxillary canine and to determinate the severity of root resorption of the adjacent teeth. **Material and methods***:* The all 230 patients of this study were referred to a CBCT scanning with Scanora machine and only the region of clinical interest was irradiated. **Results:** The age of the subjects was distributed between the ages of 11 and 42 years. From a total of 364 impacted canine teeth, 92 were side impacted canines, 97 right impacted canines and 87 were left impacted canines. **Discussion**: We report a lateral incisors root resorption present in 54.37% and 24.29% the resorption of central incisors. **Conclusions**: The use of CBCT in patients with impacted canines has potential diagnostic and may influence the planning of treatment. Also the CBCT can be a genuine method used to rectify, to diagnose and to make a suggestive treatment for the resorption of the root of incisors. **Keywords**: CBCT, IMPACTED CANINE, ROOT RESORPTION.

The impaction phenomenon is a pathological condition which is defined as the lack of eruption of a permanent tooth in the oral cavity, and was reported for the first time in 1922. The first permanent tooth impacted in the alveolar bone is third molar. After the third molar, the most frequently impacted tooth is the permanent maxillary canine, with varies from 1% to 3% (1). The canine impaction may be either vestibular or palatal and, in Europeans, the palatal impaction is 2-3 times more frequent than the vestibular impaction (2).

Most recently, in our medical practice we can used 3D investigations to locate the impacted maxillary canine and to appraisal

the root resorption of the adjacent teeth. As a starter, several studies used the CT technique, but for certain reasons it was replaced with the CBCT, owing to a reduced cone shaped X-Ray beam, size of the scanner, radiation dosage, time needed for scanning and much lower costs (3, 4, 5).

According to the CBCT imaging, a more comprehensive orthodontic diagnosis and more accurate treatment planning is possible. In 2003, Becker and Chaushu (6) announced that for patients who were younger than 20 the success of the impacted maxillary canine was 100%, while for the patients who were older than 20 the success was only 69.5%.

Therefore, the aim of this study was to use the most recently imaging technique-CBCT, to evaluate the position of the impacted maxillary canine and to determinate the severity of root resorption of the adjacent teeth.

MATERIAL AND METHODS

The sample consisted of 230 patients (132 female and 98 male, ranging in age from 11 to 42 years) from the Department of Orthodontics, University of Medicine and Pharmacy "Grigore T .Popa" Iasi, Romania and from a private Office "Oral Dent". The scanning investigation was developed at the Department of Dental Radiology, University of Medicine and Pharmacy "Grigore T. Popa" - Iasi, Romania, at "DDI" and "Explora Rx" Office.

The patients were referred to a CBCT scanning and only the region of clinical interest was irradiated, using a dentoalveolar field of view (small FOV - approximately 5 cm or less). The scan parameters were exposure time 18 seconds, voxel size resolution of 125 μm, tube current of 5mA and a tube voltage of 80 kV.

RESULTS

The age of the subjects was distributed between the ages of 11 and 42 years. From a total of 230 patients, 132 were female (57.4%) and 98 were male (42.6%). Of the 364 impacted canine teeth from the subjects, 92 were both side impacted canines (fig. 1, 2), 97 right impacted canines and 87 were left impacted canines.

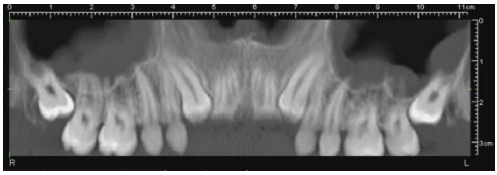

Fig. 1 Two-dimensional (2D) cone beam computed tomography image of a 19-year-old male with a bilateral impacted maxillary canine showing panoramic reconstruction for maxillary jaw.

Fig. 2. The cone beam computed tomography image of the same a 19-year-old male with a bilateral impacted maxillary canine showing axial slices, reveals a close contact with the lateral incisor and a large periapical space, highlighting an irregular outline which representing early apical resorption.

We selected the patients taking into account the age in 4 categories: group A (11-18 years), group B (19-26 years), group C (27-34 years), group D (35-42 years). We attend the position of canine, contact with incisors and incisors root resorption (tab. I).

We recorded following parameters (7, 8):

1. *the distance between the canine and*

occlusal plane in mm (fig. 3);

2. *the angle between the canine and occlusal plane;*
3. *the angle between the long axis of the canine and midline sagittal plane (fig. 3);*
4. *the angle between the long axis of the impacted canine and long axis of the central incisors in degrees (fig. 3);*
5. *relationship between the impacted canine and incisors:*

a) an existent contact- the distance between crown of the permanent canines and the adjacent incisors was less than 1 mm;

b) nonexistent contact- the distance between the crown of the permanent canines and the adjacent incisors was more than 1 mm.

6. *severity of root resorption to lateral incisors* (9)*:*

a) no resorption: intact root surfaces;

b) slight resorption (fig. 4 ,5): resorption extending up to half of the dentine thickness to the pulp;

c) moderate resorption (fig. 6): resorption midway to the pulp or more with the pulp lining being intact;

d) severe resorption (fig. 7): the pulp is exposed by the resorption.

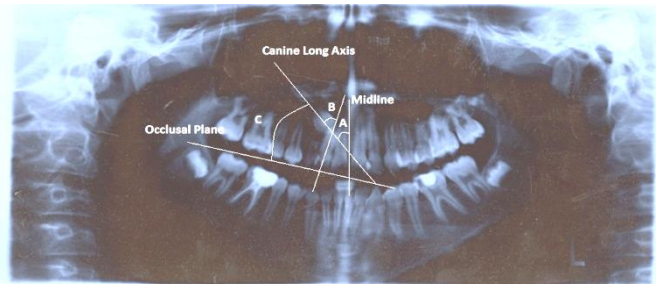

Fig. 3. Panoramic view illustrating reference lines and angular measurements. (A) Angle of impacted canine to the lateral incisor, (B) angle of the impacted canine to midline, and (C) angle of impacted canine to occlusal plane.

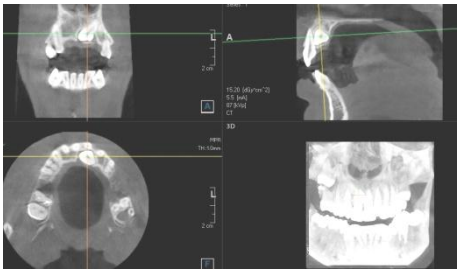

Fig. 4. The cone beam computed tomography image of a 21-year-old female with an impacted maxillary left canine showing axial slices (A); image B and C show the two-dimension (2D) reconstruction of coronal and respectively axial slices; as well as a 3D model that were used to exhibiting the left impacted maxillary canine superposed on the top of root of lateral incisor, highlighting slight root resorption of the left maxillary lateral incisor.

Fig. 5. Ortho-radial reconstruction through the center of lateral incisor shows a decrease radiopaque in the apical area, with a slightly irregular of the tip-apex which are suggestive for a slight root resorption.

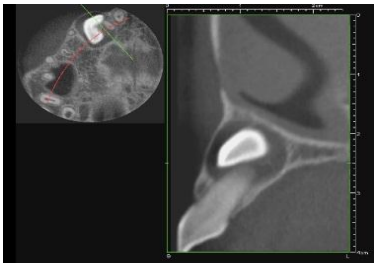

Fig. 6. Ortho-radial reconstruction tangent to the distal surface of the lateral incisor highlights the follicular sac that surrounds the lateral incisor apex, with a net reduction of apical opacity, suggesting a moderate resorption at this level.

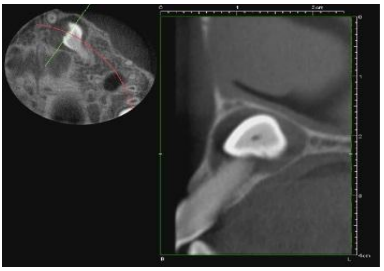

Fig. 7. Para axial reconstruction tangent to the distal surface of lateral incisor with a clear contact with the canine follicular sac, which cause resorption in the record of 1/3 of the apical area of the root, representing a severe root resorption.

TABLE I

Distribution of patients after the age in 4 categories and attend the position of canine, contact with incisors and incisors root resorption.

Age group	11-18		19-26		27-34		35-42	
	No.	%	No.	%	No.	%	No.	%
Canine localization Line of the arch	21	22.1	9	14.1	5	11.6	3	10.7
Palatally	42	44.2	30	46.8	23	53.5	14	50
Bucally	32	33.7	25	39.1	15	34.9	11	39.2
Contact with lateral incisor With contact	81	85.3	53	82.2	38	88.4	22	78.5
Without contact	14	14.7	11	17.2	5	11.6	6	21.4
Lateral incisor root resorption No resorption	42	44.2	29	45.3	23	53.5	11	39.2
Low resorption	31	32.6	21	32.8	13	30.2	13	46.4
Moderate resorption	21	22.1	11	17.2	7	16.3	3	10.7
Severe resortion	1	1	3	³.6	0	0	1	3.6
Affiliation of lateral incisor root resorption Cervical	9	9.3	4	6.2	1	2.3	2	7.2
Middle	18	19.3	16	25	7	16.2	7	25
Apical	26	27.1	15	23.4	12	27.9	8	28.6
Contact with central incisor With contact	20	10.5	14	21.8	9	20.9	7	25
Without contact	75	89.4	50	78.1	32	79.1	21	75
Central incisor root resorption No resorption	79	83.1	51	79.6	27	62.7	22	78.5
Low resorption	8	8.4	6	9.4	5	11.6	5	17.8
Moderate resorption	3	3.1	3	³.7	6	13.9	1	3.6
Severe resorption	5	5.3	5	7.8	5	11.6	0	0
Affiliation of central incisor root resorption Cervical	1	1	2	2.9	0	0	0	0
Middle	5	5.3	3	4.4	1	2.3	0	0
Apical	10	1	9	13.1	15	34.8	6	21.4

DISCUSSION

Many years 2D imaging modalities have been used in medical dentistry. Today, CBCT is the most frequently applied in

orthodontics, implantology, endodontics, maxillo-facial surgery (9).

A causative factor for the impacted canine is the lack of space. Many articles, describe that the etiology of the impaction canine remains unclear, but on the other hand, many studies sustain the fact that the impacted maxillary canines may result in several complications. It is very important to diagnosis the root resorption of the adjacent incisors when we have to plan the treatment for impacted maxillary canine. It has been demonstrated many time that with CT or CBCT it is possible to detect areas of resorption that are not detectable on the basic clinical and intraoral radiographs (7).

In this study lateral incisors root resorption were present in 54.37% and 24.29% the resorption of central incisors. If we compare with Ericson et al. study (5), the percentage of lateral incisors resorption was 38% in a CT study of 156 ectopically impacted maxillary canines lower than 54.37%, but also the reported percentage by Walker et al. (7) of 66.7% was higher than ours. On the other side, the percentage of the central root resorption was higher than reported by Ericson and Kurol (5), 9 % and Walker et al. (7), 11.1% . Walker et al. (7) evaluated all 27 patients of different ages with the NewTom QR-DVT 9000 and Ericson et al. (5) evaluated all 107 children with basic clinical and intraoral radiographic investigation which were supplemented with the computerized tomography. In the present study, radiological investigations consisted in using only CBCT. Obviously differences between studies may be explained based on the following criteria: age range and the ability and experience of using CT and CBCT images.

CONCLUSIONS

Since root resorption is asymptomatic, early detection by radiographic examination is essential for correct diagnosis and treatment. This technique is perfectly suited in cases of impacted maxillary canine and help us to reduce unnecessary radiation dose, cost and time.

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NONINVASIVE LASER THERAPY FOR OUTPATIENTS WITH CHRONIC INFLAMMATORY DISORDERS OF CERVIX

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NONINVASIVE LASER THERAPY FOR OUTPATIENTS WITH CHRONIC INFLAMMATORY DISORDERS OF CERVIX (Abstract): Chronic inflammation of the cervix can develop cervical stenosis with infertility and cervical congestion is related with the cervical cancer. We create a review of main etiological agents and methods of screening and diagnose. We also make a short brief review of modern therapeutic approach. **Conclusions**: We follow the utility of LLLT through the following aspects: evolution, indications, results of Babeş-Papanicolau screening, cytology, clinical aspects. The results of the study will allow the complex system of treatment to be used in a large category of women. We appreciate that the procedure (used in our center also) will decrease the cervical pathology, the morbidity inside the treatment, the mortality through the evolution of cervical cancer. We propose the applicability for outpatients first and then as an integrated treatment method inside hospitals for a wide access**.** **Keywords**: CERVICAL CANCER, LASER THERAPY, CHRONIC INFLAMMATION.

The evolution of chronic inflammatory disorders of uterine cervix (CIDC) has an insidious character which increases the difficulty of evaluation of symptoms and treatment. First symptoms of cervicitis as an inflammatory process are usually vaginal discharges which increase after menstruation, pruritus and congestion of external genital organs, dyspareunia, bleeding after sexual contact and/or between menstruation, dysuria, lower-back morbidity and/or hypogastric pain (usually during sexual intercourse) (2, 4, 6). In severe cases we can notice abundant vaginal discharge, with bad smell and intense pruritus and colicky abdominal pain. Chronic cervicitis can evolve for years only with abundant leucorrhea; the patient gets used with this condition, without fever, pain, menstrual disorders of damage of sexual life. Most frequently complication appears with the development of inflammation of pelvic proximity organs: parameters, salpingitis, acute or chronic infections of urinary tract.

Chronic inflammation of uterine cervix can develop in evolution a cervical stenosis, followed by infertility. Chronical cervical congestion is in relationship with cervical carcinoma (cervicitis, chronical inflammatory process uncontrolled and not treated in time, lack of prophylactic measures, external genital organs hygiene and routine checks 1-

2 times/year). Chronical cervicitis usually does not determine neoplasia but favor its development, confirming the national and international statistics (3, 7). The treatment of chronical cervicitis can be seen as a prophylactic measure in the fight against cervical cancer or other localized malignant tumors. Babeş-Papanicolau test is an integrated part of routine gynecological exam. The test is used for the identification of abnormal cervical cells. Abnormal result for this test is not quite unusual, because the cervical cells are undergoing permanent modifications (approximately 15% of women tested every year have abnormal result); only 1% of these results show constant modifications which can evolve to a cervical cancer (3, 7, 8).

Cervical diseases, their usual complications, noninvasive laser therapy treatments become an acuteness actuality and have a significant importance (5, 7) worldwide in contemporary gynecology. Actually used screening procedures, in a certain succession for those patients included in high risk categories allow precocious diagnose and best therapeutical approach. During 1990-2005 cytology was used as a screening method and allowed the diagnosis with a three times higher frequency for the cases of chronic cervical inflammation, preneoplasic stages, in situ carcinomas and stage I.

This work analyses other potential etiological factors. We can mention here the path: chronical cervicitis → cancer (direct proportion). Last year's research (1, 7, 9) demonstrate a 97% of pathogenic germs inside lesion cervix: 44,5% aerobe gram (+) germs (white hemolytic streptoccocus 18,7%, group D streptoccocus 15,8%, α hemolytic streptoccocus 4,9%, group A streptoccocus 2,9%, enteroccocus and golden stafiloccocus 0,9%); 30,5% gram (-) germs (*E. coli* 24,7%, *Haemophylus* 4,9%) and *Proteus* 0,9%); 13,8% anaerobic germs. *Trichomonas vaginalis* was diagnosed in 46,5% cases, *Candida albicans* in 48,5%, *Chlamidia trachomatis* in 13,8% and *Mycoplasma hominis* in 10,8%. Herpetic viruses: type I was diagnosed in 8.9% cases and type II in 13.8% cases (tab. I). After these last researches all women should undergo a compulsory complete screening, to balance the lack of sanitary education (1, 6, 7, 8).

TABLE I

Different pathogenic agents implicated in cervical chronic inflammation

Infectious agent	**cytology type IV**	**cytology type III**
bacterias	11.2%	2%
candida albicans	18.3%	-
trichomonas vaginalis	21.2%	21%
chlamydia trachomatis+		
mycoplasma hominis	25%	
VHS tip I - II	4.3%	4.3%

For the reduction of morbidity and mortality from cervical chronic inflammatory diseases we consider decisive the following aspects: systematic cytological and clinical checking for all women sexually active no matter age, with registration of data in health notebook; Babeş-Papanicolau routine test, recognized worldwide.

The analyze of 952 pathological cytotests promotes the idea that suspect lesions appear in 10,5% cases compare to 86,5% benign lesions and 3% malignant lesions.

The implication of microbial, parasite and viral agents in the etiology of lesional cervix with epithelial cervical disorders, benign and atipic is expressed by different cytological aspects which differ through the etiologic agent (1, 7, 9).

Some authors realized a prospective study on 101 clinical cases of lesional cervix; biological specimens were prelevated for evaluation of pathogenic agents and parasites. They notice high frequency for *Candida, Trichomonas, Chlamydia and Mycoplasma*. The protocol consisted of: correct patient history, gynecological clinical exam, routine exfoliative cytology for all patients, and association of repeated cytology with biopsy (for persistent inflammatory signs against all adapted treatment).

The elements which were followed: age – 168 (62,01%) women between 40-50 years had repeated chronical cervical inflammation distress treated many times before; place of living – 219 (67,80%) urban and 104 (32,19) – countryside; profession - 97 (30,03%) university degree, 226 (69,96%) high school; marital status - 94 (29,10%) married once, 157 (48,68) remarried, 72 (22%) married and divorced; obstetrical history – 12 (3,71%) nuligeste nulliparous, 214 (66.25%) multiparous, 97 (30.3%) primiparous; aspect of the cervix on first exam – 189 (58,51%) erosive lesions, 86 (26,62%), ulcerated lesions, 48 (14,86%) congestive lesions; stages at first exam and what treatment they have followed.

The importance of chronic inflammatory process resumes in its high prevalence worldwide, especially for cervical cancer which represents the first cause of morbidity and mortality for women in Romania also. Nowadays the most efficient method of prevention for cervical cancer is Babeş-Papanicolau cytological screening, after that cervico-vaginal cytology and correct treatment of acute and chronic cervicitis (4, 7, 8).

More and more we can speak about cervical diseases as sexually transmitted affections, no matter which is the etiology. This statement is based on solid epidemiological arguments (higher risk with the increased number of sexual partners, for teenagers with precocious sex life, incorrect treatment of cervico-vaginal infections, acute or chronic. We can also note the common risk factors for all women: lack of annual gynecological check, lack of education and information for women in the countryside, important virological new findings (the association between *Human Papiloma Virus* and chronic neoplasic alteration inside cervical cells) (6, 7, 8). Plurifactorial etiology is highlighted by the presence of other factors also, which increase the risk for cervical cancer: smoking, drinking, long term usage of oral contraceptives ($\geq$ 3-5 years).

The simptomatology for chronical cervicitis is reduced, at least in first stages and can be notable in advanced stages when becomes manifest. The alert signal for all women should be unexpected bleeding, which is in fact sign of important inflammation. In the beginning it is small bleeding, especially on intercourse. Together with bleeding can appear: leucorrhea (first white and then pale pink and finally with bad smell), vaginal discharge more and more important and with bad smell, pain in the *cul-de-sac* or pelvic pain which shows associated inflammatory signs, eventually extensive.

The correct diagnose must follow certain steps: exam of exfoliative cytology,

complete gynecological exam, biopsy – final step of the diagnosis, which brings the doubtless diagnose. Correct patient history can suggest us one specific category of women with higher risk for developing cervical cancer at certain point. From this point of view we can distinguish: 1. Minimal risk: women without sexual activity, who use barrier contraception, age more than 50 years, with repeated periodic gynecological exams and cytology. 2. Medium risk: women with usual sexual activity, with abortions and/or high number of natural deliveries before, women with persistent cervical ectropion. 3. High risk: women with early onset of sexual life (under 18), with multiple sexual partners, with many marriages, low socioeconomic status, low or absent hygiene.

When we analyze the reaction of the body to LLLT we can discover an integrated system of effects which include cellular/tisular modifications and control organism systems modifications. Usually we use two main methods of sanguine intravenous irradiation with red spectrum and simultaneous percutaneous irradiation and transmucosal, especially with infrared spectrum. We project the fascicles on targeted affected organs, in combination with magnetic flux. Laser irradiation can also be used for patients with: coronarian diseases, diabetes, leuconevraxitis, thyroid pathology, septical complications after radio/ chemotherapy for oncologic patients.

Intravenous blood irradiation was experimentally proved to have following effects: correction of humoral and cellular immunity, incensement of nonspecific immunity of the organism, adjustment of peripheral blood flow, adjustment of blood haemostasis, vasodilatation, balancing the acid-basic equilibrium, improvement of O_2 transportation, intensification of antioxidant function of blood, stimulation of erythropoiesiss, rehabilitation of damaged DNA through irradiation, rehabilitation of metabolic processes which involve proteins, lipids and carbohydrates, stimulation of rejuvenating process (tab. II).

TABLE II
LLLT therapy for cervical chronic inflammatory diseases

Recommended therapy (J/cm2) - acute - chronic	2.5 (1.8-3.3) 2.0 (1.3-3.0)
Recommended frequency (Hz) - acute - chronic	3.0 9.12
Time of therapy for max power (min.)	1.44 (acute), 1.23 (chronic)
Number of treatments in a weak - acute - chronic	Daily 3/week
Total number of treatments - acute - chronic	2 - 5 6 -12
Recommended BTL sound	(infra)red
Recommended sound power	30 mW

For the efficacy of treatment we have to follow some parameters: irradiation with optic accessory for gynecology, direct cer-

vical irradiation, laser therapy for affected areas.

CONCLUSIONS

Correct usage of LLLT through follow-up of evolution, indications, reported to Babeş-Papanicolau cytology and clinical gynecological exam has given very good results for all categories of patients.

The utilization and widening the area of LLLT usage is a goal for the future for our country too. In our medical center we have been using this technique for approximately 10 years. We appreciate that it will conduct to a decreased number of cervical inflammatory diseases. We also appreciate for the future a lower morbidity rate for this treatment and a lower mortality rate for chronic cervical inflammatory affections and also a better limitation of the evolution towards cervical cancer.

We propose the applicability in all medical centers, especially for outpatients, for a larger addressability for women from all population. These conditions have limited costs; do not induce work incapacity, usage of powerful drugs, adverse solutions, without pain.

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INFLUENCE OF PREOPERATIVE KNEE DEFORMATION ON TENSION FORCE IN POSTERIOR STABILIZED TOTAL KNEE ARTHROPLASTY

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INFLUENCE OF PREOPERATIVE KNEE DEFORMATION ON TENSION FORCE IN POSTERIOR STABILIZED KNEE ARTHROPLASTY (Abstract): Gap-dependent total knee arthroplasty involves applying a tension force to open the joint space. Intuitively, this force is dependent on the degree of joint deformity. **Aim:** The study aims at determining the amount of force and its influence on morphological parameters. **Material and methods**: In a group of 70 patients in whom total knee arthroplasty was performed ligament tension force was calculated and the influence of various individual morphological parameters was evaluated. **Results**: Statistical analysis showed that tension force is individual, dependent on knee deformation. **Conclusions**: Ligament tension force cannot be determined preoperatively, it requiring the use of appropriate tensioning devices. **Key words:** TENSOR, BALANCE, PRESSURE, PROTHESES

The two ways to place a total knee endoprosthesis are the controlled resection technique and gap-dependent technique. There are no data showing superiority of one of them, although some studies show a better positioning of the femoral component in the second case (1, 2). In both cases, the use of computer technology has made an improvement (2, 3). It helps in calculating directions and angles in the first case, and pressures/forces in the second, to prevent unstable knee (4, 5). In both cases ligamentous tension will determine the subsequent behavior of the knee. Of course, too low tension will cause laxity. Instead, there are articles showing that too much tension will cause increased imbalance (6). This is logical because if two different rigidity ligaments are stretched the tension difference increases. The question is: what the optimal ligamentous tension is. During the last period, ligament tensioning devices became available. Some of them generate a fixed force; others allow the force to be adjusted by the surgeon. However, a currently widespread idea is that a fixed force of 177-200 N regardless of the patient is necessary (7 - 13). Certainly the opening force must be applied with reduced patella. But it is curious that for different people, we create different spaces and fill them with prostheses of different sizes by applying the same force. Apparently, the greater the opening, the ligamentous tension is

even greater. Muratsu has shown that increasing the force size increases the gap (6). This paper aims at clarifying what is the balance force and the factors that determine it.

MATERIAL AND METHODS

Patients group

Seventy patients with a mean age of 73 years (range 60-90), height 1.62 ±0.07 m, weight 796±160 N, BMI 30.85 +5.93 kg/m², and a body moment (weight * height) of 1296±285 Nm entered this study. Patients were operated between July 2010 and May 2011 by the same surgeon. Exclusion criteria were the use of a surgical approach other than anterior-medial, personal history of rheumatic disease, injury, osteotomy or knee arthroplasty, conditions that could alter the capsular structure, and the absence of data. All parameters showed a normal distribution. Patients presented preoperative mechanical axis deviation of -5.65 ± 8.44 ° HKA, range 22°(varus) - 13°(valgus), flexion of 115 ± 14° (range 80-135°), and extension deficit of 4 ± 5 ° (range 0-20 °). The investigations included a preoperative conventional goniometry based on which HKA, alpha, beta angles, tibial slope, and Caton index were calculated (14, 15).

Device

We used a fourth generation E-libra® digital ligament tensor (Synvasive Technology, Inc., Reno, Nevada, USA) (fig.1). It is able to achieve ligament balancing in position of 90° knee flexion by opening the lateral side and mark this point by measuring and displaying the relative pressures in the two compartments. Balance is achieved with reduced patella. The device consists of 3 components: a femoral component, a tibial component, and ligament balance measuring system by pressure sensors. Femoral component includes two parts: a fixed part, which is applied to the distal femoral osteotomy surface, and a moving part. This allows tension adjustment by opening the lateral ligament. The adjustment is made via an actuator (screw-gear). The center of rotation in the axial plane corresponds to the geometric center of the medial femoral condyle. This allows the natural movement of rotation. Tibial component is a classic spacer thicknesses corresponding to a gap of 10, 12, 14, 16 mm. Ligament balance measurement system consists of pressure sensors disposed within each medial/lateral compartment. The processor transmits a known voltage to the two sensors, and pressure variations are converted into electric current variations. This analog signal is converted to digital and then it is processed. The signals are transmitted through a transciever to the display. The power source is batteries. All items are covered and sealed in a single device that is calibrated and sterilized by

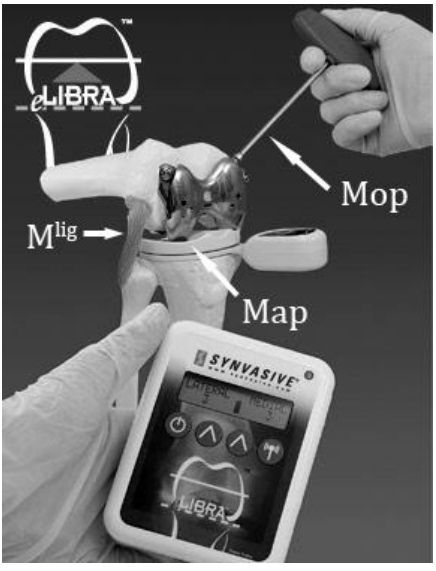

Fig. 1. E-libra www.synvasive.com

the manufacturer. The read-out unit displays the relative pressure values. Since the tensor comprises a worm-gear mechanism, the operator torque force Mop is amplified by the device to Map. To quantify the force distraction we have attached a Tochnichi torque screwdriver with a 10-100 cNm measuring scale. The assembly was calibrated using a Kirstler type force platform. The force generated by applying a progressive torque of 5 cNm was measured. For each value five determinations were made. The results revealed an excellent correlation coefficient $R^2 = 0.952$, and allowed us to calculate the multiplication factor.

METHODS

Prostheses were implanted using a technique sensor-dependent. Patients were anesthetized for ensuring complete muscle relaxation. After the tourniquet was inflated to 350 mmHg we practiced a medial parapatellar (transvastus) approach. Both cruciate ligaments were removed. The surgical sequence was the following: proximal tibial resection, distal femoral resection, measurement of femoral component, knee flexion, device insertion, knee balancing, cutting guide placement, anterior posterior femoral resection, and prosthesis implantation. All trances osteotomy were measured using a caliper to the tibial femoral contact point. We note the thickness of the tibial trances: medial tibia (MT) and lateral tibia (LT). After creating the space in extension, the surgeon considered the thickness of the meniscus (M) as the thickest that fits under a manual maximum distraction. It was inserted together with LBMS into the joint space in flexion of 90°. At that moment ligamentous tension was adjusted with a torque screwdriver. First we have achieved a maximal opening up, respectively the maximum stress on lateral collateral ligament to prevent the phenomenon of stress relaxation. Then we relaxed the device and achieved ligament balance. This was seen on the display. It noted the applied torque. The absolute force applied to the joints was calculated based on calibration.

Statistical analysis

Statistical analysis focused on the applied force. We studied the influence of various parameters on the force. These were anthropomorphic (height, weight, body moment), radiological (HKA angle, alpha, beta, tibial slope), clinical (flexion, extension), intraoperative and prosthetics (femoral and tibial size) data. Intraoperative parameters were MT, LT, M; medial / lateral opening MO / LO, where the opening is defined as the difference between the thickness of meniscus and the amount of resected bone; tibial tilt (TT) as the difference between the amount of resected bone at lateral tibia (LT) and medial tibia (MT), respectively (fig. 2). The relationship be-

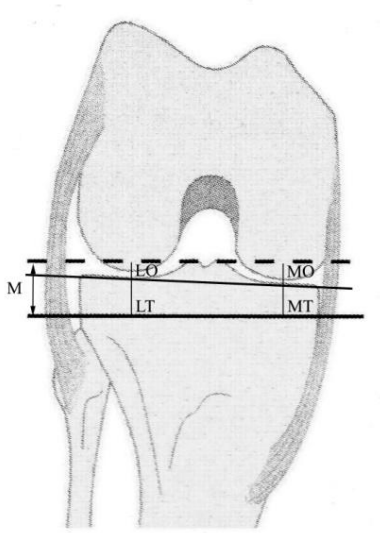

Fig. 2. Intraoperative parameters

tween the force and different parameters were analyzed using SPSS software. We used bivariate correlation tests, partial correlation, and ANOVA.

RESULTS

When placing the pressure sensor, the pressure was always higher in the medial compartment. Opening the lateral side allowed the two pressures to equalize. Balancing force was different for each patient with an average of 200 ± 124 N, range 72 to 699 N. As the values did not follow Gaussian distribution, the normalization was done by logarithms. The factors that showed a statistical correlation level of 0.01 were in the order of correlation coefficient: tibial tilt (TT), HKA angle, and femoral component size. The greater the tilt more force is required (fig.3). From the partial correlation analysis we found that this is largely due HKA angle, as between TT and HKA there is dependence.

As to the influence of joint line height, ANOVA tests revealed a significant positive/negative relationship between medial/lateral opening (MO/LO) and the force. However, the size of the meniscus (M), thus implant thickness did not correlate with the opening_force.

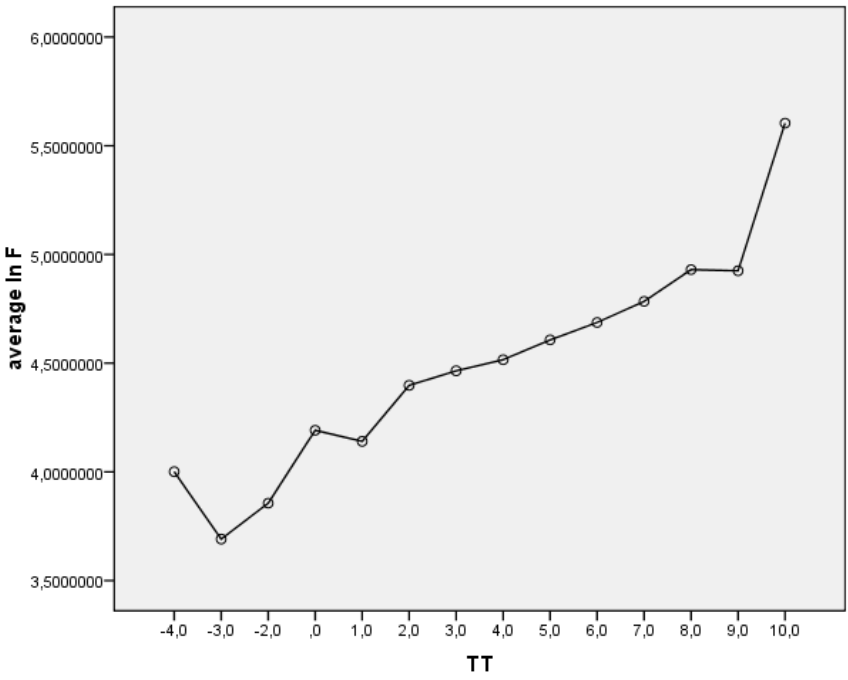

Fig. 3. Correlation between inclination of the tibia (TT) and force F

DISCUSSION

This study suggests the need of using customized force. This seems to come naturally: every individual is unique, with specific parameters. But our findings are in conflict with other studies that use fixed force. Its value derives from their experience in calculating the force needed to

open the joint space and required to insert total knee prosthesis (12). Even our study did not show an influence of implant thickness on the tension force. But the collateral ligaments have coefficients of elongation that differ from individual to individual. So, a fixed force will cause different openings in different patients. Based on these results, we aimed at finding the factors that influence the amount of force and how many of them are operator controlled or can be controlled. During surgery, the surgeon makes three balance-related gestures: ligamentous release, tibial osteotomy, and meniscus thickness choice. The first gesture is completely subjective. The other two change the joint line height and by default the size space. There are studies that show that if, in the same patient, we apply larger forces we get larger spaces (6, 16). The converse is also true: choosing the thicker meniscus ligament will generate higher tensions. Not only the meniscus thickness is important, but also the amount of resected bone. This explains the positive correlation between force and medial opening. However, lateral opening correlates negatively with force. The logic of these findings is revealed by tibial tilt. For the same elevation of the joint line, a greater tilt of the tibia suggests a greater imbalance between ligaments resulting from osteotomy. Tibia inclination reflects the joint form, a result of the wear process (HKA angle), or its anatomy (posterior - condylar angle). Partial correlation test confirms the dependence of tibia tilt (TT) and wear joint described by HKA angle. However, there is no a strong relationship between the tibia tilt and the tension, probably due to the influence of other unrated factors.

CONCLUSIONS

We find that the ligamentous tension force is individual. It is partly influenced by morphological features, but it cannot be determined preoperatively, this requiring the use of appropriate tensioning devices.

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NEWS

DEVELOPMENTAL DELAY IN FRONTAL REGIONS OF THE BRAIN IN CHILDREN WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

In a study by Shaw et al regarding the cortical anomalies underpinning attention-deficit/hyperactivity disorder (ADHD), 234 children with ADHD and 231 normally developing children were examined and 837 neuroanatomic magnetic resonance images were acquired. The purpose of the study was to see if the delayed maturation of prefrontal cortical thickness, characteristic for ADHD, also extends to the maturation of cortical surface area and gyrification. The study found that surface area developmental trajectory was delayed in ADHD. According to the researchers, the median age by which 50% of cortical vertices attained peak area in the right prefrontal cortex, was 14.6 years in ADHD, significantly later than in normally developing children at 12.7 years (Shaw P, Malek M, Watson B, Sharp W, Evans A, Greenstein D. Development of cortical surface area and gyrification in attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2012; 72(3): 191-7).

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THAPSIGARGIN-INDUCED ENDOPLASMIC RETICULUM STRESS IS NOT ACCOMPANIED BY MITOCHONDRIAL MEMBRANE POTENTIAL DISSIPATION IN MURINE PRO-B CELLS

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THAPSIGARGIN-INDUCED ENDOPLASMIC RETICULUM STRESS IS NOT ACCOMPANIED BY MITOCHONDRIAL MEMBRANE POTENTIAL DISSIPATION IN MURINE PRO-B CELLS **(**Abstract): There are almost no data concerning the involvement of endoplasmic reticulum stress (Ca^{2+} fluxes) in the apoptosis of the pro-B cell type Ba/F3. Thus, we aimed the characterization of thapsigargin-induced effects on Ba/F3 cells *in vitro*. **Material and method**: For some experiments Ba/F3 cells were treated with 1 μM thapsigargin for 24 hours. To compare, we used as positive control the effects of valinomycin 10 μM. The negative control Ba/F3 cells received no treatment for 24 hours. After that, all batches of Ba/F3 cells were incubated in the presence of 1 μM JC-1 (Sigma-Aldrich) at 37°C for 30 minutes. **Results**: From the normal Ba/F3 JC-1-control cells (20.000 events gated by flow cytometry) 77.61±2.90% are associating high and 22.39±2.90% lower mitochondrial membrane potential. In the case of thapsigargin, 75.49±1.78% of the Ba/F3 cells are associating high and 24.51±1.78% lower mitochondrial membrane potential. **Conclusions**: While mitochondrial permeability transition pore (MPTP) opening necessarily correlates with a loss of mitochondrial membrane potential (Ψ_{mt}), a decrease in Ψ_{mt} does not necessarily indicate MPTP opening. Also, endoplasmic reticulum stress regulation of high cytosolic calcium levels by thapsigargin may prompt the opening of the MPTP without necessarily triggering irreversible pore activation or subsequent apoptogenic factors in Ba/F3 cells. **Key words:** PRO-B CELLS, MITOCHONDRIAL MEMBRANE POTENTIAL, APOPTOSIS, THAPSIGARGIN, ENDOPLASMIC RETICULUM STRESS

The endoplasmic reticulum (ER) is an organelle entrusted with lipid synthesis, calcium homeostasis, protein folding, and maturation. Perturbation of ER-associated functions results in an evolutionarily conserved cell stress response, the unfolded protein response (UPR) that is also called ER stress. ER stress is aimed initially at compensating for damage but can eventually trigger cell death if ER stress is excessive or prolonged. Now the ER stress has been associated with numerous diseases (1).

The Ca^{2+}-binding protein sorcin regulates intracellular calcium homeostasis and plays a role in the induction of drug resistance in human cancers. Recently, an 18 kDa mitochondrial isoform of sorcin was reported to participate in antiapoptosis in human colorectal cancer (CRC), but information remains lacking about the functional role of the more abundant 22 kDa isoform of sorcin expressed in CRC. It was found the 22 kDa isoform to be widely expressed in human CRC cells, whether or not they were drug resistant. Its upregulation in drug-sensitive cells induced resistance to 5-fluorouracil, oxaliplatin, and irinotecan, whereas its downregulation sensitized CRC cells to these chemotherapeutic agents. Sorcin enhances the accumulation of Ca^{2+} in the ER, preventing ER stress, and, in support of this function, it was found that the 22 kDa isoform of sorcin was upregulated under conditions of ER stress. In contrast, RNAi-mediated silencing of sorcin activated caspase-3, caspase-12, and GRP78/BiP, triggering apoptosis through the mitochondrial pathway. These findings establish that CRC cells overexpress sorcin as an adaptive mechanism to prevent ER stress and escape apoptosis triggered by chemotherapeutic agents, prompting its further investigation as a novel molecular target to overcome MDR (2).

The goal of our study was represented by the characterization of the effects of thapsigargin, an endoplasmic reticulum stress inducer, on Ba/F3 cells apoptosis, using flow cytometry and JC-1 as an indicator of mitochondrial membrane potential dissipation.

MATERIAL AND METHODS

The IL-3-dependent mouse pro-B cell line Ba/F3 was maintained in RPMI 1640 (Sigma-Aldrich) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated fetal bovine serum and 10% WEHI-3-conditioned media as a source of murine IL-3, in an atmosphere with 5% CO_2 and at 37°C (3).

Cells were grown at a density of around 5 x 10^5 per ml before treatment. For some experiments (in triplicate) Ba/F3 cells were treated with 1 μM thapsigargin for 24 hours. To compare, we used as positive control the effects of valinomycin 10 μM, a well known inducer of dissipation of mitochondrial membrane potential, also in triplicate. The negative control Ba/F3 cells received no treatment for 24 hours. After that, all batches of Ba/F3 cells were incubated in the presence of 1 μM JC-1 (Sigma-Aldrich) at 37°C for 30 minutes. JC-1 [5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide or $CBIC_2$(3)] is a very sensitive marker for mitochondrial membrane potential (Ψ_{mt}). To demonstrate the involvement of mitochondrial permeability transition pore (MPTP) activation we administered the inhibitor cyclosporine A (CsA), also administered in parallel in 1 μM concentration for 24 hours.

Ba/F3 cells were washed three times with phosphate-buffered saline (PBS, Sigma-Aldrich) at 300 x g for 5 minutes each and for the determination of JC-1 loading in mitochondrial membrane we used a FACS Calibur (Beckton-Dickinson) which was set up for JC-1 fluorescence.

The final concentration for dimethyl sulfoxide (DMSO) in the medium, used as a drug solvent, did not exceed 0.1%, having no cellular effects at this concentration.

The statistical significance of test results was highlighted using the Variance One-Way ANOVA, All Pairwise Multiple Comparison Procedures (Holm-Sidak method) and the results were expressed as

mean ± S.E.M (n = 3). Value of $p<0.05$ was considered statistically significant always.

RESULTS

From the normal Ba/F3 JC-1-control cells (20.000 events gated) 77.61±2.90% are associating high and 22.39±2.90% lower mitochondrial membrane potential as shown by JC-1in Fig. 1 (triplicate experiment).

The mitochondrial membrane potential variation is evident if we compare these cells with the negative control ones (no treatment, Fig. 2). For the negative control cells, the vast majority is found in the bottom left region, that means 99.45±0.55% (n=3).

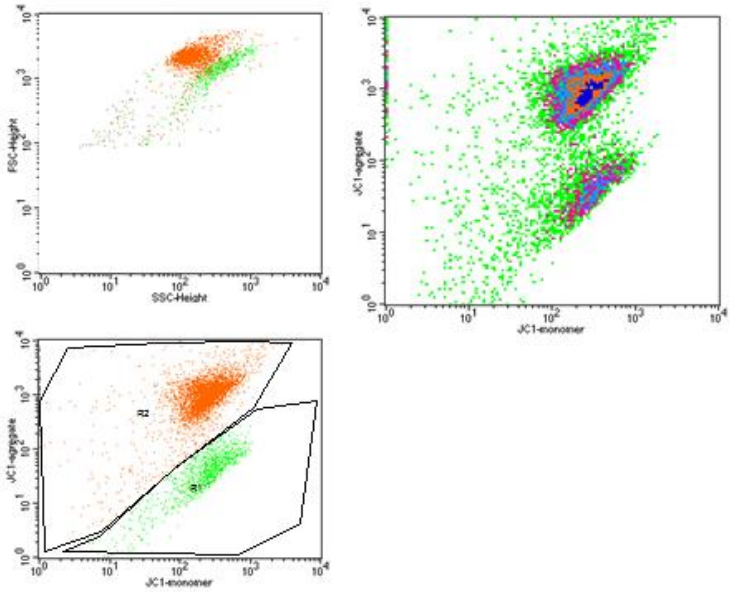

Fig. 1. Representative flow cytometry recordings of $1x10^6$ Ba/F3 cells/ml in the presence of 1 μM JC-1at 37°C for 30 minutes. As shown, 77.61±2.90% of the cells are associating high (R2 region) and 22.39±2.90% lower mitochondrial membrane potential (R1 region). $p<0.001$ as compared to control cells with no JC-1, data are expressed as mean ± S.E.M (n = 3).

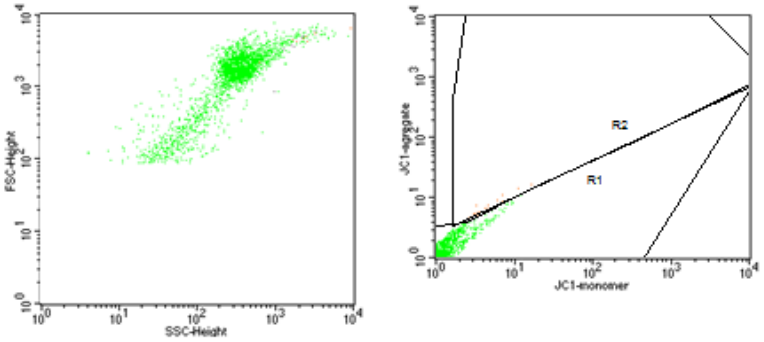

Fig. 2. Representative flow cytometry recordings of $1x10^6$ negative control Ba/F3 cells/ml. The vast majority is found in the bottom left region, that means 99.45±0.55%, data are expressed as mean ± S.E.M (n = 3).

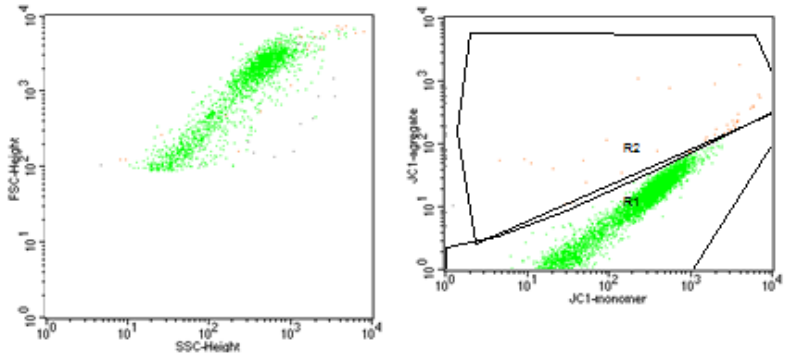

Fig. 3. Representative effects of 24 h treatment with valinomycin 10 μM on 1x10^6 Ba/F3 positive control cells/ml. The cells were stained with 1 μM JC-1 at 37°C for 30 minutes. As shown, only 1.69±0.95% of cells have mitochondria with high membrane potential (live cells, the R2 region), 98.31±0.95% being apoptotic (the R1 region). p<0.001 as compared to cells with only JC-1 and to cells with no JC-1, data are expressed as mean ± S.E.M (n = 3).

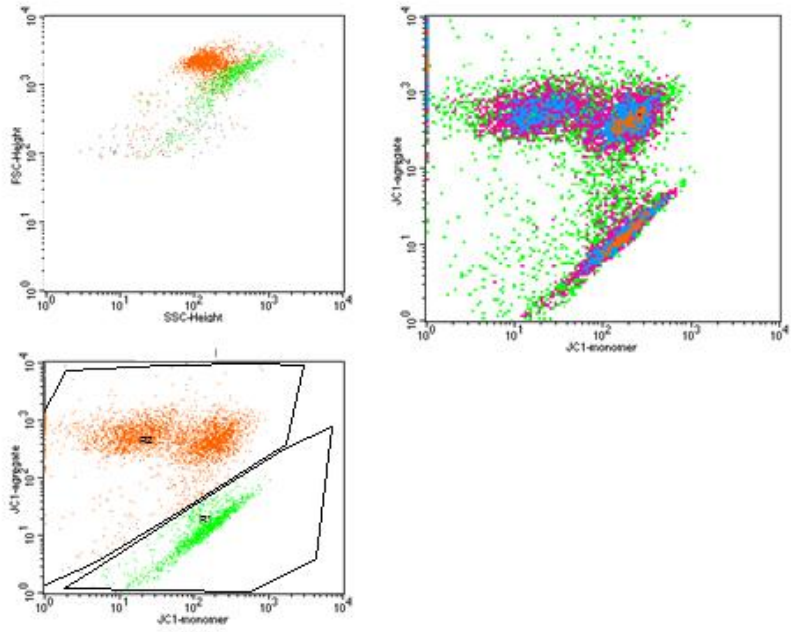

Fig. 4. Representative effects of 24 h treatment with 1 μM thapsigargin in culture medium on 1x10^6 Ba/F3 cells/ml. The cells were stained with 1 μM JC-1 at 37°C for 30 minutes. In this case, 75.49±1.78% of the Ba/F3 cells are associating high and 24.51±1.78% lower mitochondrial membrane potential. p<0.001 as compared to cells with only JC-1 and to cells with no JC-1, data are expressed as mean ± S.E.M (n = 3).

For comparison we used as positive control the effects of valinomycin 10 μM, a well known inducer of mitochondrial membrane potential dissipation, also in triplicate. After 24 h of treatment the R2 region contains only 1.69±0.95% of cells having mitochondria with high membrane potential (live cells), more than 98% being apoptotic (the R1 region, Fig. 3, n=3).

For some experiments (in triplicate) Ba/F3 cells were treated with 1 μM thapsigargin in culture medium for 24 hours (Fig. 4). In this case, 75.49±1.78% of the Ba/F3 cells are associating high and 24.51±1.78% lower mitochondrial membrane potential as shown by JC-1.

To demonstrate the involvement of MPTP activation we used the inhibitor cyclosporine A (CsA), also administered in parallel in 1 μM concentration for 24 hours. CsA dramatically reduced the valinomycin apoptotic effects on Ba/F3 cells, having no influence on thapsigargin treatment (data not shown).

DISCUSSION

The mechanisms regulating neutrophil apoptosis are basically unaffected by the aging process. However, a significant impairment of cell survival occurs in elderly individuals following neutrophil challenge with pro-inflammatory stimuli, such as granulocyte-macrophage colony-stimulating factor (GM-CSF). Treatments with the specific inhibitor of cytosolic calcium reuptake, thapsigargin, and/or the calcium mobilizing agonist, N-formyl-methionyl-leucyl-phenylalanine (fMLP), induced mitochondrial membrane depolarization, caspase activation, phosphatidylserine (PS) externalization, and DNA fragmentation in leukocytes from both young and elderly volunteers, although such effects were much more evident in aged leukocytes. Importantly, melatonin treatment substantially preserved mitochondrial membrane potential, reversed caspase activation, reduced PS exposure and forestalled DNA fragmentation in leukocytes from both age groups. In conclusion, melatonin is able to delay endoplasmic reticulum stress-induced apoptosis in aged leukocytes and may counteract, at the cellular level, age-related degenerative phenomena linked to oxidative stress (4,5).

The proteasome has been recognized as a druggable target in cancer cells, and this has led to searches for pharmacologic agents that target this cellular organelle for cancer therapeutic purposes. Syrbactins are a group of microbial metabolites consisting of two related families, the glidobactins and the syringolins. Some members of this group have revealed cytotoxic efficacy in tumor cells, and more recently it was discovered that they exert proteasome-inhibitory function. Some results show that two of these novel compounds are able to inhibit proteasome activity in the nanomolar range, reduce the expression of anti-apoptotic proteins survivin and Mcl-1, and cause severe ER stress, resulting in pronounced tumor cell death (multiple myeloma, Waldenstrom's macroglobulinemia, and lymphocytic leukemia). These anticancer effects can be synergistically enhanced when the agents are combined with thapsigargin, which further aggravates ER stress by a different mechanism. Taken together, the findings support the notion that syrbactin analogs may provide a structural platform for the development of novel cancer therapeutics, and that their efficacy may be further increased when complemented with other agents that trigger ER stress (6).

Autocrine motility factor/phosphoglucose isomerase (AMF/PGI) promotes cell survival by the pAkt survival pathway. Its receptor, gp78/AMFR, is an E3 ubiquitin

ligase implicated in ER-associated protein degradation. It was demonstrated that AMF/PGI also protects against thapsigargin- and tunicamycin (TUN)-induced ER stress and apoptosis. AMF/PGI protection against the ER stress response is receptor mediated as it is not observed in gp78/AMFR-knockdown HEK293 cells. However, AMF/PGI protection against the ER stress response by thapsigargin and TUN was mediated only partially through PI3K/Akt activation. AMF/PGI reduction of the elevation of cytosolic calcium in response to either thapsigargin or inositol 1,4,5-trisphosphate receptor activation with ATP was gp78/AMFR-dependent, independent of mitochondrial depolarization and not associated with changes in ER calcium content. These results implicate regulation of ER calcium release in AMF/PGI protection against ER stress and apoptosis. By regulating ER calcium release, AMF/PGI interaction with gp78/AMFR therefore protects against ER stress identifying novel roles for these cancer-associated proteins in promoting tumor cell survival (7).

CONCLUSIONS

While mitochondrial permeability transition pore (MPTP) opening necessarily correlates with a loss of mitochondrial membrane potential (Ψ_{mt}), a decrease in Ψ_{mt} does not necessarily indicate MPTP opening. Also, endoplasmic reticulum stress regulation of high cytosolic calcium levels by thapsigargin may prompt the opening of the MPTP without necessarily triggering irreversible pore activation or subsequent apoptogenic factors in Ba/F3 cells.

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THE USE OF BETA BLOCKER *NEBIVOLOL* IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN ASSOCIATION WITH ARTERIAL HYPERTENSION

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THE USE OF BETA BLOCKER *NEBIVOLOL* IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN ASSOCIATION WITH ARTERIAL HYPERTENSION (Abstract): There were investigated 22 patients with chronic obstructive pulmonary disease in association with arterial hypertension initially and after 5 weeks of treatment with beta-blocker *Nebivolol* 5 mg/day. There were not detected any negative changes on bronchial permeability after treatment. Considerable improvement of endothelial function (from 7-12% to 17-35%, $p<0.05$) in parallel with decreasing of pulmonary artery, systolic (44.5±5.5 mmHg vs. 32.8±4.1 mmHg, $p<0.05$) and mean pressure (26.3±4.2 mmHg vs. 21.1±2.1 mmHg, $p<0.05$) – were observed after treatment. Thus, *Nebilet* is well-tolerated and highly effective in patients with chronic obstructive pulmonary disease in association with arterial hypertension. **Key words**: BRONCHIAL PERMEABILITY, ARTERIAL HYPERTENSION, BETA-BLOCKER, ENDOTHELIAL FUNCTION, MEAN PRESSURE.

Beta blockers hold one of the main positions in the structure of the complex treatment of patients with arterial hypertension and congestive heart failure (1, 2, 3, 4, 5, 9, 12, 13). Beta blockers reduce the cardiac output, change the susceptibility of the bar receptor reflex and block the peripheral adrenoreceptors (6, 7, 8, 10). Some beta blockers also depress the rennin secretion. Despite the above mentioned positive aspects, manifested by blocking beta II – receptors of the bronchial muscles, they also induce bronchoconstriction, increase the reactivity of respiratory tract and decrease or block the effects of the beta II inhaled agonists (9, 10). Therefore, there shall be prudence or avoidance of the use of beta blockers in patients with chronic obstructive pulmonary disease. That is why the international guidelines have established that bronchial asthma and chronic obstructive pulmonary disease are definitely contraindicated to the use of beta blockers in the treatment of arterial hypertension. *Nebivolol* (*NB, Nebilet, Berilin – Chemie, Menarini Group*) represents a superselective third generation beta blocker. The cardioselective index is of 293 (the proportion of beta blocker I adrenoreceptors and beta blocker II – adrenoreceptors). In order to make a comparison, one can observe the cardioselective index of athenol is of 15, of metoprolol – 25, bisoprolol – 119 (1, 2). The therapeutic dose of NB is of 5 mg/day. The compound has a prolonged action (more than 24 hours, in some cases, it reaches 30-32 hours), stimulates synthesis and discharge of nitric oxide

from the vascular endothelial cells, and has an antiproliferative effect against the smooth muscle cells which leads to physiological vasodilatation (3). The study of the effects of NB over the mechanics of respiration, the lung circuit and the endothelial function is of high interest.

The study of the efficiency and security of using NB in patients with chronic obstructive pulmonary disease in association with arterial hypertension.

MATERIALS AND METHODS

There were investigated 22 male patients with chronic obstructive pulmonary disease of moderate seriousness (Tiffeneau index<70%, 50%≤ VEMS <80%) in association with second degree arterial hypertension, 45-56 of age (average 52.6±2.54 years) and duration of illness of 7 to 20 years (average 14.3±7.7 years) – initially and after 5 weeks of treatment with NB. There were performed: spirometry (maximum and forced expirogram with the registration of the flux-volume and volume-time curves); standard electrocardiogram, Doppler echocardiography (to estimate the systolic pressure in the lung artery) – initially and after treatment with NB.

The endothelial function was estimated with the help of computerized photoplethysmography by making the test of reactive hyperemia. The endothelial dysfunction was considered when the index of the endothelial function was below 10%.

RESULTS AND DISCUSSION

The analysis of the safety and efficiency of the use of NB in patients with chronic obstructive pulmonary disease and arterial hypertension has not revealed aggravation of the evolution of chronic obstructive pulmonary disease and bronchial obstructive syndrome during the 5 weeks of treatment. There have been no significant changes of the bronchial permeability while studying the dynamics of the spirometry indexes (tab. I). There have been revealed certain tendencies of the increase of the forced expiratory volume per second (FEV1), of the forced expiratory flow in the middle of the forced vital capacity (FEF 25/75) and of the forced vital capacity (FVC).

The index of the endothelial dysfunction (after being tested with reactive hyperemia) increased considerably from 7-12% to 17-35% ($p<0.05$). A positive dynamic of the level of arterial pressure and FCC have also been observed: from the initial 156.33±15.5 mmHg to 136.57±18.12 mmHg and 86.72±7.4 heart beats per minute versus 70.56±3.88 heart beats per minute ($p<0.05$). The Doppler echocardiography revealed a moderate dilatation of the right parts of the heart (the telediastolic volume of the right ventricle - 28.6±0.11 mm) and light biventricular hypertrophy (thickness of the right ventricle anterior wall– 5.5± 0.3mm, and of the left ventricular posterior wall – 1.27±0.07). There was significant dynamics of pressure in the pulmonary artery (5-12%): systolic - from 44.5±5.5 mmHg initially versus 32.8±4.1 mmHg $p<0.05$) after 5 weeks of treatment; average – from 26.3±4.2 mmHg versus 21.1±2.1 mmHg and of the time of increase of the pulmonary flux – 100.5±11.4msec initially and 126.1±10.5 msec after the treatment with NB ($p<0.05$).

By „endothelial dysfunction" we understand a lack of balance of the endothelial factors which equilibrate the process of homeostasis, proliferation and vascular tonus. Nowadays, the endothelial dysfunction holds a main part in the pathogenic of pulmonary hypertensions both primary and secondary. Among the pathogenic reactions

that lead to the appearance of pulmonary hypertension, the impact of hypoxemia is first of all realized by the action of the latter over the structural and functional state of the endothelium of the small and medium pulmonary vessels.

TABLE I

Mechanics of breathing in patients with obstructive lung disease after the treatment with *Nebilet*

Indications	Actual value Initially/after treatment	Proper value Initially/after treatment	% of the Proper value Initially/after treatment
FVC, 1	3.73±0.5/ 3.85±0.7	4.16±0.7/ 4.22±0.4	89.66±11.2/ 91.22±10.7
FEV1, 1	2.20±0.3/ 2.26±0.5	3.46±0.7/ 3.50±0.7	63.58±9.9/ 64.57±7.7
PEF, peak expiratory flow, l/min	2.13±0.3/ 2.22±0.5	6.43±0.2/ 6.51±0.2	33.11±8.8/ 33.31±6.0
FEF 25/75 %, l/min	1.34±0.1/ 1.40±0.1	32.2±11.5/ 33.3±9.6	32.2±11.5/ 33.3±9.6
FEV1/FVC, Tiffeneau index	68.5±11.5/ 69.0±9.9	75.0±9.9/ 75.0±9.7	91.33±10.0/ 92.05±11.3

Based on photoplethysmography, the dynamics of the endothelial function was positive after treatment (tab. II): the cumulating values of the endothelial dysfunction (after being tested with reactive hyperemia) increased considerably from the initial 7-12% to 17-35% ($p<0.05$) and even doubled in some patients. The index of rigidity of the pulse wave decreased from 7.91±0.33% ($p<0.05$), the reflection index remained the same, 48.1±2.07 versus 47.7±2.4m/s. Similar tendencies were noticed after tests with reactive hyperemia, in comparison with initial data.

TABLE II

Dynamics of photoplethysmography parameters after treatment with *Nebilet*

Indexes	Initial	After treatment
Amplitude 1 (mm)	52.5± 4.1 (52.7± 4.4)	64.1±3.3 (72.7±3.0)
Amplitude 2 (mm)	25.0± 2.0 (23.2 ±4.5)	30.3±1.8 (28.3±2.2)
T (sec)	0.23±0.001 (0.23±0.01)	0.23±0.01 (0.23±0.01)
Height (mm)	1.82±0.05 (1.82±0.07)	1.64±0.03 (1.64±0.05)
Reflection index %	48.1±2.07 (44.1±2.2)	47.7±2.4 (39.0±2.7)
Rigidity index, m/s	7.91±0.35 (7.91±0.37)	7.45±0.33 (7.13±0.1)
Endothelial dysfunction index (%)	8.6±1.1	17.9±2.5

Legend: between brackets – data after the test with reactive hyperemia

The dynamic supervision of the indexes of rigidity and reflexion in patients with chronic obstructive pulmonary disease allows us to estimate the efficiency of the treatment, the state of the process of vascular remodeling and the diagnosis of the basic illness.

CONCLUSIONS

In patients with the bronchial obstructive syndrome in association with arterial hypertension the use of beta blocker *Nebilet* does not create the aggravation of bronchial permeability and improves considerably the endothelial function, the state of the central and pulmonary hemodynamic.

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CERCETĂRI PRIVIND INCIDENŢA MALFORMAŢIILOR CONGENITALE ÎN SPITALUL CLINIC "CUZA VODĂ" IAŞI

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INCIDENCE OF CONGENITAL MALFORMATIONS IN THE IAŞI "CUZA VODĂ" MATERNITY HOSPITAL (Abstract): **Aim**: To update the data on the incidence of congenital malformations in our area and emphasize the importance of prenatal diagnosis. **Material and methods**: The study was conducted over a period of ten years and included 2211 cases with congenital malformations. Such parameters as the incidence of malformations over these years, gestational age at the moment of diagnosis, and mortality from congenital malformations were followed. Statistical and graphical data were processed with the help of SPSS 16.0 and MS Excel software. **Results**: The total prevalence of malformations was 3.52%, comparable with the data in the literature. Over this period a number of 262 cases (11.8%) ended in spontaneous miscarriage or termination of pregnancy for fetal abnormalities. A significant percentage of these pregnant women gave birth to their child, many of them dying shortafter. **Conclusions**: Congenital malformations remain the leading cause of mortality in neonates. In Romania, the advanced techniques of prenatal diagnosis being used only in specialized centers, and a large number of cases may escape early diagnosis. This study underlines the importance of a correct assessment of fetuses so that the discovery of fetal abnormalities at birth not to be a surprise. **Key words**: CONGENITAL MALFORMATION, INCIDENCE, MORTALITY

În România, în anul 1984 s-a realizat „Registrul naţional de malformaţii congenitale şi boli genetice" şi fişe de evidenţă pentru urmărirea evoluţiei cazurilor cu malformaţii. Cu toate acestea, registrul naţional de malformaţii nu aduce date suficiente privind valorile de incidenţă deoarece unele cazuri subclinice rămân nediagnosticate (bicuspidia valvelor aortice), altele nu sunt înregistrate (malformaţiile grave care au determinat moartea intrauterină a fătului) (1, 2).

Studiul s-a realizat în cadrul Spitalului Clinic „Cuza Vodă" Iaşi. Am înregistrat atât sarcinile cu malformaţii congenitale fetale decelate ecografic prenatal, cât şi nou-născuţii cu anomalii care nu au fost detectate la examenul prenatal. Scopul studiului a fost de a actualiza datele privind incidenţa cazurilor de malformaţii congenitale, în perioada 2001-2010 şi sublinierea importanţei diagnosticului prenatal.

MATERIAL ŞI METODĂ

În studiu am inclus 2211 cazuri cu malformaţii care au fost diagnosticate în unita-

tea noastră în perioada 1 ianuarie 2001 - 31 decembrie 2010. Parametrii urmăriţi au fost: incidenţa malformaţiilor raportată la numărul total de naşteri, mediul de provenienţă şi vârstă gestaţională a gravidelor, mortalitatea datorată malformaţiilor congenitale. Datele au fost preluate din condicile de naştere, condicile de mortalitate şi morbiditate neonatală, din foile de observaţie şi condicile de necropsii şi au fost introduse într-o bază de date realizată în acest scop cu programul Excel. Prelucrarea statistică şi grafică a datelor a fost efectuată cu programul *SPSS 16.0* şi MS Excel.

Malformaţiile congenitale majore şi minore au fost grupate în 8 categorii în funcţie de localizare: sistem nervos central, cord, toraco-pulmonare, tub digestiv şi perete abdominal, genito-urinare, ale membrelor, ale feţei şi sindroame genetice. Au fost considerate malformaţii majore anomaliile letale sau incurabile şi condiţiile asociate cu handicapuri severe sau care au necesitat intervenţie chirurgicală în primul an de viaţă (3).

REZULTATE ŞI DISCUŢII

1. Incidenţa totală a malformaţiilor în perioada 1 ianuarie 2001-31 decembrie 2010. În perioada studiată, în Spitalul clinic de obstetrică şi ginecologie "Cuza-Vodă" Iaşi, au fost internaţi 62709 nou-născuţi dintre care 2211 copii cu malformaţii. Incidenţa totală a malformaţiilor a fost de 3,52%, valoare comparabilă cu cele existente în literatura de specialitate (2) şi puţin mai mare decât cea evidenţiată într-un studiu anterior efectuat în acelaşi spital în perioada 1 ianuarie 1997- 31 decembrie 2001 (4). Incidenţa malformaţiilor a crescut de la 6,4% în 2001 la 15,6% în anul 2007, după care a prezentat o scădere semnificativă până la 7,3% în 2010 (fig. 1). Această constatare poate fi explicată prin creşterea factorilor de risc pentru malformaţii (5, 6). Din luna februarie 2005, unitatea noastră e devenit centru terţiar de diagnostic şi tratament, având ca efect şi creşterea adresabilităţii gravidelor cu suspiciune de anomalii fetale.

2. Repartiţia pe sexe a lotului luat în studiu în perioada 2001-2010. În cei 10 ani luaţi în studiu, din lotul de 2211 copii cu malformaţii congenitale, 916 (41,4%) au fost de sex feminin, 1295 (58,6%) masculin (fig. 2); 1042 (47,1%) au provenit din mediul urban şi 1169 (52,9%) din rural. Se remarcă o uşoară preferinţă pentru sexul masculin. Vârsta maternă a fost cuprinsă între 18 şi 46 ani, cu o medie de 31,2 ani.

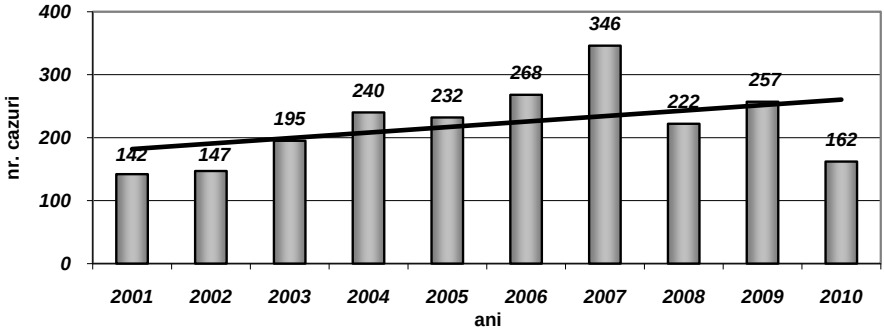

Fig. 1. Distribuţia pe ani a cazurilor cu malformaţii. Se remarcă o creştere a constantă numărului de cazuri până în anul 2007

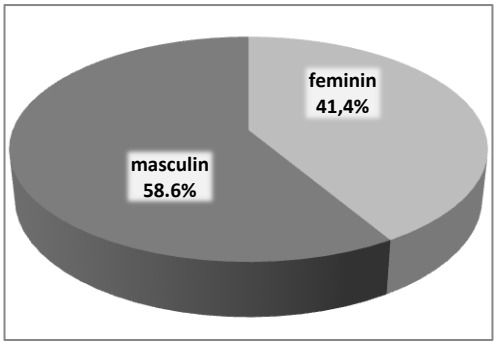

Fig. 2. Structura lotului pe sexe. Se observă o afectare preponderentă a sexului masculin comparativ cu cel feminin

3. Repartiţia pe mediul de proveniență a gravidelor din lotul studiat. Urmărind structura lotului pe medii de proveniență se observă diferenţe semnificative statistic (χ^2 = 22,824, p= 0,007 < 0,05) între cei 10 ani în privinţa structurii lotului pe medii de proveniență. Astfel, în anii 2004, 2005, 2007 şi 2008 majoritatea cazurilor au provenit din mediul urban, cu un procentaj maxim de 53,3% în anul 2004, în timp ce în ceilalţi ani majoritatea cazurilor au provenit din mediul rural, cu un maxim înregistrat în anul 2010 – 64,2% (fig. 3, 4).

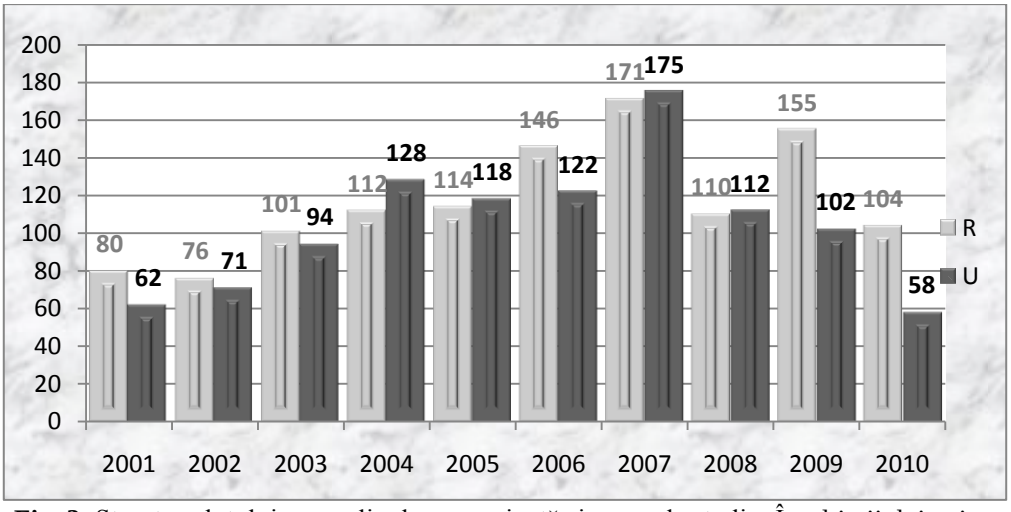

Fig. 3. Structura lotului pe mediu de proveniență şi pe an de studiu. În ultimii doi ani se observă o creştere semnificativă a cazurilor din mediul rural.

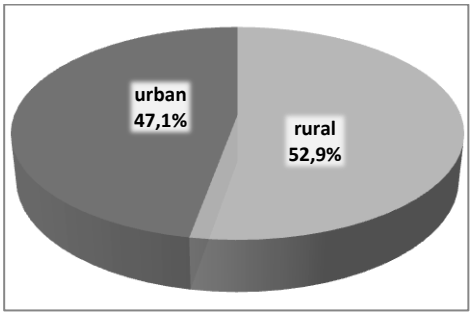

Fig. 4. Structura lotului pe mediu de proveniență

4. Analiza repartiţiei, în relaţie cu vârsta gestaţională. În perioada luată în studiu, din cele 2211 cazuri de malformaţii înregistrate în 262 cazuri (11,8%) sarcina s-a terminat prin avort spontan sau terapeutic pentru malformaţii grave incompatibile cu supravieţuirea. În restul cazurilor sarcina s-a încheiat prematur într-un procent de 47,5%. La termen s-au născut 41% dintre feţii cu malformaţii (fig. 5).

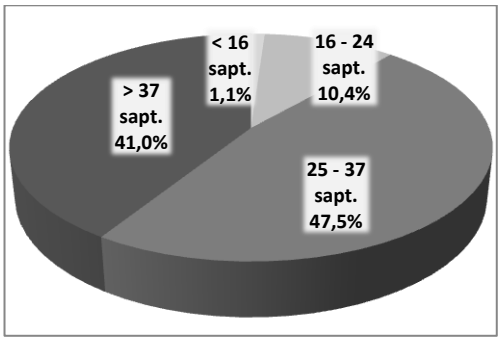

Fig. 5. Structura lotului după vârsta gestaţională

5. Repartiţia în relaţie cu modalitatea de finalizare a sarcinii Odată cu creşterea cazurilor cu malformaţii a crescut şi rata diagnosticului fapt evidenţiat prin creşterea constantă a cazurilor cu avort terapeutic (fig. 6).

6. Distribuţia cazurilor în relaţie cu tipul de anomalie congenitală. Anomaliile au variat de la minore la incompatibile cu viaţa. *Anomalii izolate* s-au înregistrat la 2032 (91,9%) din cazuri, cele mai frecvente fiind cele cardiace, genito-urinare şi scheletice. În 174 cazuri diagnosticul a fost sindrom plurimalformativ. S-au înregistrat 178 malformaţii de sistem nervos central, 640 malformaţii de cord, 59 toracice şi pulmonare, 125 de tub digestiv şi perete abdominal, 419 de aparat genito-urinar, 326 ale membrelor şi scheletului, 153 ale feţei, 174 cazuri cu sindrom plurimalformativ şi 152 de sindroame genetice (fig. 7).

7. Decesele înregistrate la copiii cu anomalii congenitale. În perioada 2001-2010, au decedat 185 copii (9,5%) din totalul de 2211 de cazuri cu malformaţii.

Dintre aceştia, 13% prezentau anomalii ale SNC, 53.5% prezentau anomalii cardiace, 3.8% malformaţii toracice şi pulmonare, 2.7% malformaţii de tub digestiv şi perete abdominal, 7% malformaţii genito-urinare, 3.2% malformaţii ale membrelor şi scheletului, 2.2% malformaţii ale feţei, 4.9% sindroame genetice, 23.3% sindroame plurimalformative şi 2.7% alte tipuri de malformaţii (fig. 9).

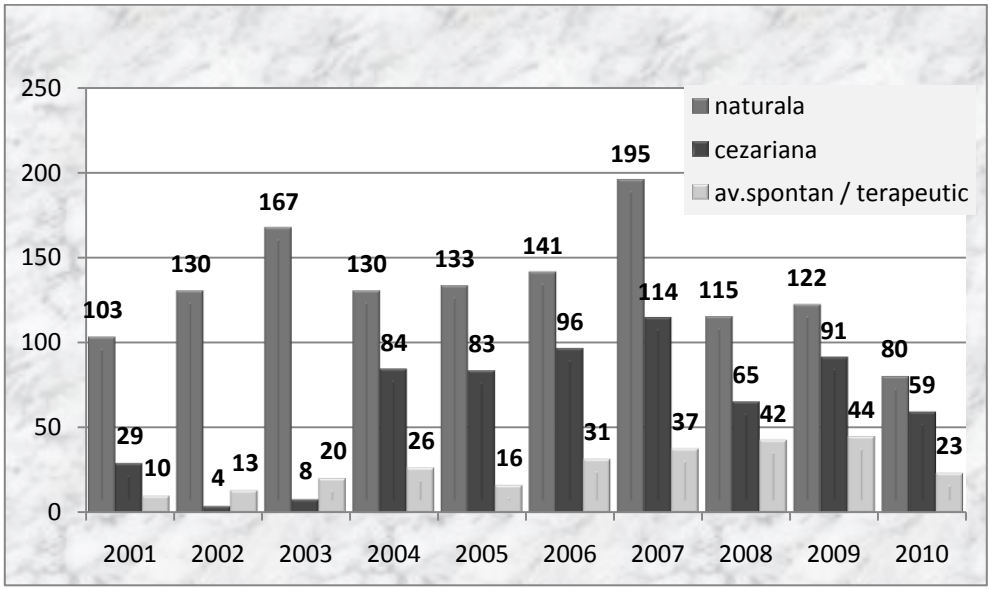

Fig. 6. Structura lotului după modul de finalizare a sarcinii pe ani de studiu

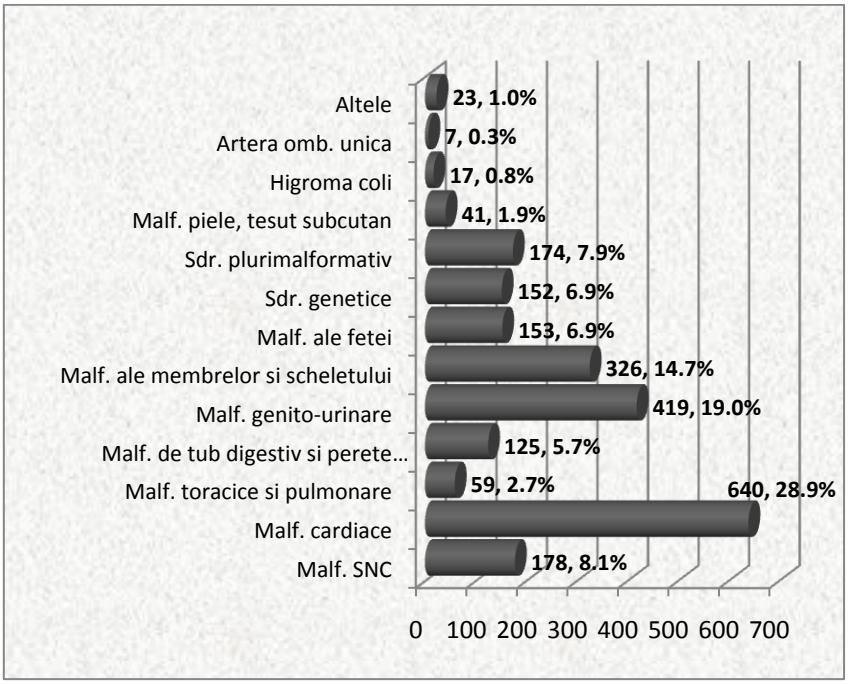

Fig. 7. Prevalenta principalelor grupe de malformaţii incluse în studiu

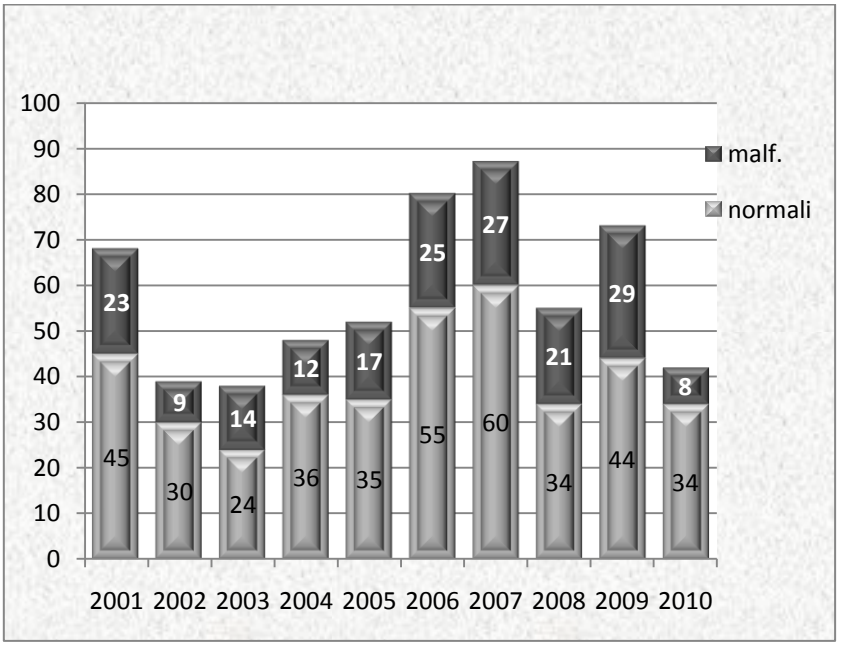

Fig. 8. Diagrama reflectă raportul între decesele neonatale şi decesele prin malformaţii comparativ pe anii 2001-2010.

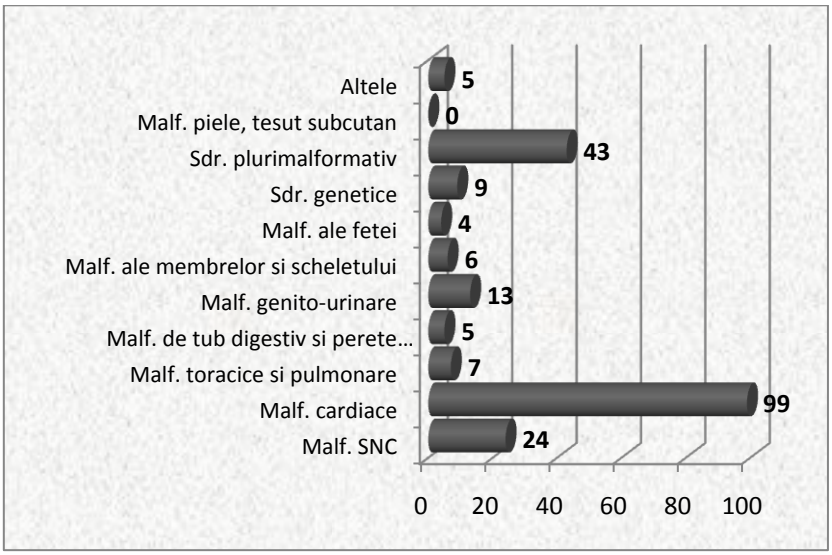

Fig. 9. Distribuţia deceselor neonatale pe categorii de malformaţii. Se observă numărul ridicat de decese la cazurile cu malformaţii cardiace.

CONCLUZII

Malformaţiile congenitale reprezintă una din principalele cauze de mortalitate la nou-născuţi. Singura modalitate de reducere a incidenţei lor rămâne dispensarizarea corectă a sarcinilor şi creşterea calităţii diagnosticului prenatal (7). În România, utilizarea tehnicilor avansate de diagnostic prenatal este limitată numai în centre specializate, drept urmare, un număr important de cazuri nu sunt diagnosticate precoce. Acest studiu subliniază importanţa unei evaluări responsabile a sănătăţii fătului, astfel încât să nu mai existe surpriza descoperirii unor anomalii fetale la naştere.

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MODELAREA MATEMATICĂ A FENOMENULUI DE DROGODEPENDENŢĂ

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MATHEMATICAL MODELLING OF DRUG ADDICTION (Abstract). **Aim:** To describe and assess the phenomenon of drug addiction by the use of a system of linear differential equations. **Material and methods:** For analytical purposes, the study population (1020 individuals) was divided into three groups: likely to use drugs, drug users, and recovered (S-D model). **Results:** The number of individuals likely to use drugs decreases with the increase in the number of infected ones; thus, in about 10 days, the number of infected individuals matches the number of those likely to use drugs. If the global parameters of the study model are changed, a more rapid increase in the number of infected individuals is noticed. Also noticed is the fact that the number of recoveries is likely to decrease with the increase in the number of infected individuals. **Conclusions:** Although numerical techniques are often preferable, the analytical ones have some advantages. Thus, an analytical approach may provide a deeper understanding of the mechanism of a given phenomenon, so it is more convenient to describe the state variables in terms of parameters than in terms of numerical approximation. To assess to role of hazard and uncertain associations in the future statistical methods will be used. **Key words:** DRUG ADDICTION, MATHEMATICAL MODEL

Un avantaj al modelării matematice a dependenței de droguri îl constituie claritatea şi precizia formulării matematice. Un model ce utilizează ecuații diferențiale, integrale sau funcții diferențiale nu este ambiguu sau vag. Desigur, parametrii trebuiesc definiți precis şi fiecare termen al ecuațiilor trebuie explicat în termenii mecanismului, dar rezultatul modelului este o exprimare clară a principiilor de bază implicate. Odată ce formularea matematică este completă există mai multe tehnici matematice pentru determinarea limitelor, echilibrului, soluțiilor periodice şi stabilității lor globale. Mai mult informații cu privire la model pot fi obținute prin simulări numerice ale ecuațiilor. Analiza matematică şi simulările computerizate pot să identifice combinații importante ale parametrilor şi aspecte esențiale sau variabile ale modelului respectiv. Pentru a alege şi utiliza eficient modelul epidemiologic pentru o boală specifică trebuie să înțelegem comportamentul formulărilor disponibile şi a celor particulare. Singurele date disponibile sunt din grupurile de consumatori deja descoperiti sau din incidența endemică. Din păcate aceste date nu sunt complete deoarece multe cazuri nu sunt raportate. Deoarece de multe ori nu există date concrete,

simulările computerizate trebuie utilizate pentru a elabora teorii experimentale utilizând diferite valori ale parametrilor.

MATERIAL ŞI METODĂ

În formularea unui model matematic pentru o dependentă de un drog este necesară determinarea factorilor ce trebuie incluşi sau omişi. Modelele simple au avantajul că sunt formate din mai puţini parametri dar au dezavantajul că sunt mai puţin realiste. Modelele mai complexe pot fi mai realiste dar pot conţine mai mulţi parametri pentru care nu pot fi obţinute valori estimative. Este necesară luarea unor decizii în formularea modelului astfel încât acesta să fie cât mai simplu posibil şi cât mai adecvat problemelor considerate. Cea mai dificilă etapă în modelare este găsirea combinaţiei perfecte între datele existente, întrebările de interes şi modelul matematic ce conduce la răspunsuri. Se poate crea un model în care se considera au considerat o populaţie fixă împărţită în trei compartimente: susceptibili de a consuma droguri **S(t),** consumatori **D(t)** şi recuperaţi **R(t**). Compartimentele folosite în acest model definesc trei clase:

- **S(t**) reprezintă numărul indivizilor ce nu au consumat droguri la momentul de timp *t* sau sunt susceptibili pentru a consuma un drog specific;
- **D(t)** reprezintă numărul indivizilor ce consuma regulat droguri şi care au capacitatea de a atrage noi indivizi (dacă sunt considerati consumatori de risc mare) în rândul populaţiei susceptibile;
- **R(t)** reprezintă compartimentul din care fac parte indivizi ce au consumat droguri şi s-au recuperat. Aceştia nu mai sunt pasibili de a consuma droguri sau de a atrage noi consumatori.
- Numărul reproductiv de bază R_0 este definit ca fiind numărul mediu de consumatori atraşi în grup (consumatori de droguri usoare) de indivizii care consumă regulat droguri într-o populaţie complet susceptibilă. Reţinem faptul că R_0 este denumit şi raţia reproductivă de bază. Numărul de contacte **e** este definit ca fiind numărul mediu de contacte produse de un consumator regulat pe întreaga perioadă studiată. Numărul de înlocuiri **R** este definit ca find numărul mediu de consumatori atraşi în grup (consumatori de droguri uşoare) de indivizii care consumă regulat droguri de-a lungul perioadei studiate. Parametrul **R** se modifică în funcţie de timpul **t** odată cu evoluţia dependenţei de droguri după apariţia unui consumator în grupul studiat.Modelul propus şi dezvoltat în această lucrare include un sistem de ecuaţii diferenţiale liniare. Principial, rezolvarea poate fi analitică, ca în cazurile foarte simple,sau numerică, în cazurile complexe, astfel încât să se obţină o parametrizare cât mai apropiată de modelul real, în condiţii rezonabile de resurse de calcul şi timp.

Pentru o evaluare analitică să presupunem un model compartimental (1), de tip S-D pentru o populaţie constantă, unul din cele mai simple modele. Considerăm condiţiile iniţiale de forma:

$$\begin{cases} S(0) = S_0 \\ D(0) = D_0 \end{cases}$$

Deoarece populaţia grupului studiat ramâne constantă, vom avea:

$$\begin{cases} S(t) + D(t) = S_0 + D_0, \forall t \in \Re \\ S_0 = n, D_0 = 1 \end{cases}$$

Legea conservării masei, exprimată prima dată de Guldberg în 1864 postulează că "rata" unei reacţii chimice este direct proporţională cu produsul concentraţiei efective a fiecărei molecule participante. Conform acestei legii, susceptibilii devin

consumatori la o rată proporţională cu produsul mărimii celor două populaţii. Deci, dacă $b > 0$ reprezintă constanta de contact, atunci $bS(t)D(t)$ este numărul de susceptibili deveniţi consumatori pe unitatea de timp. Astfel putem scrie:

$$\begin{cases} \dfrac{dS}{dt} = -bS(t)D(t) \\ \dfrac{dD}{dt} = bS(t)D(t) \end{cases},$$

$$\begin{cases} S(t) > 0, D(t) > 0, b > 0 \\ \dfrac{dS}{dt} \le 0, S(t) descrescatoare \\ \dfrac{dD}{dt} \le 0, S(t) descrescatoare \end{cases}$$

Fiind dat b>0 şi $D(t) > 0$ pentru toţi t, sistemul de ecuaţii atinge echilibrul dacă şi numai dacă : S(t) = 0. Atunci epidemia se opreşte şi toţi susceptibilii devin consumatori. Ecuaţiile pot fi analizate analitic. Se obţine

$$\begin{cases} \dfrac{dS}{dt} = -bS[(n+1) - S] \\ S(t) = \dfrac{(n+1)e^{-Cbt(n+1)}}{1 - e^{-Cbt(n+1)}} \end{cases}$$

Impunând condiţiile iniţiale anterioare, obţinem soluţia generală:

$$S(t) = \frac{n(n+1)e^{b(n+1)t}}{n + e^{b(n+1)t}} .$$

REZULTATE

Abordările de rezolvare numerică sunt variate: ecuaţii cu diferenţe, metode Monte Carlo şi Runge-Kutta, metoda lui Galerkin în ecuaţii integro-diferenţiale pentru modelele bazate pe difuzie. Modelele compartimentale pentru dependenţă stochastică sunt în mod obişnuit inatacabile matematic, când sunt studiate prin metode numerice tradiţionale. Ecuaţiile diferenţiale din modelele compartimentale sunt transformate în ecuaţii cu diferenţe cu pas finit, pentru calcule care au fost implementate în Java . Alte metode numerice implică calculul numeric al unor integrale prin metode aproximative. Pasul de integrare, şi metoda numerică de integrare aleasă va determina efortul computaţional şi precizia de aproximare faţă de un calcul analitic. În modelarea infecţiilor şi dependentei de droguri au fost propuse tehnici şi algoritmi care includ curbe statistice de aproximare, curbe de extrapolare statistice şi modele dinamice compartimentale. Unele lucrări tratează aspectul haotic al sistemelor dinamice şi ca atare sunt analizate periodicitatea, atractorii haotici. Deşi tehnicile numerice sunt de multe ori preferabile, în obţinerea soluţiilor pentru aceste sisteme dinamice, soluţiile analitice au şi avantaje. De exemplu, o abordare analitică, poate furniza o mai profundă înţelegere asupra mecanismului dependentei, şi deci este mai convenabil să descriem variabilele de stare în termen de parametri, decât o aproximare numerică.

$$\begin{cases} \dfrac{dS}{dt} = -bS[(n+1) - S] \\ S(t) = \dfrac{(n+1)e^{-Cbt(n+1)}}{1 - e^{-Cbt(n+1)}} \end{cases}$$

Modelul clasic dependent de timp este alcătuit din trei ecuaţii diferenţiale ordinare, iar în modelul studiat ,grupul studiat este dependent atât de timp cât şi de spaţiu, generând un sistem de ecuaţii neliniare ce trebuie rezolvat pentru fiecare interval de timp.

Având în vedere o populaţie fixă de 1020 persoane, se observă că numărul persoanelor susceptibile scade odată cu creşterea numărului persoanelor infecţioase astfel încât în aproximativ zece zile numărul celor infectaţi, respectiv al susceptibililor are aceeaşi valoare (fig.1).

Dacă modificăm parametrii globali ca-

racteristici modelului S-D, se observă o creştere mai rapidă a indivizilor infecţioşi, corespunzător unui punct de echilibru raportat la curbele S/I mai mic (fig.2).

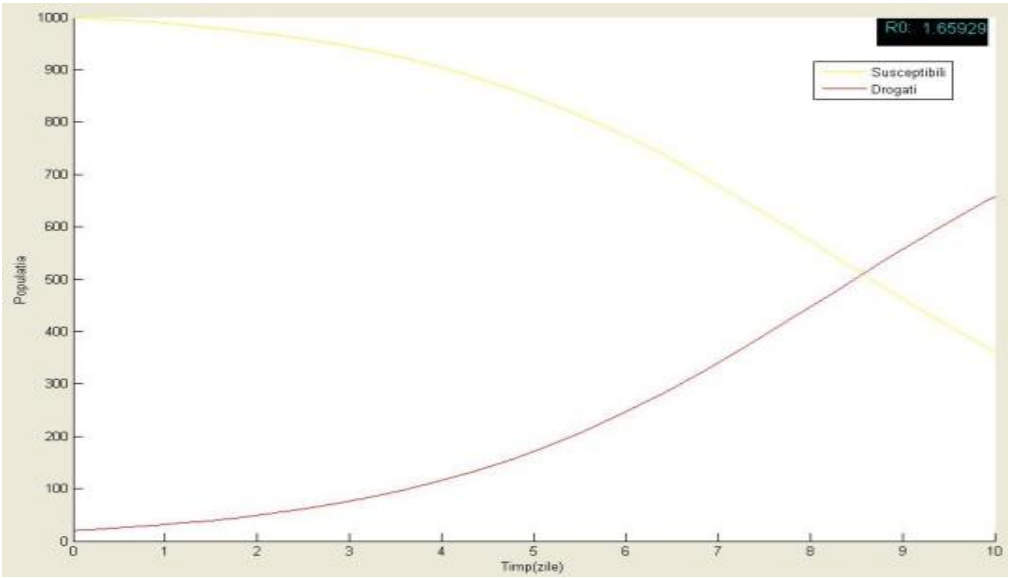

Fig. 1. Simularea soluției pentru n=1020,b=5

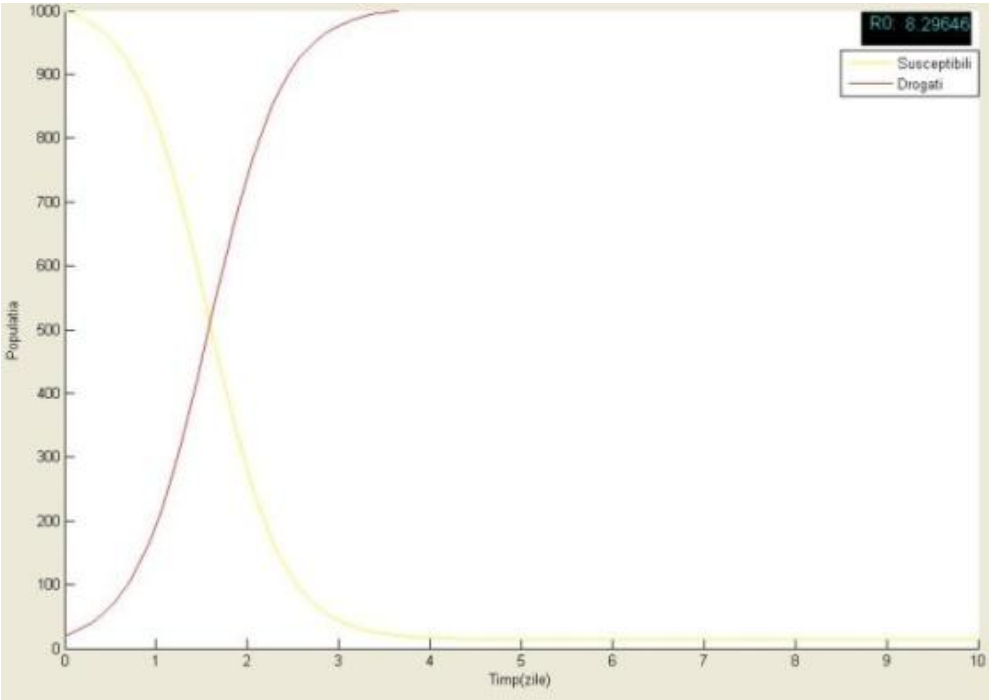

Fig. 2. Simularea soluției pentru n=1020,b=4

De asemenea se observă că o variație mare a perioadei infecțioase (de la trei la zece zile) determină o variație proporțională a numărului reproductiv de bază. Un număr mic de contacte a indivizilor cu grupul studiat de consumatori , păstrând aceleaşi condiţii pentru perioada studiată , va conduce la o creştere în timp mai lentă a numarului de consumatori concomitent cu o descreştere lentă a susceptibililor, cu un maxim al indivizilor recuperați.

Mecanismul de dinamică a dependenței în cadrul unui grup poate fi studiată cu ajutorul metodei Newton şi implementată în Matlab. Grupul se va mişca în spațiu datorită unor multitudini de nevoi, tendința lor fiind de dispersie sau de grupare. Dispersia are un caracter aleator. Dacă grupul şi durata de timp sunt suficient de mari atunci modelarea dispersiei are la bază principiile legii de deplasare a lui Fick. Modelul de dispersie în cazul S-D este de forma (2):

$$\begin{cases} \dfrac{\partial S}{\partial t} = -aSD, S(x,0) = S_0, 0 \le x \le L \\ \dfrac{\partial D}{\partial t} = aSD - bD + U\dfrac{\partial^2 D}{\partial x^2}, D(x,0) = D_0 \\ \dfrac{\partial D}{\partial t}(0,t) = \dfrac{\partial D}{\partial x}(L,t) = 0 \end{cases}$$

Pentru a rezolva ecuația diferențială caracteristică populației consumatoare de droguri, ce are în componența sa o variație unidirecțională, trebuie să impunem condiții la limită specifice:

$$\frac{\partial D}{\partial x}(0,t) = \frac{\partial D}{\partial t}(L,t) = 0$$

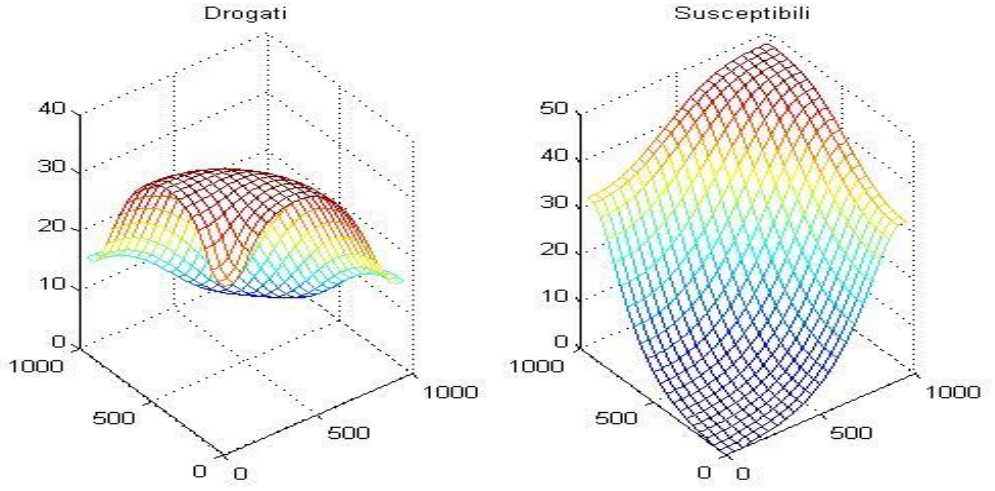

Fig. 3. Aproximarea numerică a soluției în cazul unidimensional

Modelul S-D poate fi modificat în două moduri (3). Un prim mod este să presupunem că cei care consumă droguri nu se recuperează şi în cele din urmă părăsesc grupul, cu o rată –bD.

Al doilea mod presupune faptul că populația grupului de consumatori este dispersată în două direcții potrivit legii lui Fick iar populația susceptibilă de a consuma droguri nu se dispersează.

Astfel rezultă că populația este dependentă atât de timp cât şi de spațiu în două

direcții, cu valori de concentrație S(x,y,t)și D(x,y,t), conform relațiilor (4) :

$$\begin{cases} \dfrac{\partial S}{\partial t} = -aSD, D(x,y,0) = D_0 \\ \dfrac{\partial D}{\partial t} = aSD - bD + U\dfrac{\partial^2 D}{\partial x^2} + U\dfrac{\partial^2 D}{\partial y^2} \\ \dfrac{\partial D}{\partial x}(0,y,t) = \dfrac{\partial D}{\partial x}(L,y,t) \\ S(x,y,0) = S_0, 0 \le x, y \le L, \\ \dfrac{\partial D}{\partial x}(x,0,t) = \dfrac{\partial D}{\partial y}(x,L,t) \end{cases}$$

Prin discretizarea ecuațiilor sistemului, vom obține o aproximare numerică a soluției de forma:

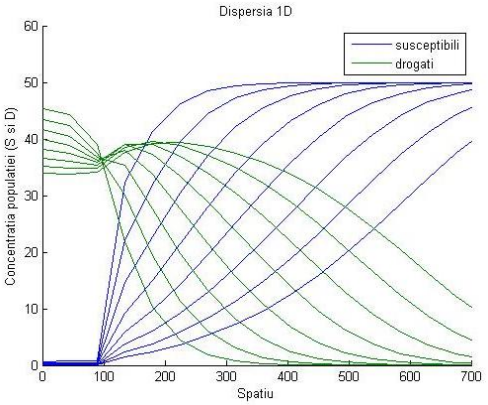

Fig. 4. Aproximarea numerică a soluției în cazul bidimensional

CONCLUZII

Metodele matematice şi informatice au devenit unelte importante în analiza şi controlul consumului de droguri. Parteneriatul între informaticieni, matematicieni, bioingineri, experții în sănătate publică şi biologi este extrem de importantă în lupta împotriva acestui flagel. În modelul prezentat în acest articol am considerat populația constantă. Această presupunere este rezonabilă, dacă grupul de consumatori devine destul de mare în mai puțin de un an. Este de asemenea o presupunere rezonabilă, în modelarea consumului de droguri de-a lungul mai multor ani ca numărul de noi consumatori care intră în grupul studiat să compenseze numărul celor care renunță la droguri. Dacă însă această balanță nu este realizată, atunci mărimea populației este o funcție de timp. Modelarea este crucială în studiul fenomenului de drogodependență deoarece în cele mai multe cazuri nu se pot realiza experimente. Pentru a evalua rolul hazardului şi a asocierilor incerte ,în viitor vom utiliza metode statistice. Rolul acestora devine mult mai complicat cu trecerea timpului datorită existenței unui volum mare de informații şi dezvoltarea unor noi tehnici şi metode computaționale mult mai performante.

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RP-HPLC ANALYSIS METHOD OF BISOPROLOL FUMARATE IN A NEW TABLET FORMULATION

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RP-HPLC ANALYSIS METHOD OF BISOPROLOL FUMARATE IN A NEW TABLET FORMULATION (Abstract): **Aim:** Development of a new HPLC method for the quantitative determination of bisoprolol fumarate in a novel sustained release tablet formulation. **Material and methods:** The sustained release matrix tablets with bisoprolol fumarate were formulated using an innovative combination of hydroxypropyl methylcellulose and Precirol ATO5 as excipients. The HPLC separation was done using an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 µm) and a mobile phase consisting of acetonitrile 10 mM and pH 4.5 phosphate buffer solution (10:90, v/v) at a flow rate of 1.0 mL/min. The method was validated according to ICH guidelines in terms of accuracy, precision, linearity and robustness. **Results:** Bisoprolol fumarate was separated in less than 4 minutes with good resolution and without any tailing effect or excipient interference. The limit of detection and the limit of quantification were 2.79 and 3.07 µg/mL, respectively. The relative standard deviation evaluated for the precision of the method was lower than 1%. **Conclusions:** The HPLC method developed for the determination of bisoprolol fumarate in a novel sustained release tablet formulation was specific and simple and it could also be used for *in vitro* studies.
Keywords: HPLC METHOD, BISOPROLOL FUMARATE, PRECIROL, HYDROXYPROPYL METHYLCELLULOSE, NEW TABLET FORMULATIONS.

Hypertension continues to be one of the leading causes of death in the world. The management of hypertension requires long-term treatment that may result in poor patient compliance with conventional dosage forms due to greater frequency of drug administration (1). For this disease the most suitable administration route is the oral route. The advantages of oral administration and oral dosage forms are convenience, ease of use and lower costs.

Bisoprolol fumarate (BF) is a synthetic β_1-selective adrenoceptor blocking agent used extensively in the management of hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. It may be used alone or in combination with other antihypertensive agents, particularly thiazide diuretics (2, 3). A number of factors might affect the pharmacokinetic profile of BF and thereby contribute to the changes in therapeutic efficacy or modify

the adverse events profile. A quick and efficient way to eliminate these problems is the development of an appropriate dosage form in order to improve patient compliance. The release rate of BF, a highly water-soluble drug can be modulated using hydroxypropyl methylcellulose and Precirol ATO5 as excipients (4, 5, 6).

In order to increase the bioavailability of a drug, one of the options found in the arsenal of formulators today is melt granulation. In general, melt granulation is done by melting an appropriate polymer (Precirol) and then homogenizing the active ingredient and other formulation components into the melt (4). The aim of the procedure is the homogeneous distribution of the active ingredient in the resulting solid, which can be processed further into a solid dosage form. The advantage of melt granulation technique in comparison to conventional granulation is that no water or organic solvents are needed. Because there is no drying step, the process is less time consuming and uses less energy than wet granulation (5).

Over the last decade, high performance liquid chromatography (HPLC) has proven to be the predominant technology used in pharmaceutical laboratories worldwide in order to analyze various drug substances.

The aim of the study was the development and validation of a Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method for the determination of BF, formulated into new oral tablets with modified release. Thorough analysis of specialized literature revealed that there were numerous determination methods of bisoprolol in bulk, tablet dosage forms and biological samples, such as HPLC (7, 8), wave voltammetry (9, 10), UV-VIs spectrophotometry (11, 12), LC-MS/MS (13, 14). The reported methods are sensitive, but they are quite complicated and time consuming, thus unsuitable for routine analysis, and require expensive or sophisticated instruments.

MATERIAL AND METHODS

The substances used were: bisoprolol fumarate - 99.97 % reference substance (Unichem Laboratories LTD India), HPLC-grade acetonitrile (Merck Germany), hydrogen peroxide, ortho-phosphoric acid (Chemical Company Romania), sodium hydroxide, hydrochloric acid (Merck Germany). All chemicals and solvents used in the assay procedures were of analytical reagent grade.

Apparatus. An Agilent Technologies 1100 High Performance Liquid Chromatograph equipped with Diode Array Detector, a Kern 770 analytical balance and a Cole Parmer ultrasonic bath were used.

Preparation of modified release tablets. The 20 mg bisoprolol fumarate modified release matrix tablets had been formulated previously using an innovative combination of hydroxypropyl methylcellulose and Precirol ATO5 as excipients through melt granulation technique (4, 6).

Chromatographic conditions.The chromatographic separation of bisoprolol was performed using isocratic elution on an Agilent Eclipse XDB-C18 reverse phase stainless steel column (150 mm x 4.6 mm, 5 µm). The temperature of the column and auto sampler was maintained constant at 25°C. A 50 µL sample loop injector was used. The detection was done spectrophotometrically at 227 nm. The mobile phase was a mixture of acetonitrile and pH 4.5 phosphate buffer solution (10:90, v/v) at a flow rate of 1 mL/min

Preparation of stock and standard solutions: A 100 µg/mL BF stock solution was prepared using Milli-Q water. Aliquots of

the stock solutions were diluted using mobile phase to yield BF standard solutions in the concentration range 5-80 µg/mL.

Procedure for quantitative determination of BF from the original 20 mg tablets: 20 tablets were accurately weighed, crushed and finely powdered. An amount of powder equivalent to 10 mg BF was weighed and transferred into a 50 mL volumetric flask with methanol and ultrasonicated for 15 minutes. The solutions were filtered and further diluted using mobile phase to achieve the final concentration of 50 µg/mL BF.

The described method has been validated for the assay of BF according to ICH guidelines (15).

System suitability test of the HPLC system was performed through six replicate injections of BF standard solution. The capacity factor, tailing factor, theoretical plates and symmetry were also evaluated (16).

Linearity, LOD and LOQ: The standard solution was injected in triplicate into the HPLC system and calibration graphs were obtained by plotting the area of the peaks versus corresponding concentration levels. Regression data analysis was performed using least squares linear regression for the statistical analysis. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations: LOD = 3.3×standard deviation/ slope and LOQ = 10×standard deviation/slope (17, 18).

Precision study was performed to evaluate the intra- and inter-day variation. It was carried out by estimating the corresponding responses three times on the same day and on three different day (first, second and fifth day) for three different concentration levels of BF (25, 50, 75 µg/mL).

The accuracy of the analytic method was assessed as the percentage relative error. The recovery was assessed at three different concentration levels (15).

The robustness was determined by analyzing the same sample under a variety of conditions, such as: variations in flow rate, detection wavelength and mobile phase composition.

Stability studies indicated that the samples were stable while kept at room temperature and 4°C for 12 (short-term) and 48 hours (long-term).

RESULTS AND DISCUSSION

The objective of the chromatographic method was to separate the peaks of BF and its impurities in a short run time with good system suitability parameters. The majority of the ionizable pharmaceutical compounds can be very well separated on C18 (17), thus, bisoprolol could be satisfactorily separated using reversed phase chromatography. Several parameters like pH and the composition of the mobile phase, flow rate, column temperature, wavelength were examined for the optimization of HPLC analysis of bisoprolol. According to the results it was established that the optimum mobile phase was a mixture of acetonitrile and pH 4.5 phosphate buffer solution (10:90, v/v) and the retention time for the pharmaceutical substance was 3.52 min (fig. 1). Detection wavelength was established at 227 nm (fig. 2).

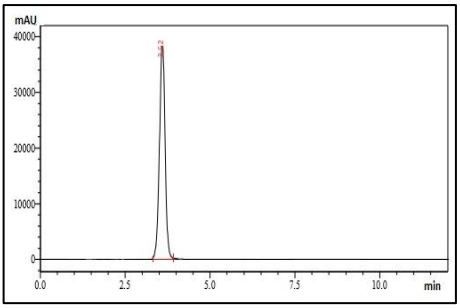

Fig. 1. Retention time

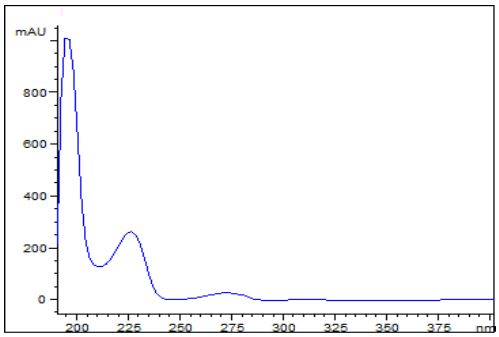

Fig. 2. BF absorption spectra

System suitability was assessed by injecting six replicates of standard solution into the apparatus. It was established through six replicate injections of standard solution that the tailing factor was less than 2.0 (18, 19) and the theoretical plates were greater than 5,000 (tab. I).

TABLE I

System suitability parameters

Capacity factor	8.02
Tailing factor	1.72
Theoretical plates	8400
Symmetry	0.91

For the linearity study the graph (fig. 3) showed a good linearity in the 5-80 µg/mL BF concentration range with a correlation coefficient (r) of 0.9994. The statistical parameters of the developed method are summarized below (tab. II).

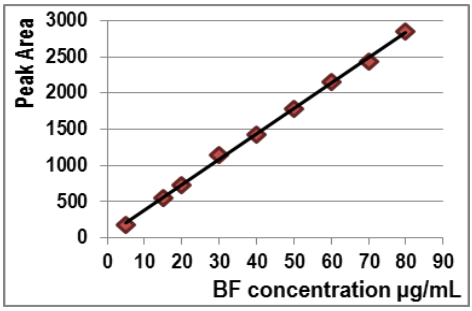

Fig. 3. Calibration curve

TABLE II

Statistical parameters

Parameter	Value
Retention time	3.52 min
Linear range	5-80 µg/mL
Limit of detection	2.79 µg/mL
Limit of quantification	3.07 µg/mL
Slope	35.096
Intercept	32.681
Correlation coefficient (r)	0.9994

Values for accuracy and precision of the method are listed in table III. All experimental results are in the range of the acceptability for accuracy (97.0-103.0%). The % RSD values found in precision study showed that the proposed method provides acceptable intra- and inter-day variations for BF determination.

TABLE III

Accuracy and precision studies

Theoretical conc. (µg/mL)	Accuracy		Precision			
			Intra-day		Inter-day	
	Mean recovered conc. of BF	Mean % recovery	Mean*±SD	% RSD	Mean*±SD	% RSD
25	24.78	99.12	24.98±0.3141	0.71	24.86±0.3051	0.75
50	50.05	100.10	50.09±0.2856	0.63	50.06±0.2924	0.59
75	75.05	100.06	75.04±0.3015	0.68	75.10±0.2963	0.72
** average concentration (µg/mL) for three determinations*						

The results of robustness study (tab. IV) showed that there were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, proving that the method was robust. The % RSD of bisoprolol fumarate was lower than 2.0 %.

The results of the stability studies are given the percent ratios within the acceptance range of 90-110% (tab. V).

TABLE IV
Robustness Study

Parameter	Optimized	Variations	Retention time (min)	Conclusion
Flow rate (±0.2 mL/min)	1.0 mL/min	0.8 mL/min	3.59	robust
		1.0 mL/min	3.52	robust
		1.2 mL/min	3.33	robust
Detection wavelength (±5 nm)	227 nm	223 nm	3.52	robust
		227 nm	3.52	robust
		230 nm	3.58	robust
Mobile phase composition (acetonitrile: pH 4.5 phosphate buffer solution) (±0.5 %)	10:90 (v/v)	5:95 v/v	3.61	robust
		10:90 v/v	3.52	robust
		15:85 v/v	3.55	robust

TABLE V
Stability studies of BF in solution

Added, µg mL^{-1}	Intra-day		Inter-day	
	Room temperature (12 h)	Refrigerator (4°C, 12 h)	Room temperature (12 h)	Refrigerator (4°C, 12 h)
25	98.11±3.85	99.61±3.78	99.01±3.16	99.31±2.16
50	99.17±2.37	98.93±2.17	100.02±0.66	99.92±1.66
75	98.67±2.37	99.83±1.17	101.02±3.66	99.95±2.66

The validated method was applied to the determination of BF in original tablet formulations. The result of the assays (n = 6) are presented in table VI. No interference of the tablet excipients was detected.

TABLE VI
Analysis of BF in developed tablets

Label claim (mg/tablet)	% found ± SD (n=6)	RSD (%)
20	100.12±0.6942	0.6879
20	100.32±0.87.52	0.8556

CONCLUSIONS

We have developed a new HPLC method with UV detection for the estimation of bisoprolol fumarate in sustained release tablets. The advantages of our method are short analysis time, a simple procedure for sample preparation, specificity, and ability to separate the drug from formulation excipients. No extraction procedure is necessary, and the mobile phase composition is quite simple to prepare and is economical. The method is suitable for routine analysis of BF in bulk or in tablet dosage forms and can be also used during *in vitro* studies.

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CONSIDERAŢII EPIDEMIOLOGICE ŞI MEDICO-LEGALE ASUPRA FRECVENŢEI ŞI CAUZELOR PRODUCERII DECESELOR PRIN VIOLENŢĂ, ÎN JUDEŢUL IAŞI, ÎN PERIOADA 1997-2006

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EPIDEMIOLOGICAL AND FORENSIC MEDICAL CONSIDERATIONS REGARDING FREQUENCY AND CAUSES OF VIOLENT DEATHS IN IASI COUNTY, BETWEEN 1997-2006 (Abstract)**:** Authors present some epidemiological aspects of violent deaths in Iasi County, with certain comparisons with Romania, during the period 1997-2006. **Material and method:** The study included information collected by Institutes of Forensic Medicine at the national level. **Results**: In Romania, violent deaths registered values between 2,6 and 4,5‰, with yearly non-significant statistical differences when reported to causes of death, excepted years 2005 and 2006. Violent deaths situated Iasi County over the national level (8-11 / 2.7‰). Homicides registered 9.3‰, as compared with much more reduced values in other 12 Romanian counties, in the same period of study. **Conclusions**: Violent death in Romania and Iasi County highlighted a dependence of difficulties to carry out efficient programmes of prevention and emergency assistance. **Key words**: VIOLENT DEATH, EPIDEMIOLOGY, FORENSIC MEDICINE

Violenţa a fost şi va fi mereu parte a experienţei umane, cu un impact diferit în variate arii geografice. Anual, la nivel mondial, violenţa ucide peste un milion de oameni, îndeosebi din grupurile de vârsta 15-44 ani (1,2,3,4).

Eteroviolenţa este semnalată în istoria existenţei umane, atribuindu-i-se numeroase definiţii, între care, recent, se face referire la cea elaborată de OMS, după care: „violenţa reprezintă utilizarea intenţionată a puterii sau a forţei fizice propriu-zise sau prin ameninţare, îndreptate împotriva propriei persoane sau a alteia sau împotriva unui grup de persoane sau a unei comunităţi, care, fie are ca rezultat sau este probabilă a avea ca rezultat afectarea integrităţii fizice sau psihice, decesul, împiedicarea dezvoltării normale" (7,8).

În ciuda faptului că violenţa a fost mereu prezentă, umanitatea nu o acceptă ca fiind parte inevitabilă a condiţiei sale. Prevenţia şi combaterea violenţei se află, astăzi, în preocupările unor sisteme religioase, filosofice, juridice şi comunitare (1,2,3,7).

Lucrarea de faţă îşi propune să evidenţieze unele aspecte privind frecvenţa deceselor prin cauze violente, în judeţul Iaşi, cu unele referiri şi la situaţia din România, pentru perioada 1997-2006.

MATERIAL ŞI METODE

În studiu au fost incluse informaţiile furnizate de către Institutele de Medicină Legală şi acumulate la nivel naţional. Prelucrarea şi

TABELUL I

Evoluţia anuală a frecvenţei cazurilor de moarte violentă, în România, în perioada 2000-2006

Anul	‰	% din total decese
2000	4,5	61,3
2001	4,4	59,0
2002	4,0	57,2
2003	3,6	61,4
2004	2,8	62,1
2005	3,1	59,4
2006	2,6	63,3

interpretarea datelor s-au realizat prin metode epidemiologice şi statistico-matematice.

REZULTATE ŞI DISCUŢII

1. Aspecte din evoluţia frecvenţei cazurilor de moarte violentă în relaţie cu decesele prin cauze non-naturale. În România, în perioada 2000-2006, frecvenţa morţii violente a înregistrat valori de 2,6 la 4,5‰, iar raportat la totalul deceselor, au fost valori de 57,2 la 63,3%. În ambele aspecte, diferenţele anuale au avut o semnificaţie statistică redusă ($p < 0,05$) (tab. I).

Pentru perioada 1997-2006, moartea prin violenţă din total decese prin cauze non-naturale a înregistrat valori anuale cu diferenţe nesemnificative ($p < 0,05$) (57,4 – 61,9%), cu excepţia anilor 2005 şi 2006, când valorile au crescut la 83,1 şi 84,7%, cu o diferenţă puternic semnificativă ($p < 0,01$) (fig. 1).

Sub toate aspectele, evaluările asupra frecvenţei cazurilor de moarte violentă arată valori ridicate, explicate, cel puţin parţial, prin situaţia socio-economică, cu modificări, adesea, brutale şi creşterea ponderii unor structuri demografice cu importante dificultăţi de inserţie socială.

2. Situaţia anuală, comparativă, între România şi judeţul Iaşi, a frecvenţei deceselor prin violenţă, altele decât sinuciderile. Perioada 2000-2006 s-a remarcat, în România şi în judeţul Iaşi, prin înregistrarea unor valori ridicate ale deceselor prin violenţă, altele decât sinuciderile. Astfel, în cei 7 ani luaţi în studiu, acest tip de deces a înregistrat valori anuale de 2,7 în 2006 şi 4,5‰ în 2000 şi 2001, cu diferenţe semnificative statistic ($p < 0,05$), care se păstrează când se compară situaţia de la nivel naţional cu cea din judeţul Iaşi, unde valorile de frecvenţă au fost de 8,0 în 2006 şi 11,0 în 2000 sau 9,3‰ în 2003. Rezultatele din judeţul Iaşi pot fi explicate prin situarea acestuia într-o arie geografică unde se înregistrează importante dificultăţi socio-economice, care îşi pun amprenta negativă pe natura comportamentului uman (fig. 2).

TABELUL II

Evoluţia anuală numerică a cazurilor de moarte prin omucidere, în România, în perioada 1997-2006

Anul	Cazuri	
	Nr.	‰
1997	1047	0,47
1998	870	0,39
1999	863	0,39
2000	1024	0,46
2001	947	0,43
2002	873	0,39
2003	782	0,35
2004	617	0,28
2005	672	0,30
2006	563	0,25

3. Aspecte privind evoluţia frecvenţei omuciderilor în România şi judeţele din Moldova. În perioada 1997-2006, decesele prin omucidere au înregistrat valori relativ crescute, cu variaţii de la 0,25 – 0,28 în 2006 şi 2004 şi între 30 şi 40‰, în ceilalţi ani. S-a observat o scădere moderat semnificativă, îndeosebi în intervalul 2002-2006, dar nu poate fi vorba de o tendinţă, întrucât valorile anuale au înregistrat modificări nesemnificative ($p > 0,05$) (tab. II).

Situaţia omuciderilor raportate la 100.000 locuitori, comparativ în România şi judeţele din Moldova arată, îndeosebi pentru judeţul Iaşi, valori peste media ţării,

TABELUL III
Situaţia omuciderilor la 100.000 / loc., în România şi judeţele din Moldova, în perioada 2003-2006

Anul	România	Iaşi	Vaslui	Vrancea	Suceava	Neamț	Galați	Botoşani	Bacău
2003	3,5	9,3	5,6	4,3	4,3	3,9	6,5	3,2	7,8
2004	2,8	4,0	4,3	4,3	3,1	4,3	3,4	3,2	5,6
2005	3,1	10,1	5,2	4,0	3,5	3,5	3,7	2,8	4,3
2006	2,6	8,0	3,4	2,8	1,7	2,1	4,0	2,8	2,6

TABELUL IV
Analiza unor cauze şi circumstanţe în care s-a produs moartea violentă, în România, comparativ în anul 1998 şi 2006

Cauze şi circumstanțe	% din total cazuri de moarte violentă	
	1998	*2006*
Lovire cu un corp dur	30,3	25,8
Politraumatisme	29,3	53,4
Sinucideri	26,3	27,7
Accidente rutiere	22,2	23,3
Intoxicații	10,1	10,8
Spânzurare	6,9	19,9
Arsuri	4,3	3,4
Accidente feroviare	4,2	2,6
Sugrumare	4,2	6,1
Accidente casnice	2,9	11,7
Accidente de muncă	2,4	2,9
Electrocuții	1,7	2,0
Plăgi înțepate / tăiate	1,5	2,2
Împuşcare	1,0	0,3

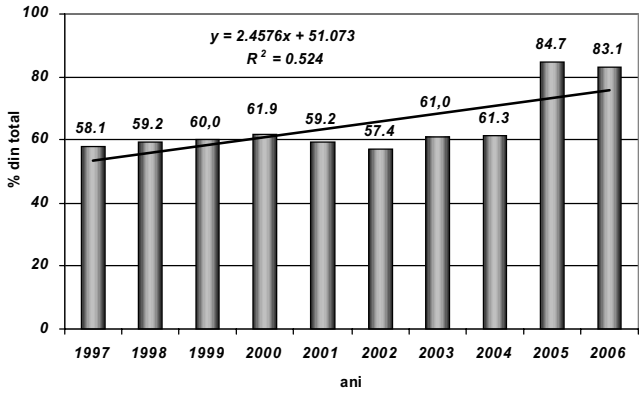

Fig. 1. Evaluări anuale ale raportului dintre moartea prin violenţă din total decese prin cauze non-naturale, în România, în perioada 1997-2006

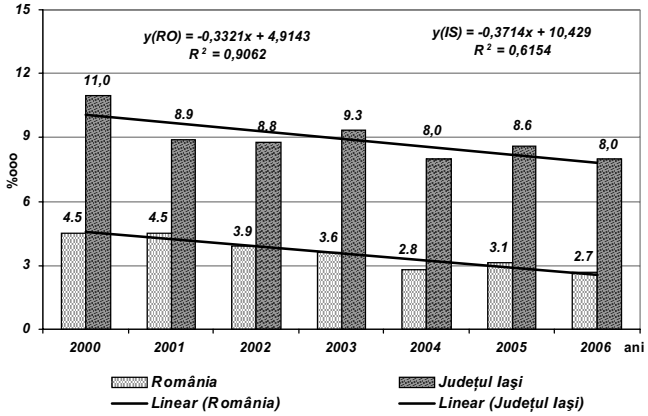

Fig. 2. Evoluţia anuală a frecvenţei deceselor prin violenţă, altele decât sinuciderile, în România şi comparativ în judeţul Iaşi, în perioada 1997-2006

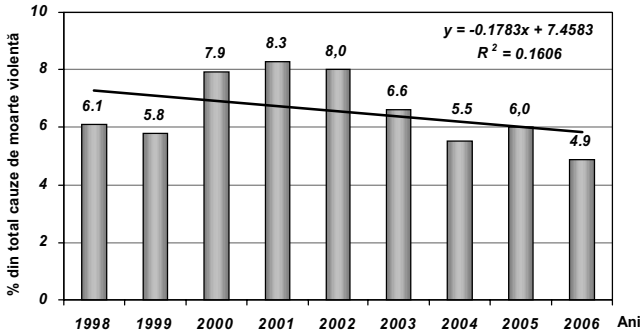

Fig. 3. Evoluţia anuală a frecvenţei cazurilor de omucideri în relaţie cu totalul cauzelor de moarte violentă, în România, în perioada 1998-2006

cu creşteri de aproximativ 3-4 ori, în intervalul 2003-2006 ($p < 0,05$). Diferenţele sunt prezente, fără această amploare, şi în celelalte judeţe din Moldova, aceste aspecte fiind concludente pentru zona geografică, unde există structuri demografice marcate de comportamente care predispun la producerea omuciderilor (tab. III, fig. 3).

Valorile frecvenţei omuciderilor, în judeţul Iaşi, comparativ cu alte 12 judeţe din România, îl situează pe acesta pe primul loc cu 9,3‰ cazuri şi între primele 6, cu valori situate peste media ţării (4,5‰) (fig. 4).

4. Aspecte privind cauzele şi circumstanţele în care s-a produs moartea violentă. Analiza comparativă, între anul 1998 şi 2006, pentru un eşantion de 12 cauze şi circumstanţe producătoare de moarte violentă, în România, a situat între primele locuri loviriile cu un corp dur (30,3, respectiv 25,8%) din total cazuri de moarte violentă, urmată de politraumatisme (29,3 / 53,4%); sinucideri (26,3 / 27,7%); accidente rutiere (22,2 / 23,3%) şi intoxicaţii (10,1 / 10,8%), urmate de celelalte cauze, cu proporţii variabile, dar mai reduse (tab. IV).

Variabilitatea de cauze şi circumstanţe evidenţiază polimorfismul acestora, dependent de multitudinea intervenţiilor umane şi, deci, de dificultăţile în a realiza programe eficiente de prevenţie şi asistenţă de urgenţă. Ca şi în cazul celorlalte aspecte care evidenţiază particularităţile condiţiilor şi circumstanţelor producerii morţii violente, factorii socio-economici, inclusiv cei educaţionali,

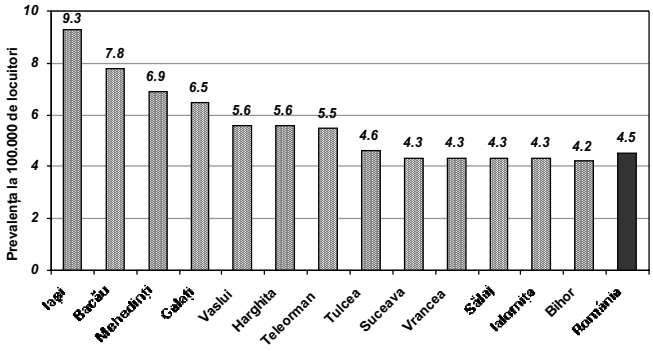

Fig. 4. Valorile frecvenţei cazurilor de omucideri în judeţul Iaşi, comparativ cu alte zone din România, în anul 2003

explică diferenţele valorilor crescute ale frecvenţei morţii violente, privite temporal şi spaţial geografic.

Din toate aceste date, rezultă necesitatea elaborării şi implementării unor măsuri complexe, cu caracter de permanenţă, cu diferenţieri pe structuri populaţionale, pe cauze şi circumstanţe implicate în determinarea morţii violente.

CONCLUZII

În România, în perioada 1997-2006, frecvenţa morţii violente a înregistrat valori de 2,6 - 4,5‰, iar, raportat la total decese prin cauze non-naturale, valorile anuale au avut diferenţe nesemnificative (57,4-61,9%), cu excepţia anilor 2005 şi 2006 (83,1-84,7%). Mortalitatea anuală, în perioada 2000-2006, a deceselor prin violenţă, comparativ, în România şi judeţul Iaşi, l-a situat pe acesta din urmă la un nivel deosebit de ridicat (2,7- 4,5 în România şi 8-11‰ în judeţul Iaşi), situaţie explicată prin dificultăţile socio-economice, cu amprentă negativă asupra naturii comportamentelor umane.

În intervalul 2003-2006, frecvenţa omuciderilor în judeţele Moldovei a situat judeţul Iaşi pe primul loc 9,3‰, la fel când situaţia a fost comparată cu alte judeţe din România. Analiza cauzelor şi circumstanţelor producătoare de moarte violentă, în România, a evidenţiat varietatea acestora, dependent de multitudinea intervenţiilor umane şi, deci, şi de dificultăţile în a realiza programe eficiente de prevenţie şi asistenţă cu caracter de urgenţă.

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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *VERBASCUM BLATTARIA* L.

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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *VERBASCUM BLATTARIA* L. (Abstract): **Aim**: The HPLC-ESI-Q-TOF-MS phytochemical profile and *in vitro* antioxidant activity of extracts obtained from the aerial parts of *Verbascum blattaria* were investigated. **Material and methods:** Phytochemical screening was performed by a hyphenated LC-MS technique, whereas total phenolic content was assessed by Folin-Ciocalteu method. Antioxidant activity was evaluated using four different *in vitro* tests: hydroxyl and DPPH radicals scavenging, ferrous ion chelating and ferric ion reducing assays. **Results and discussion**: A total of 17 secondary metabolites (carboxylic acids, flavonoids, iridoids, phenylethanoids and saponins) were identified in *V. blattaria* extracts. Among the four samples, *n*-butanol extract was the most potent scavenger of the hydroxyl and DPPH radicals, with EC_{50} values of 224.14 and 591.67 µg/mL, respectively. Additionally, the same extract exhibited an EC_{50} of 193.51 µg/mL in the ferric ion reducing assay. **Conclusions**: The current work allowed concluding that *n*-butanol extract of *V. blattaria* is the most promising in relation to antioxidant potential; the *in vivo* significance of its antioxidant effects needs to be further evaluated. **Keywords**: *VERBASCUM BLATTARIA*, IRIDOIDS, ANTIOXIDANT, HYDROXYL RADICAL, LC-MS.

With more than 350 species worldwide distributed, *Verbascum* L. is the largest genus of Scrophulariaceae family (1). In the traditional folk medicine, the roots, leaves and flowers have been used since ancient times mainly as remedies for respiratory tract disorders, such as bronchitis, cough, tuberculosis or asthma (2). *Verbascum* species (mulleins) attracted researchers' attention in the past 30 years, as they were found to be a very rich source of bioactive constituents (iridoids, phenylethanoids, saponins, flavonoids) with confirmed anti-inflammatory, antioxidant, antimicrobial or cytotoxic properties (3). Despite the fact that numerous mullein species have been already scientifically approached (1), new species are continuously discovered, whilst some known ones are currently very little investigated. It is the case of *Verbascum blattaria* L. (moth mullein), for which literature is not very abundant. In one study, 11 iridoid glycosides have been identified by thin-layer chromatography (4), whereas in another one, four iridoid and flavonoid glycosides have been isolated (5). The aim of this study was

to perform the phytochemical screening of various extracts obtained from the aerial parts of *V. blattaria* using different polarity solvents and evaluate for the first time their *in vitro* antioxidant activities.

MATERIAL AND METHODS

Plant material

The aerial parts of *Verbascum blattaria* L. were collected in Răscăieți (Ștefan Vodă County, Republic of Moldova) in June 2014. The plants were dried, milled to powder and deposited in the Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania (voucher specimen VB2806/2014).

Extraction

Five grams of powdered plant material were subjected to ultrasound extraction for three cycles of 30 minutes each, with 50 mL of solvent. Extracts were filtered, combined and dried under reduced pressure. *n*-Hexane (HE, 0.015 g, yield: 0.15%), ethyl acetate (EAE, 0.025 g, yield: 0.26%), *n*-butanol (BE, 0.19 g, yield: 3.80%) and aqueous (AQE, 0.840 g, yield: 16.8%) extracts resulted.

HPLC-ESI-Q-TOF-MS analysis

LC-MS analysis was performed using an Agilent 1260 HPLC equipped with a G1312C binary pump, G1322A degasser, G1316A column oven, G1315 D diode array detector (DAD), G1320B autosampler and a G6530B quantum-time-of-flight-mass spectrometer (Q-TOF-MS) with an electrospray ionization (ESI) source. Separation was carried out on a Zorbax RP-18 (150 × 2.1 mm, 3.5 μm) column. The mobile phase consisted of: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A linear gradient varying from 0% B (0 min) to 55% B (40 min) was used, at a flow rate of 0.2 mL/ min. Analyses were performed in negative ionization mode with the additional parameters: nitrogen gas flow rate 12 L/min; nebulizer pressure 40 psig; vaporizer temperature 300°C; capillary voltage 4000 V; fragmentor 140 V; skimmer 65 V; 100–1700 m/z; auto-tandem MS (MS/MS) acquisition mode; collision induced dissociation 20 eV.

Total phenolic and flavonoid contents

Total phenolic and flavonoid contents were estimated using Folin-Ciocalteu assay (6) and the method of Ozsoy *et al*. (7), respectively. Results were expressed as mg gallic acid equivalents (GAE)/g extract for total phenolics and mg catechin equivalents (CE)/g extract for flavonoids.

In vitro antioxidant assays

DPPH radical scavenging and ferric ion reducing assays were performed as described in Aprotosoaie *et al*. (8), hydroxyl radical scavenging as in Cretu *et al*. (9) and ferrous ion chelating assay as in Luca *et al*. (10). DPPH radical-scavenging activity (%) was calculated according to the following equation: % = [(absorbance of DPPH solution – absorbance of DPPH solution with sample after 5 min)/absorbance of the DPPH solution] × 100. Ferrous ion chelating and hydroxyl radical scavenging activities (%) were calculated as follows: % = [(absorbance of control − absorbance of sample)/absorbance of control] × 100.

Statistical analysis

All experiments were conducted in triplicate. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Student's t test; $p < 0.05$ was considered statistically significant. The efficient concentration 50 (EC_{50}) values were calculated by linear interpolation between absorbances above and below 0.5000 in ferric ion reducing assay and activities above and below 50% in all other tests.

RESULTS AND DISCUSSION

HPLC-ESI-Q-TOF-MS analysis

The four extracts of *V. blattaria* by using different polarity solvents (*n*-hexane, ethyl acetate, *n*-butanol and water) were subjected to a rapid HPLC-ESI-Q-TOF-MS analysis to systematize the information regarding their phytochemical composition. Two compounds (verbascoside and luteolin-7-*O*-glucoside) were identified by additionally injecting the corresponding commercially available standards. The other constituents were tentatively identified by comparing their MS data with those reported in the literature (11) and METLIN database (12). A total of 17 secondary metabolites were identified (fig. 1), grouped into several classes: carboxylic acids (compounds **1**–**5**), flavonoids (compounds **7** and **10**), iridoids (compounds **6**, **9**, **11**–**15**), phenylethanoids (compound **8**) and saponins (compounds **16** and **17**). Acylated derivatives of 6-*O*-rhamnosylcatalpol, such as *p*-coumaroyl acetyl 6-*O*-rhamnosylcatalpol and cinnamoyl acetyl 6-*O*-rhamnosylcatalpol, were the most abundant iridoids. This subcategory of compounds is known to be well represented in *Verbascum* species, with more than 30 congeners reported up to date (1). Moreover, verbascoside and luteolin-7-*O*-glucoside are among the most frequent constituents, being identified in almost all investigated mullein species (1). Several noteworthy qualitative differences between the four types of *V. blattaria* extracts were observed. In HE (the least polar extract) very few metabolites were identified, whereas AQE showed the highest number of polar constituents (aliphatic carboxylic acids, such as malic acid, citric acid, quinic acid). EAE and, especially, BE contained metabolites with medium polarity (including here iridoids, phenylethanoids and saponins) that are known to possess intrinsic antioxidant, cytotoxic and anti-inflammatory properties (1).

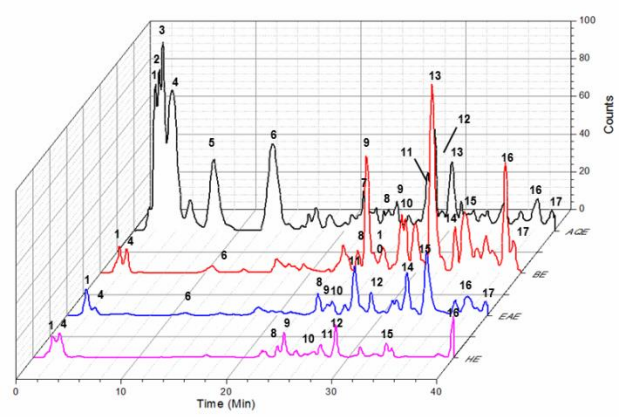

Fig. 1. Base peak chromatograms of extracts obtained from the aerial parts of *Verbasucm blattaria*. 1 – caffeic acid glucoside (Rt 1.8 min); 2 – malic acid (Rt 2.3 min); 3 – citric acid (Rt 2.6 min); 4 – quinic acid (Rt 3.2 min); 5 – hydroxyvaleric acid glucoside (Rt 5.4 min); 6 – ajugol (Rt 10.0 min); 7 – apigenin pentoside (Rt 22.2 min); 8 – verbascoside (Rt 23.2 min); 9 – *p*-coumaroyl 6-*O*-rhamnosylcatalpol (Rt 24.0 min); 10 – luteolin-7-*O*-glucoside (Rt 25.0 min); 11, 12 – *p*-coumaroyl acetyl 6-*O*-rhamnosylcatalpol isomers (Rt 27.3 and 28.9 min); 13 – di-*p*-coumaroyl 6-*O*-rhamnosylcatalpol (31.1 min); 14 – cinnamoyl acetyl 6-*O*-hamnosylcatalpol (Rt 32.6 min); 15 – *p*-coumaroyl diacetyl 6-*O*-rhamnosylcatalpol (33.2 min); 16 – mulleinsaponin IV (Rt 38.4 min); 17 – ilwensisaponin C (Rt 39.4 min).

Total phenolic and flavonoid contents

Total phenolic content of *V. blattaria* extracts varied between 1.53±0.21 mg GAE/g in HE and 52.41±1.13 mg GAE/g in BE. No significant differences were observed between total phenolic content in EAE and AQE (tab. I). These values were very similar to those reported in the aqueous extracts of *V. phlomoides* (51.3±0.3 mg GAE/g) and *V. thapsus* (52.3±0.3 mg GAE/g), but lower than those found in various extracts of other *Verbascum* species (74.1±0.4 –135.0±2.1 mg GAE/g) (13).

TABLE I

Total phenolic and flavonoid contents of extracts obtained from the aerial parts of *V. blattaria*

Sample	TPC (mg GAE/g extract)	TFC (mg CE/g extract)
HE	1.53±0.21[a]	–
EAE	43.90±1.86[b]	11.11±0.21[a]
BE	52.41±1.13[c]	26.34±0.32[b]
AQE	43.88±1.18[b]	9.66±0.38[c]

AQE aqueous extract; BE *n*-butanol extract; CE catechin equivalents; EAE ethyl acetate extract; GAE gallic acid equivalents; HE *n*-hexane extract; values in each row with different superscripts are significantly different ($p < 0.05$).

With regard to flavonoid content, BE possessed the highest amount (26.34±0.32 mg CE/g), whereas EAE and AQE had contents 2.3–2.8 times lower No flavonoids were quantified in HE (tab. I). Mihailovic *et al* (13) reported a flavonoid content of 10.2±0.2–53.4±0.2 mg rutin equivalents/g in *V. nigrum*, *V. phlomides* and *V. thapsus*, whilst Karamian and Ghasemlou (14) showed that *V. nudicaule*, *V. sinuatuam* and *V. speciosum* contained 4.83±0.13–5.77±0.23 mg rutin equivalents/g.

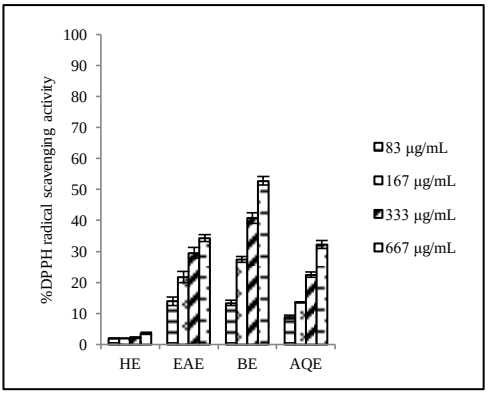

Fig. 2. DPPH free radical scavenging activity of extracts obtained from the aerial parts of *V. blattaria*

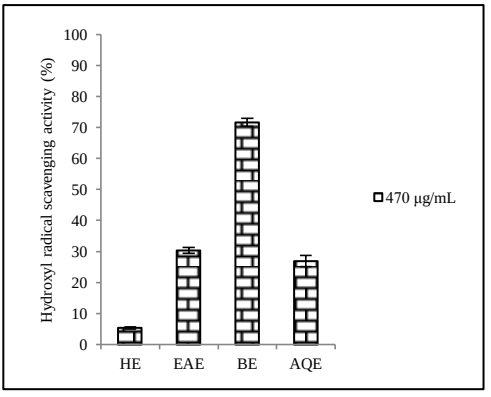

Fig. 3. Hydroxyl radical scavenging activity of extracts obtained from the aerial parts of *V. blattaria*

DPPH free radical scavenging activity

V. blattaria extracts showed moderate scavenging activity against DPPH radical. At the highest tested concentration (667 µg/mL), EAE, BE and AQE scavenged 34.29±1.07%, 52.78±1.31% and 32.25± 1.28% of the radical, respectively. At the same concentration, HE had very weak scavenging effects (3.59±0.28%). With respect to the EC_{50} values, BE was the most active extract (591.67±25.58 µg/mL). This activity was, however, lower than that reported in literature for the methanolic and aqueous extracts of *V. thaspsus*, *V. phlomoides* and *V. nigrum* (EC_{50} = 60.7±1.7–257.0±4.6 µg/mL) (13).

Hydroxyl radical scavenging activity

Among the four extracts of *V. blattaria*, BE was the most active, with a hydroxyl scavenging activity of 71.61±1.34% at 470 µg/mL. At the same concentration, EAE and AQE were two times less efficient (31.22±0.93% and 26.91±1.81%, respectively), whereas HE was almost inactive (Fig. 3). EC_{50} value was possible to be subtracted only for BE (224.14±10.35 µg/mL). As compared to the *n*-butanol extract of *V. ovalifolium* with a reported EC_{50} of 490.49±2.57 µg/mL (11), BE of *V. blattaria* was a more efficient hydroxyl radical scavenger. However, the methanolic and water extracts of *V. thapsus*, *V. nigrum* and *V. phlomoides* generated lower EC_{50} values (3.3±0.7–171.6±5.0 µg/mL) (13).

Ferrous ion chelating activity

The extracts obtained from *V. blattaria* exhibited poor ferrous ion chelating effects. At the concentration of 8 mg/mL, the most active extract, BE (11.69±1.29%), was followed by EAE (10.56±0.94%), AQE (8.17±0.77%) and HE (2.37±0.29%) (Fig. 4). Using a slightly different experimental protocol, Karamian and Ghasemlou (14) found that the methanolic extracts of *V. nudicaule*, *V. sinuatum* and *V. speciosum* exhibited chelating activities of 19.06±8.80%, 14.44±4.89% and 5.42± 0.72%, respectively, at 1 mg/mL.

Ferric ion reducing activity

EAE and BE catalyzed the reduction of iron (III) to iron (II) in a linear dose-dependent manner, the absorbance (700 nm) increasing from 0.1725±0.0106 and 0.3153±0.0114, respectively, at 30 µg/mL, to 0.5949±0.0076 and 1.0611±0.0306, respectively, at 240 µg/mL (R^2=0.9999 and 0.9945, respectively). At the highest tested concentration, HE and AQE showed an absorbance of only 0.1633±0.0170 and 0.1344±0.0100, respectively (Fig. 5). Therefore, EC_{50} values were calculated only for EAE (EC_{50}=193.51±6.68 µg/mL) and BE (EC_{50}=73.59±3.48 µg/mL). These values suggest that BE was approximately 2.5-fold more efficient in reducing iron (III). At a similar concentration (250 µg/mL), the methanolic extract of *V. thapsus* showed an absorbance of 1.162 at 700 nm, very close to that of BE (13). However, as compared to *n*-butanol extract of *V. ovalifolium* with a reported EC_{50} of 15.56±0.47 µg/mL (11), BE of *V. blattaria* was 4.5 times less active.

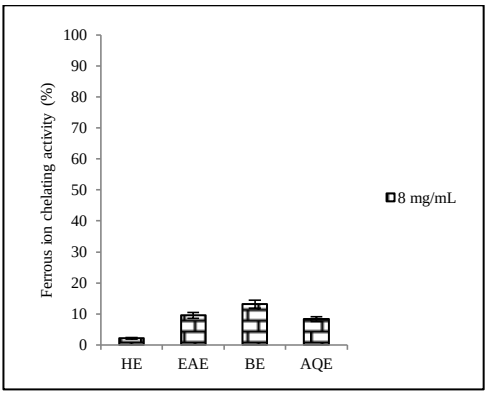

Fig. 4. Ferrous ion chelating activity of extracts obtained from the aerial parts of *V. blattaria*

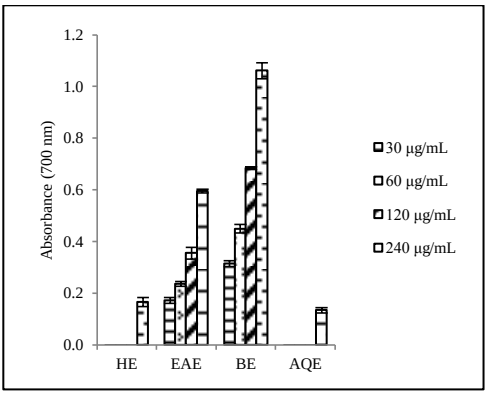

Fig. 5. Ferric ion reducing activity of extracts obtained from the aerial parts of *V. blattaria*

CONCLUSIONS

In the current work, four extracts obtained from the aerial parts of *V. blattaria* by extraction with different polarity solvents were phytochemically screened by HPLC-ESI-Q-TOF-MS. Base peak chromatograms, together with evaluation of total phenolic and flavonoid contents, suggested that ethyl acetate, *n*-butanol and aqueous extracts contained the main metabolites responsible for the potential health benefits of this species. Indeed, *in vitro* antioxidant activity allowed concluding that *n*-butanol was the most potent DPPH and hydroxyl radicals scavenger, as well as the most efficient ferric ion reducing agent. In most tests, ethyl acetate, and aqueous extracts exhibited similar antioxidant activities, but significantly lower. Therefore, solvents with medium polarities, such as *n*-butanol, might have the ability to extract a higher number of mullein bioactive constituents, as compared to non-polar (*n*-hexane, ethyl acetate) and polar (water) solvents.

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NEWS

CIPROFLOXACIN - ALLY OR ENEMY?

Aortic dissection is the most common fatal disease process of the aorta and is precede in most cases by the aneurysm formation. Ciprofloxacin is one of the most often used antibiotic and it is prescribed to treat a broad spectrum of bacterial infections, including infected aortic aneurysm. Results of recent clinical studies revealed that treatment with fluoroquinolone is associated with an increased risk of acute aortic events. In a recent study, LeMaire *et al*., have induced a sporadic aneurysm formation in mice with high-fat diet and low-dose angiotensin infusion, the effects of ciprofloxacin on smooth muscle cells and frequency of acute aortic events were studied. The results were stunning, incidence of aortic dissection and rupture was almost 10 times higher in the group with ciprofloxacin and this outcome was linked to the increased level and activity of matrix metalloproteinase. Thus, this drug should be used with caution in patients with aortic dilatation and those at high risk for acute aortic events (Scott A. LeMaire, Lin Zhang, Wei Luo, *et al*. Effect of Ciprofloxacin on Susceptibility to Aortic Dissection and Rupture in Mice. *JAMA Surg*. doi:10.1001/ jamasurg. 2018.1804).

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EVALUARE COMPARATIVĂ A COSTURILOR TEHNICII CHIRURGICALE LAPAROSCOPICE ÎN SISTEMELE DE SĂNĂTATE PUBLIC ŞI PRIVAT

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COMPARATIVE EVALUATION OF SURGICAL LAPAROSCOPIC TEHNIQUES COSTS IN PUBLIC AND PRIVATE HEALTH SYSTEMS (Abstract): Due to the fact that in Romania there are two health systems, public and private, some questions have been raised regarding the efficiency of a certain system, taking into account the quality but also the costs of provided services. **Material and method**: The study compares the costs of laparoscopic surgical techniques, analysing the causes of the differences that were found, if that were some advantages offered by one of the system to another and if there is a possibility of complementarity perspective between those two medical assistance organisation methods. **Key words**: LAPAROSCOPIC SURGERY, PUBLIC HEALTH SYSTEM, PRIVATE HEALTH SYSTEM

O chestiune puţin analizată în literatura de specialitate este perspectiva actuală (2010) asupra cheltuielilor legate de managementul pacientului chirurgical din punct de vedere al calculelor de eficienţă economică. Aceste cheltuieli implică spitalizarea efectivă, tehnica operaturie, medicaţia aferentă fiecărui pacient chirurgical la care s-a intervenit operator. Obiectivul principal este de a evidenţia, dacă există, diferenţele de costuri între abordarea endoscopică în spital de stat şi privat.

Scopul studiului este de a evalua sistemul public şi sistemul privat de sănătate prin prisma cheltuielilor de tratament (spitalizare, tehnică operatorie, medicaţie) în ceea ce priveşte tehnica chirurgicală laparoscopică.

Obiectivul principal este de a calcula diferenţele de costuri între abordarea endoscopică în spital de stat şi privat.

Obiective secundare:

- Stabilirea criteriilor de comparabilitate între cele două tipuri de intervenţie
- Descrierea intervenţiilor chirurgicale evaluate
- Stabilirea parametrilor de comparat în termeni de costuri şi eficienţă economică
- Compararea costurilor public vs privat

MATERIAL ŞI METODĂ

În prezentul studiu s-a ţinut cont de următoarele criterii de comparabilitate:

- Vârsta. Toţi pacienţii incluşi în studiu au avut vârsta mai mare de 18 ani.
- Sexul. Au fost incluşi pacienţi de ambele sexe.
- Tip afecţiune. Au fost selectaţi pacienţii cu aceeaşi afecţiune de bază fără diagnostice asociate sau complicaţii şi doar pacienţii internaţi şi operaţi în regim de programare.

1. Stabilirea parametrilor de comparat în termeni de costuri şi eficienţă economică

Pentru compararea costurilor privind pacientul chirurgical s-au luat în considerare următorii parametri:

- Medicaţia administrată pe parcursul internării
- Investigaţiile efectuate
- Numărul zilelor de spitalizare
- Materialele sanitare utilizate
- Manopera chirurgicală efectuată (endoscopică sau clasică)

Aceşti parametri au fost luaţi în considerare la compararea costurilor intervenţiilor endoscopice în sistem public vs privat.

Din punct de vedere al medicaţiei, atât în sistem public, cât şi în sistem privat s-au administrat aceleaşi clase de medicamente. Deasemenea s-au efectuat aceleaşi investigaţii de laborator, seturi de analize, ţinând cont de afecţiunea de bază.

În cazul zilelor de spitalizare, costul a fost raportat pe zi de spitalizare.

În ceea ce priveşte costul materialelor sanitare, la calcularea acestuia s-au luat în calcul toate materialele sanitare necesare pentru fiecare tip de intervenţie (colecistectomie laparoscopică, tratamentul herniei hiatale prin tehnica laparoscopică şi ovarectomia laparoscopică).

Manopera chirurgicală a fost calculată în funcţie de durata intervenţiei şi de numărul personalului medical din echipa operatorie.

Culegerea datelor a fost de tip exhaustiv.

2.Tehnica de eşantionare

S-a folosit eşantionul simplu randomizat.

Culegerea datelor de tip eşantion reprezentativ permite inferenţa statistică spre populaţia generală.

3.Evaluare statistică

Am aplicat pentru studiul statistic al datelor statistica descriptivă şi statistica inferenţială.

Statistica descriptivă. Prezentarea descriptivă a datelor se face prin înregistrarea datelor culese în tabele care să reprezinte în mod sintetic valorile măsurate.

Statistica descriptivă permite :

- Aprecierea prezenţei erorilor sau confuziei
- Reprezentarea datelor în tabele permite evaluarea fiecărui pacient şi dacă au existat pierderi din studiu
- Reprezentarea grafică a datelor pentru evaluarea mai eficientă a datelor culese despre pacienţii înrolaţi

S-au calculat indicatori statistici pentru descrierea repartiţiilor cazurilor luate în studiu pe grupe distincte, care studiate ne-au permis statuarea de concluzii pertinente relativ la scopul tezei. Pe baza datelor statistice astfel calculate am emis ipotezele inferenţei statistice cu ajutorul căreia am studiat diferenţele de care am avut nevoie în atingerea obiectivului studiului.

Statistica inferenţială. Variabilele folosite sunt continue. Diferenţierea variabilelor din punctul de vedere a distribuţiei se face folosind testul de normalitate Kolmogorov – Smirnov.

Pentru evaluarea lotului, precum şi pentru comparare de medii s-a utilizat testul t pentru un singur eşantion cu prag de semnificaţie $\alpha = 0,05$.

Pentru fiecare test statistic s-au verificat condiţiile de aplicare ale acestuia.

Interpretarea rezultatelor statistice a inclus evaluarea valorii p returnată prin aplicarea testului de inferenţă. Practic această valoare arată semnificaţia asocierii / semnificaţiei / diferenţei dintre variabilele studiate, scoţând în evidenţă diferenţa statistică prin compararea valorii p cu valoarea lui α. Am statuat din punct de vedere statistic următoarele : dacă $p > \alpha$ ($p > 0,05$) nu există semnificaţie statistică, dacă $p < \alpha$ ($p < 0,05$) există semnificaţie statistică.

Pornind de la premisa că semnificaţia statistică nu impune cu necesitate semnificaţia ştiinţifică şi viceversa am discutat rezultatele la limită $p \Leftrightarrow \alpha$ în funcţie de experienţa ştiinţifică a autorului.

Toate analizele statistice au fost realizate cu ajutorul programului Graphpad Prism 5.0 Trial pentru Windows.

TABELUL I
Colecistectomia laparoscopică

Colecistectomie laparoscopică	Sistem public	Sistem privat	Valoare p
Medicaţie	67,55	50,00	p > 0,05
Investigaţii	46,59	124,00	p < 0,05
Zi spitalizare	126,6	350,0	p < 0,05
Materiale sanitare	279,8	274	p > 0,05
Manoperă	90	950	-
Cost total	1149	2448	p < 0,05

* datele prezentate sunt superpozabile celor publicate in literatura de specialitate

REZULTATE

În prezentul studiu am evaluat abordarea laparoscopică din punct de vedere economico-financiar în sistemul public şi privat. Pentru fiecare tip de intervenţie s-au evaluat costurile medicaţiei, investigaţiilor de laborator, materialelor sanitare, costul raportat la o zi de spitalizare şi costul manoperei.

În cazul colecistectomiilor laparoscopice nu s-a observat o diferenţă semnificativă statistic în ceea ce priveşte costul medicaţiei. În sistem public, costul mediu al medicaţiei a fost de 67,55 ± 30,67 RON, faţă de 50 RON în sistemul privat. Costul mai scăzut în sistemul privat poate fi explicat prin evitarea poligragmaziei întâlnită des în sistemul public şi prin politica de preţuri diferită, adoptată atât de distribuitorii de medicamente, cât şi de furnizorul de servicii medicale private. O diferenţă semnificativă statistic s-a observat în cazul costurilor investigaţiilor de laborator pentru colecistectomia laparoscopică în sistemul privat faţă de cel public. Costul mediu al investigaţiilor de laborator în sistemul public a fost de 46,59 ± 40,77 RON faţă de 124 RON în sistemul privat. Această diferenţă de cost poate fi explicată prin modalitatea de amortizare a aparaturii de laborator adoptată de sistemul privat cât şi de eventualul „exces" în investigare, în argumentaţia unei eventuale complicaţii postoperatorii (tab. I).

Din punct de vedere al costului raportat la o zi de spitalizare, costul a fost mai crescut în sistemul privat, observându-se o diferenţă semnificativă din punct de vedere statistic. Costul mediu pentru o zi de spitalizare a fost de 350 RON în sistemul privat şi un cost mediu de 126,6 ± 49,31 RON pentru cel public. Un cost mai crescut în sistemul privat poate fi explicat de gradul sporit de confort oferit şi prin nivelul de salarizare al personalului implicat mai aproape de valoarea reală.

În cazul materialelor sanitare necesare, costul a inclus amortizarea echipamentelor şi a instrumentarului utilizat. S-a observat un cost mai ridicat în sistemul public, dar diferenţa nefiind semnificativă din punct de vedere statistic. Media costului materialelor sanitare necesare pentru sistemul public a fost de 279,8 ± 14,99 RON şi de 274 RON pentru sistemul privat.

Costul manoperei a fost evaluat în funcţie de durata tipului de intervenţie chirurgicală efectuată (laparoscopică sau clasică) şi de componenţa echipei operatorii.

În studiul de faţă în evaluarea costului manoperei s-a luat în considerare că echipa operatorie este formată din trei medici chirurgi şi o asistentă, un medic şi o asistentă ATI, iar durata unei intervenţii a fost considerată în medie de 36 minute pentru colecistectomia laparoscopică.

Costului manoperei pentru colecistectomia laparoscopică în sistem public a fost de 90 RON şi de 950 RON în sistem public. Costul mai ridicat în sistem privat poate fi

TABELUL II
Hernia hiatală

Cura laparoscopică a herniei hiatale	Sistem public	Sistem privat	Valoare p
Medicaţie	339	418	p > 0,05
Investigaţii	155,9	113	p < 0,05
Zi spitalizare	174,8	350	p < 0,05
Materiale sanitare	293,2	286	p > 0,05
Manoperă	120	2100	-
Cost total	2689	3967	p < 0,05

explicat prin posibilitatea oferirii de către angajatorul privat a unei remunerări a echipei medicale mai aproape de realitate.

În cazul colecistectomiei s-a observat o diferenţă semnificativă din punct de vedere statistic între costul total din sistemul public faţă de sistemul privat, costul total mediu fiind de 1149 ± 321,7 RON în sistemul public şi de 2448 RON în cel privat.

În cazul herniilor hiatale s-a observat o diferenţă nesemnificativă statistic în ceea ce priveşte costul medicaţiei. În sistemul public, costul mediu al medicaţiei a fost de 339 ± 67,26 RON, faţă de 418 RON în sistemul privat. Costul ridicat poate fi explicat prin medicaţia mai modernă şi mai „safe" utilizată (tab. II).

S-a observat o diferenţă semnificativă statistic în cazul costurilor investigaţiilor de laborator pentru hernia hiatală în sistem public faţă de cel privat. Costul mediu al investigaţiilor de laborator în sistem public a fost de 155,9 ± 25,09 RON şi 113 RON pentru sistemul privat. Această diferenţă de cost poate fi explicată prin investigaţiilor mai amănunţite în vederea unui stabilirii unui diagnostic pozitiv şi efectuării unuia diferenţial, cât şi examenului asociat cât mai minuţios în scopul evitării unor „surprize" neplăcute atât pre, per, intra şi postoperatorii.

Din punct de vedere al costului raportat la o zi de spitalizare, costul a fost mai crescut în cazul sistemului privat, obţinându-se o diferenţă semnificativă din punct de vedere statistic. Costul mediu pentru o zi de spitalizare a fost de 174,8 ± 13,38 RON pentru sistemul public şi un cost mediu de 350 RON pentru sistemul privat. Un cost mai crescut în sistemul privat poate fi explicat gradul de confort sporit şi de nivelul de salarizare mai aproape de realitate al personalului medical.

În cazul materialelor sanitare necesare, costul a inclus amortizarea echipamentelor şi a instrumentarului utilizat. S-a observat un cost mai ridicat în cazul sistemului public, diferenţa fiind nesemnificativă din punct de vedere statistic. Media costului materialelor sanitare necesare pentru hernia hiatală în sistemul public a fost de 293,2 ± 27,34 RON şi de 286 RON în cel privat.

Costul manoperei a fost evaluat în funcţie de durata tipului de intervenţie chirurgicală efectuată (laparoscopică sau clasică) şi de componenţa echipei operatorii.

În studiul de faţă în evaluarea costului manoperei s-a luat în considerare că echipa operatorie este formată din trei medici chirurgi şi o asistentă, un medic şi o asistentă ATI, iar durata unei intervenţii a fost considerată în medie de 48 minute pentru hernia hiatală prin tehnica laparoscopică.

Valoarea medie a costului manoperei pentru hernia hiatală prin în sistem public a fost de 120 RON şi de 2100 RON pentru sistemul privat. Costul mai ridicat în sistem privat poate fi explicat

În cazul herniilor hiatale s-a observat o diferenţă semnificativă din punct de vedere statistic între costul total în sistem public faţă de cel privat, costul total mediu pentru public fiind de 2689 ± 1 RON şi de 3967 RON pentru sistemul privat.

TABELUL III
Ovarectomia laparoscopică

Ovarectomie laparoscopică	Sistem public	Sistem privat	Valoare p
Medicaţie	101,7	235	p > 0,05
Investigaţii	75,25	50	p < 0,05
Zi spitalizare	157,2	350	p < 0,05
Materiale sanitare	302,6	123	p > 0,05
Manoperă	90	2200	-
Cost total	1577	3621	p < 0,05

În cazul ovarectomiilor s-a observat o diferenţă semnificativă statistic în ceea ce priveşte costul medicaţiei. În sistem public, costul mediu al medicaţiei a fost de 101,7 ± 8,86 RON, faţă de 235 RON. Costul ridicat poate fi explicat prin medicaţia mai modernă şi mai „safe" utilizată (tab. III).

În ceea ce priveşte costul investigaţiilor de laborator s-a observat o diferenţă semnificativă statistic pentru sistemul public faţă de cel privat. Costul mediu al investigaţiilor de laborator pentru sistemul public a fost de 75,25 ± 6,43 RON şi 50 RON pentru sistemul privat.

Din punct de vedere al costului raportat la o zi de spitalizare, costul a fost mai crescut pentru sistemul privat, observându-se o diferenţă semnificativă din punct de vedere statistic. Costul mediu pentru o zi de spitalizare a fost de 157,2 ± 2,47 RON pentru sistemul public şi un cost mediu de 350 RON pentru sistemul privat. Un cost mai crescut în sistemul privat se explică atât prin gradul sporit de confort oferit, cât şi prin nivelul de salarizare mai aproape de salarizarea reală a personalului medical.

În cazul materialelor sanitare necesare, costul a inclus amortizarea echipamentelor şi a instrumentarului utilizat. S-a observat un cost mai ridicat în sistemului public, diferenţa fiind semnificativă din punct de vedere statistic. Media costului materialelor sanitare necesare pentru ovarectomia laparoscopică în sistem public a fost de 302,6 ± 13,39 RON şi de 123 RON în sistem privat.

În evaluarea costului manoperei s-a luat în considerare că echipa operatorie este formată din trei medici chirurgi şi o asistentă, un medic şi o asistentă ATI, iar durata unei intervenţii a fost considerată în medie de 36 min pentru colecistectomia laparoscopică.

Valoarea medie a costului manoperei pentru ovarectomia laparoscopică în sistem public a fost de 90 RON şi de 2200 RON pentru sistemul privat. Costul mai ridicat în sistem privat poate fi explicat prin posibilitatea negocierii unui contract mai mare de către chirurg la angajare.

În cazul ovarectomiei s-a observat o diferenţă semnificativă din punct de vedere statistic între costul total în sistem public faţă de cel privat, costul total mediu în sistem public fiind de 1577 ± 2,07 RON şi de 3621 RON în sistem privat.

DISCUŢII

În prezentul studiu, s-a observat un cost crescut al medicaţiei în sistemul public în comparaţie cu sistemul privat privind colecistectomia laparoscopică, dar diferenţa nefiind una semnificativă, costul mai scăzut în sistemul privat poate fi explicat prin evitarea poligragmaziei întâlnită adesea în sistemul public şi prin politica de preţuri diferită, adoptată atât de distribuitorii de medicamente, cât şi de furnizorul de servicii medicale private. În cazul celorlalte intervenţii, hernia hiatală prin tehnica laparoscopică, deşi s-a observat un cost crescut al medicaţiei în sistemul privat în comparaţie cu

sistemul public, nu s-a putut arăta o diferenţă semnificativă. În ceea ce priveşte costul medicaţiei pentru ovarectomia laparoscopică, s-a observat un cost mai ridicat în sistemul privat faţă de sistemul public, diferenţa fiind una semnificativă.

Din punct de vedere al costului investigaţiilor de laborator, acesta a fost mai crescut în sistemul privat în cazul colecistectomiilor laparoscopice, diferenţa fiind semnificativă, şi mai crescut în sistem public în cazul herniei hiatale şi ovarectomiilor prin tehnica laparoscopică, diferenţa fiind deasemenea semnificativă. Toate acestea se datoresc atât investigaţiilor mai amănunţite în vederea unui stabilirii unui diagnostic pozitiv şi efectuării unuia diferenţial, cât şi examenului asociat cât mai minuţios în scopul evitării unor „surprize" neplăcute atât pre, per, intra şi posoperatorii.

În ceea ce priveşte costul raportat pe zi de spitalizare, acesta a fost mai ridicat în sistem privat pentru toate intervenţiile studiate, diferenţa fiind una semnificativă faţă de sistemul public.

În cazul materialelor sanitare necesare, costul a fost evaluat pentru fiecare intervenţie (colecistectomie, tratamentul herniei hiatale şi ovarectomii prin tehnică laparoscopică). S-a observat un cost mai ridicat în sistemul public, diferenţa nefiind una semnificativă în cazul colecistectomiilor şi tratamentului herniei hiatale şi semnificativă în cazul ovarectomiilor.

Costul manoperei a fost evaluat în funcţie de durata tipului de intervenţie chirurgicală efectuată şi componenţa echipei operatorii. S-a observat un cost mai crescut în sistemul privat faţă de cel public, diferenţa fiind una semnificativă, având în vedere calificarea, expertiza şi corolar nivelul de salarizare al echipei medicale atrase în sectorul privat.

CONCLUZII

Evaluând costul total al intervenţiilor laparoscopice studiate s-a observat un cost crescut în sistemul privat faţă de cel public, diferenţa fiind semnificativă în sensul componentelor costului mai sus-amintite.

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SERUM LIPOPROTEIN (a) LEVELS IN PATIENTS WITH ARTERIAL HYPERTENSION

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SERUM LIPOPROTEIN(A) LEVELS IN PATIENTS WITH ARTERIAL HYPERTENSION (Abstract): Lp(a) is capable of deleteriously altering the balance between the procoagulant and anticoagulant, proinflammatory and anti-inflammatory, and vasorelaxing and vasoconstricting properties of the endothelium. **Material and method**: The purpose of this study was to investigate the serum concentration of Lp(a) and the main parameters of lipid profile in three groups of subjects: a control group that included 16 healthy subjects, 20 patients with arterial hypertension and dyslipidemia and 20 patients with arterial hypertension without dyslipidemia. Using B-mode ultrasonography, we evaluated carotid intima-media thickness (IMT) and flow mediated vasodilation (FMD) on brachial artery. **Results**: We found significant higher Lp(a) concentrations in hypertensive patients with dislipidemia (70 ± 55.95 mg/dL, $p < 0.001$) and in hypertensive patients without dislipidemia (69 ± 52.33 mg/dL, $p < 0.001$), comparative with the control group (19 ± 14.64 mg/dL). In hypertensive patients with dislipidemia we found a strong negative correlation between Lp(a) and carotid IMT ($R^2 = -0.75$, $p < 0.001$) and a moderate negative correlation between Lp(a) and FMD ($R^2 = -0.38$, $p < 0.001$). Lp(a) level wasn't correlated with the main parameters of lipid profile. **Conclusions**: These results indicated that serum Lp(a) values could play an important role in essential hypertension pathogenesis and could be considered as an individual risk factor in hypertensive patients. **Key words**: HYPERTENSION, SERUM LIPOPROTEIN(A), INTIMA-MEDIA THICKNESS, FLOW–MEDIATED VASODILATION

Lipoprotein(a) [Lp(a)] is a unique, complex macromolecule consisting of a particle of low density lipoprotein (apo B100) linked to apolipoprotein(a), a molecule that is highly homologous with plasminogen (1). Elevated Lp(a) levels might promote cardiovascular events not by inducing or enhancing atherosclerosis, by stimulation of smooth muscle cell migration and proliferation (2) or by inducing endothelial chemoatractant activity (3), but mainly by its prothrombotic properties. Additionally, Lp(a) has been associated with high blood pressure (4), diabetes mellitus (5, 6) and dyslipidemia (7).

In hypertensive patients, a modification of the lipid profile has been observed, with increased levels of total cholesterol, triglycerides and LDL-C and low blood concentrations of HDL-C (8).

Carotid artery intima-media thickness (IMT), measured noninvasively by high-resolution B-mode ultrasonography, has been

associated with the risk of coronary artery disease, stroke, and myocardial infarction, and it predicts the progression of coronary artery disease (9). A positive associations between Lp(a) and IMT, a well-established surrogate index of coronary atherosclerosis, was reported in some studies (10, 11).

Ultrasonographical measurement of flow-mediated dilation (FMD) of the brachial artery allows direct and noninvasive assessment of endothelial-dependent vascular function. An impairment of FMD has been demonstrated in subjects with uncomplicated hypertension (12).

Because endothelial dysfunction occurs at a preclinical stage of disease, its presence serves as a highly sensitive disease marker that may help clarify the pathogenic role of Lp(a) in hypertensive patients.

The present study was conducted in order to measure Lp(a) levels, the main parameters of lipid profile, IMT and FMD values in hypertensive patients with or without dyslipidemia, with no clinical signs of associated pathologies or organ damage.

MATERIAL AND METHOD

In a 24 months period, fifty-six subjects from the IVth Medical Clinic of the University of Medicine and Pharmacy Victor Babes Timişoara were included in the study that comprised 20 patients with arterial hypertension and dyslipidemia (45% males, 55% females), 20 patients with arterial hypertension without dyslipidemia (25% males, 75% females), and 16 healthy subjects (31% males, 69% females), aged between 50-60 years old and sex ratio 45 men (66%) and 23 women (34%).

Venous blood samples were withdrawn in the morning, after an overnight fast. Serum total cholesterol triglycerides, high density lipoprotein – cholesterol (HDL – C) and fasting glucose concentrations were determined in all patients (Abbott photometry). The low density lipoprotein – cholesterol (LDL – C) concentration was calculated using Friedewalds' equation (13). Lp(a) was measured by a commercially available sandwich ELISA (Mercodia AB, Uppsala, Sweden).

Clinic blood pressure was recorded following the recommendations of the 2007 European Society of Hypertension/European Society of Cardiology guidelines (14). Dyslipidemia was based on the current European Society of Cardiology guidelines on cardiovascular disease prevention in clinical practice (15).

Assessment of vascular function

IMT was measured by high-resolution B-mode ultrasonography with an ultrasonographical apparatus with linear transducer of 7.5 MHz (Model ProSound SSD-4000, Aloka Co., Ltd., Tokyo, Japan), in both carotid arteries, on the distal straight 1 cm of the common carotid arteries, the carotid bifurcations, and the proximal 1 cm of the internal carotid arteries. The carotid IMT was determined as the average of 12 measurements (both sides 6 measurements each from the near and far wall of each of the three segments). A value >0.9 mm was considered abnormal.

Endothelial function was assessed by means of FMD on the brachial artery. Smooth muscle dilation was tested by 400 μg of sublingual nitroglycerin followed by imaging at 3 min after oral absorption (NTG).

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2000.

Data analysis

Continuous variables were expressed as means $\pm$ SD. Means were compared using analysis of variance or the Student t-test. The correlations between Lp(a) and all the remaining parameters studied were checked using the Spearman correlation test, taking Lp(a) as an independent variable. Statistical significance was defined as two–sided $p < 0.05$. All statistical analyses were performed using Excel Microsoft Office 2003.

RESULTS

The baseline characteristics of the subjects from the three groups are summarized in table I. The three groups were similar in terms of age, sex, family history of cardiovascular disease, smoking habits, and body mass index. The average of systolic and diastolic blood pressure values was higher in hypertensive patients, with or without dislipidemia, comparative with control group.

Table II describes serum lipid and lipoprotein profiles of the patients in the study groups. The plasma concentration of total cholesterol, triglycerides, LDL-cholesterol and non HDL-cholesterol were higher in hypertensive group with or without dislipidemia than in the controls ($p < 0.001$).

The most elevated concentrations of Lp(a) were found in hypertensive group with dislipidemia (70 ± 55.95 mg/dL, $p < 0.001$) comparative with hypertensive group without dislipidemia (69 ± 52.33 mg/dL, $p < 0.001$), and with the control group (19 ±

TABLE I

Baseline characteristics, FMD and IMT in the study groups

Characteristics	Control group	Hypertensive group with dislipidemia	Hypertensive group without dislipidemia	*p*
Patients (n)	16	20	20	12
Age (y)	56 ± 4.25	58 ± 3.15	55 ± 4.75	0.18
Sex M/F (%)	31/69	45/55	25/75	68
FH (+) (%)	43	45	35	0.51
Smokers (%)	31	24	45	0.18
BMI (kg/m^2)	22.85±1.85	22.93 ± 2.06	23.88 ± 1.15	0.22
Systolic BP (mmHg)	120 ± 7.27	165 ± 4.99	165± 5.5	<0.001
Diastolic BP (mmHg)	77 ± 7.96	103 ± 4.83	102 ± 3.40	<0.001
FMD (%)	11.88 ± 2.02	7.04 ± 3.12	6.65 ± 3.43	<0.001
NTG (%)	17.20 ± 1.21	16.54 ± 1.30	17.10 ± 1.32	0.18
IMT (mm)	0.68 ± 0.06	0.79 ±0.16	0.85 ± 0.19	0.003

Note: dates are presented as mean ± standard deviation. FH = family history of cardiovascular disease, BP = blood pressure, BMI = body mass index

TABLE II

Serum lipid and lipoprotein profiles of the patients

Characteristics	Control group	Hypertensive group with dislipidemia	Hypertensive group without dislipidemia	*p*
TC (mg/dL)	177 ± 8.88	179 ± 8.66	234 ± 50.78	<0.001
Triglycerides (mg/dL)	115 ± 21.80	128 ± 23.08	169 ± 14.68	<0.001
LDL cholesterol (mg/dL)	107 ± 6.77	105 ± 6.88	165 ± 47.55	<0.001
HDL cholesterol (mg/dL)	46 ± 4.15	48 ± 5.69	34 ± 5.12	<0.001
Lipoprotein (a)	19 ± 14.64	70 ± 55.95	69 ± 52.33	<0.001

Note: dates are presented as mean ± standard deviation

TABLE III

Correlations between Lp(a) levels and other parameters in hypertensive group with dislipidemia

Parameters	R^2 values	*p*
IMT (mm)	0.75	Strong and significant
FMD (%)	-0.38	Moderate and significant
TC (mg/dL)	0.05	n.s.
Triglycerides (mg/dL)	0.28	n.s.
LDL cholesterol (mg/dL)	0.10	n.s.
HDL cholesterol (mg/dL)	0.03	n.s.

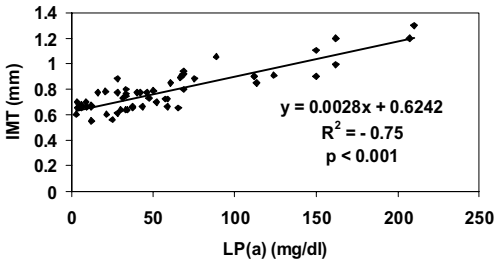

Fig. 1. Correlation between Lp(a) and IMT in hypertensive group with dislipidemia

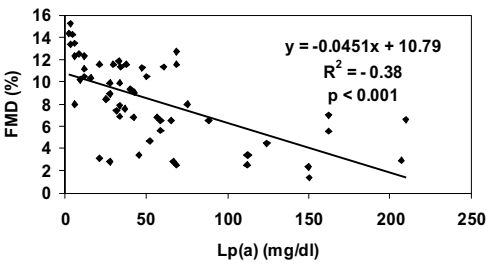

Fig. 2. Correlation between Lp(a) and FMD in hypertensive group with dislipidemia

14.64 mg/dL).

In table III are described the correlations between Lp(a) levels and other parameters in hypertensive group with dislipidemia. The Lp(a) levels wasn't correlated with the main parameters of lipid profile.

In hypertensive group with dislipidemia we found a strong positive correlation between Lp(a) and carotid IMT ($R^2 = 0.75$, $p < 0.001$) (fig. 1), and a moderate negative correlation between Lp(a) and FMD ($R^2 = -0.38$, $p < 0.001$) (fig. 2).

DISCUSSIONS

In our study that included patients with essential arterial hypertension with or without dislipidemia, with no clinical signs of pathologies or organ damage and with no family histories of cardiovascular disease, we measured the plasma concentration of Lp(a), the main parameters of lipid profiles and we assessed ultrasonographically the IMT and FMD values. Our results indicated that patients with arterial hypertension with or without dislipidemia have increased levels of Lp(a). Elevated concentrations of Lp(a) in hypertensive patients could constitute a first pathological sign, in the absence of noticeable organ damages and could be considered a negative factor of prognosis. Some authors indicated a positive correlation between high Lp(a) concentration and a positive family history of cardiovascular disease in hypertensive patients (16).

In vitro experiments have also illustrated that oxidized Lp(a) is able to impair the arterial endothelium-dependent dilation, and suggested a possible role of Lp(a) in the genesis of essential hypertension (17, 18). The relationship between Lp(a) and FMD was evident in our study, the subjects with increased levels of Lp(a) presented a markedly reduced FMD.

Previously, several studies have found Lp(a) to be related to early structural changes of the carotid arteries as measured by IMT (19). The localization of Lp(a) within the arterial wall suggests a direct causative role for Lp(a) in the initiation or progression of atherosclerosis. In our study, in the group of patients with arterial hypertension and dislipidemia, subjects with elevated levels of Lp(a) presented increased values of IMT.

Understanding of the relationship between Lp(a) and other cardiovascular disease risk factors through larger studies will be useful in understanding the mechanism by which Lp(a) participates in the atherosclerotic process (20).

CONCLUSIONS

We can say that hypertension and dislipidemia, which are two of the main risk factors for vascular disease, are often associated. Elevated Lp(a) values seems to be an important independent coronary risk factor that aggravates the coronary risk exerted by elevated LDL cholesterol, low HDL cholesterol, hypertension or the combined effects of multiple risk factors. Lp(a) should play a role as a screening tool in the quotidian clinical practice.

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EFECTELE ADMINISTRĂRII UNOR AMINOACIZI NONPOLARI TIP VALINĂ ŞI LEUCINĂ ASUPRA PERETELUI ARTERIAL SUPUS UNEI DIETE HIPERCOLESTEROLEMICE

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THE EFFECTS OF SOME NONPOLAR AMINOACIDS – VALINE, LEUCINE – ADMINISTRATION ON THE ARTERIAL WALL ALREADY EXPOSED TO A HYPERCHOLESTEROLEMIC DIET(Abstract) : Hypercholesterolemia plays an important role in atherosclerosis. The dietary supplementation of proteins or specific aminoacids seems to enhance the immune status in animals and humans with a complex pathological status, decreasing morbidity and mortality. **Aim** : In the present study we intended to analyse the effects of some nonpolar aminoacids- valine and leucine on cholesterol blood levels in a high-fat-diet in rats. In the same time we evaluated the vascular walls impact produced by the hypercholesterolemic diet. **Material and methods** : Our experiment was realised on 32 male Wistar rats, which were fed with cholesterol, valine and leucine for 8 weeks. At the end of experiment we analysed serum levels of cholesterol and also histopathological features of hypercholesterolemia on the arterial wall. **Conclusion** : The results of our study indicate that valine and leucine decrease the serum cholesterol and therefore the hypercholesterolemic-induced prooxidant status of the body being useful in reducing atherosclerosis. **Key words** : VALINE, LEUCINE, PLASMA CHOLESTEROL, HYPERCHOLESTEROLEMIA, ATHEROSCLEROTIC LESIONS

VEMURAFENIB BASED HYDROGELS AS POTENTIAL TOPICAL BIOFORMULATIONS FOR THE TREATMENT OF MELANOMA

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VEMURAFENIB BASED HYDROGELS AS POTENTIAL TOPICAL BIOFORMULATIONS FOR THE TREATMENT OF MELANOMA (Abstract): One of the most aggressive forms of cancer, cutaneous melanoma, occurs as a result of uncontrolled division of melanocytes. The efficacy of the used treatments can be diminished in terms of targeted action, increased toxicity and resistance to treatment. In the present study we have investigated an antitumor agent from the BRAFV600 inhibitor class, namely vemurafenib, currently approved by the FDA and EMA for oral administration only. Due to its high toxicity, we evaluated the possibility of cutaneous administration for the treatment of early-stage melanoma or post-surgical resection. **Materials and methods**: In that regard, we considered hydrogels based on chitosan, β-cyclodextrin and polyvinyl alcohol in various concentration ratios from which, after a careful analysis of the previously published data, we chose two matrices to be loaded with vemurafenib. **Results:** The inclusion complexes were obtained by PVA gelation through repeated freeze-thaw cycles and subsequently characterized by FTIR, scanning electron microscopy and swelling capacity at various pH and temperature values. **Conclusions**: The obtained results were promising, as the synthesized formulations proved their capability of controlled release for vemurafenib. **Keywords:** CHITOSAN, β-CYCLODEXTRIN, POLYVINYL ALCOHOL, HYDROGEL, VEMURAFENIB, SMART DRUG DELIVERY.

INTRODUCTION

In view of the increasing number of malignant pathologies being diagnosed, the medical world is showing a keen interest in the study of new systems for the transportation and targeted delivery of active substances with selectivity towards cancer cells, minimal toxicity to healthy tissue and fewer side effects. In that regard, numerous transport systems have been synthesized, including smart hydrogels that have gained notoriety in recent years in medical research. Invasive melanoma accounts for 1% of all skin cancer cases with very poor

prognosis, being the least common but the deadliest type of skin cancer in the incidence of which, the main involved factors are UV irradiation, prolonged sun exposure, light skin tone, family history of melanoma, higher numbers of melanocytic nevi and chronic immunosuppression. Death occurs as a result of metastasis, a stage characterized by increased tumor cell invasion and migration of cancer to other organs. The main method of treatment is surgical resection with excellent long-term prognosis, but in advanced melanoma (stage III-IVB), that approach is not sufficient and must be complemented by other therapeutic methods considering the spread of cancer cells to several organs (1, 2). Although surgical resection remains a gold standard for the treatment of melanoma, certain cases in which that approach is not possible, such as those in which patients are not suitable surgery-candidates depending on tumor/patient's characteristics, must also be considered as a second-line therapy. In that context, two possible options are the topical application of a specific drug or the radiation therapy, being the only ones that have proven a certain therapeutic efficacy. Other therapeutic approaches such as liquid nitrogen cryotherapy, electrodesiccation, curettage, laser surgery, and fluorouracil 5% cream have been linked with a high risk of recurrence and are not recommended. Currently, the only cream available on the pharmaceutical market but used off-label for the treatment of melanoma is imiquimod 5%, as adjuvant therapy (3).

Targeted therapy of cutaneous melanoma has developed based on the observation that more than 66% of malignant cases presented somatic missense mutations of protein kinase b-raf (BRAF). 69 BRAF mutations result in high serine/threonine kinase activity and mediate cell proliferation through the regulation of the mitogen-activated protein kinase (MAPK) signaling pathway. The most widespread variant is a val-to-glu point mutation at codon 600 (V600 E), which benefits from the treatment with VEM, which has been designated an orphan drug by the FDA for the treatment of BRAF V600E mutation-positive stage IIB to IV melanoma and it is not indicated for use in patients with wild-type BRAF. When evaluating physico-chemical properties of numerous antitumor drugs, specifically their solubility, we observed that most of them had low solubility in water, raising bioavailability issues (6). Attempts on the topical application of VEM have also been made in other studies, e.g., in the form of a solid-in-oil nanodispersion as an efficient and safe way to deliver VEM in early-stage melanoma (7). Peuvrel *et al.* (8) reported the appearance of skin rashes as a toxic response to a prolonged dermal treatment with VEM. That problem could be solved through formulation related strategies. One of them could be the inclusion of the drug within a biopolymeric matrix which can improve targeted drug delivery and diminish its cutaneous toxicity. This could not only target better VEM to the site but also to buffer its skin toxicity.

Analyzing the option of topical application, we have considered the synthesis of a chitosan (CS)-based hydrogel combined with β-cyclodextrin (β-CD) to allow controlled and targeted release of VEM and to counteract its limitations imposed by low water solubility and by its toxicity.

Due to its structural properties, in particular the presence of amino groups on the polysaccharide chain, CS is a biopolymer that exhibits certain properties that make it

suitable for biomedical applications such as high biocompatibility, low immunogenicity and allergenicity. CS is obtained by the deacetylation of chitin and it is a polysaccharide biomaterial composed of β-(1-4)-2-acetamido2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose. The combination of CS with β-CD can provide unique benefits, particularly when administering hydrophobic medications. Cyclodextrins are produced by *Bacillus macerans* and from a chemical point of view they are a particular category of oligosaccharides obtained from the enzymatic degradation of starch. β-CD is a derived oligosaccharide with electron-rich glycosidic oxygen atoms, that has a hydrophobic central cavity that can integrate hydrophobic molecules such as VEM in the presence of water and thus forming inclusion complexes with various drugs and bioactive molecules. Moreover, its ability to improve the solubility of certain drugs and the permeability of some macromolecular substances have also been demonstrated (9).

As Wang *et al.* (10) demonstrated in their study, the copolymer chitosan-grafted β-cyclodextrin (CS-g-β-CD) showed an improved hydrophilicity as its porous structure is advantageous for the complete exposure of polar functional groups (amino and hydroxyl groups) on its surface, being a smart drug carrier for etoposide, another anticancer drug.

The present study aimed to develop a method that combined CS and β-CD as hydrogels within an optimal composition to include VEM as the active substance. The obtained formulation was intended for topical delivery of VEM, the matrix being analyzed as a potential alternative to the topical preparations that are currently used. The preparation of the CS/β-CD complex is based on CS's ability to act as a cationic polymer to generate hydrogels (11,12) and to modulate the complex's properties, such as controlled release of drugs (13) by varying the external stimuli (i.e., pH), leading to smart delivery systems. An additional crosslinking was necessary because of the requirement that the biopolymeric matrix must be resistant to enzyme activity and other physicochemical factors. A non-invasive crosslinking strategy is physical crosslinking with the help of polyvinyl alcohol (PVA) involving the hydrogen bonding process, leading to a strong gel without external crosslinking agents. That type of gelation may provide biocompatible hydrogels of very high purity, with enhanced mechanical strength, with recently discovered inherent self-healability, that are extremely useful for post-surgery applications (14-16). The present study had its premises in a previous work done by Cheaburu *et al.*, where the behavior of an antimycotic drug with unfavorable pharmaco-toxicological profile for oral and/or parenteral administration was studied by loading it into a similar CS-based hydrogel, namely a CS-g-PNIPAAm/PVA copolymer, intended for topical mucosal application, with promising results (17).

MATERIALS AND METHODS

Materials. In our experiment, we have used the following materials: vemurafenib (VEM), chitosan (CS, 75-80% degree of deacetylation), β-cyclodextrin (β-CD), polyvinyl alcohol (PVA, 99% hydrolyzed with Mw of 89,000-98,000 Da), and phosphate buffered saline tablets pH = 7.4 (PBS), all purchased from Sigma-Aldrich, St. Louis, MO, USA. Other materials not mentioned were of analytical grade and used without further purification. All aque-

ous solutions were prepared using ultrapure water (Milli-Q, Millipore).

Preparation of Freeze-Thaw Hydrogels CS/β-CD/PVA. For the preparation of the hydrogels, we synthesized chitosan and β-cyclodextrin complexes (CS/β-CD) by solution mixing of 2 wt.% solution of CS in 0.5% acetic acid solution and 2 wt.% solution of β-CD in various ratios to obtain complexes with various compositions. Freeze-thaw hydrogels were prepared by mixing the prepared solutions of CS/β-CD with 5 wt.% PVA solution. Gelation strategy involved the freeze-thaw technique conducted by exposing the product to freezing temperatures (approximately -20°C) for 20 hours, and then allowing it to thaw at room temperature (RT) for 4 hours. The process of freezing and thawing (F-T cycles) was repeated three times. The number of F-T cycles was determined considering the previously obtained results of texture profile and rheological analyses of the grafted chitosan crosslinked with PVA being suitable for topical application and requiring low hardness and good adhesion properties. Additionally, freeze-thaw hydrogels chitosan/PVA and β-CD/PVA were prepared as reference matrices with the same compositions. Table I details the list and the precise composition of each prepared hydrogel.

TABLE I.
List of synthetized hydrogels

CODE	FORMULATION
CS/β-CD/PVA 25/70/5 + VEM	Hydrogel CS/β-CD physically crosslinked with PVA (25/70/5 combination ratio); loading with VEM was done by solution mixing after hydrogel preparation
CS/β-CD/PVA 25/70/5	Hydrogel CS/β-CD physically crosslinked with PVA (25/70/5 combination ratio), without VEM
CS/β-CD/PVA 20/75/5 + VEM	Hydrogel CS/β-CD physically crosslinked with PVA (20/75/5 combination ratio); loading with VEM was done by solution mixing after the hydrogel preparation
CS/β-CD/PVA 20/75/5	Hydrogel CS/β-CD physically crosslinked with PVA (20/75/5 combination ratio), without VEM
CS/β-CD/PVA 45/50/5	Hydrogel CS/β-CD physically crosslinked with PVA (45/50/5 combination ratio), without VEM
CS/β-CD/PVA 5/90/5	Hydrogel CS/β-CD physically crosslinked with PVA (5/90/5 combination ratio), without VEM
β-CD/PVA 95/5	Hydrogel containing only β-CD with PVA (95/5 combination ratio)by three cycles of −20/25°C freeze-thaw, without VEM
where: CS/β-CD - chitosan and β-cyclodextrin complexes; PVA - polyvinyl alcohol; VEM - Vemurafenib.	

Preparation of Vemurafenib loaded hydrogels. The biopolymeric matrices' characteristics were analyzed by means of FT-IR spectroscopy and swelling tests. The most suitable hydrogels for VEM loading designated for topical application were determined to be CS/β-CD/PVA 25/70/5 and CS/β-CD/PVA 20/75/5.

The drug loading was done by dissolving 1.5 mg VEM in 100 μL of DMSO (due to low water solubility) and mixed with β-CD solution and then complexed with CS and crosslinked with PVA. The proposed reaction mechanism is illustrated in figure 1.

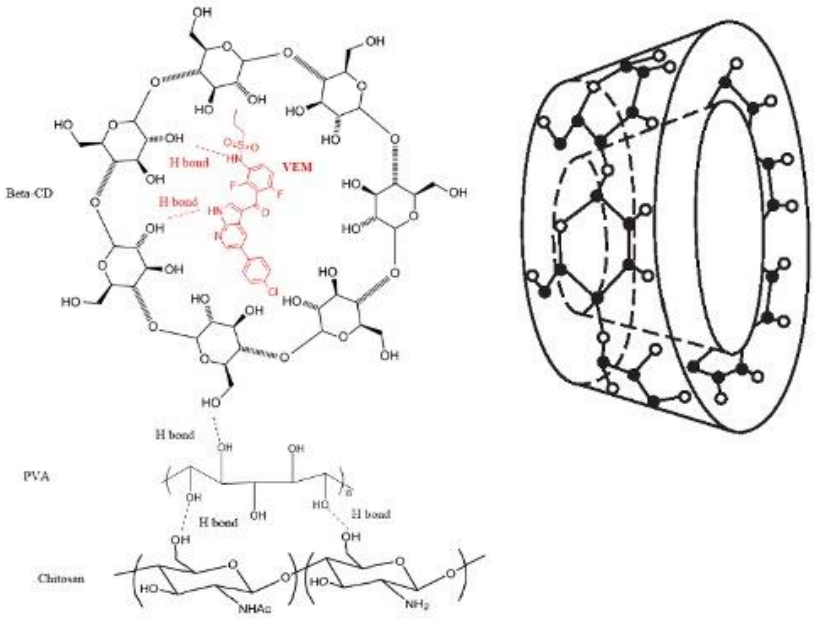


Fig. 1. Proposed preparation mechanism of biopolymeric based hydrogels

Characterization methods

The prepared complexes with various compositions in CS and β-CD with and without VEM and finally gelified by cross-linking with PVA were analyzed by means of structural identity, ability to swell on a substrate, morphological observations and drug loading capacity.

1. FTIR Spectroscopy

The chemical characterization of the obtained final hydrogels and individual components was accomplished by employing a Perkin Elmer Spectrum-100 instrument (Shelton, CT, USA), through reflection on a diamond crystal with a 45° angle, 4 cm^{-1} resolution of and over the 4000-400 cm^{-1} range

2. Hydrogel swelling behavior

The swelling behavior of the freeze-dried hydrogels was evaluated by direct immersion of formulations in acidic medium, water, and phosphate buffer saline (PBS, pH = 5.5) at various temperature values to simulate medium conditions for further *in vitro* experiments. The samples were periodically removed from the solution, wiped with a soft tissue to remove surface solution, weighed, and then carefully placed back into the beaker as quickly as possible.

The swelling degree (Q%) was calculated according to Equation (1):

$$Q\% = \frac{w_t - w_d}{w_d} \times 100 \quad (\mathbf{1})$$

where w_t is the weight of the swollen samples at time t and w_d is the weight of the dry sample.

3. Morphological observations characterization of the hydrogels

The scanning electron microscopic (SEM) images of cross-sections of the lyophilized hydrogels were captured using a Hitachi SU 1510 Scanning Electron Mi-

croscope (Hitachi Company, Japan), while the samples were set on an Aluminum stub and coated with a 7 nm thick gold layer using a Cressington 108 device before observation, at various magnification levels.

4. Drug loading capacity (DL) and drug entrapment efficiency (DEE%) of the formulations

The amount of VEM loaded in the hydrogels was determined by using a high-pressure liquid chromatography (HPLC) system Shimadzu Nexera LC-40-XR (Shimadzu, Kyoto, Japan) equipped with a serial dual-plunger pump, an autosampler (SIL 40 XR), a SPD-40V series UV-Vis, and a RF-20Axs fluorescence detector. The chromatographic separation of VEM was performed on a Waters CORTECS C18 column (2.1 × 100 mm, 2.7 μm) using as mobile phase a mixture of: A (water/formic acid - 99.9/0.1, *v/v*), B (acetonitrile) and C (methanol). Before use, the solvents were filtered through a 0.22 μm filter and degassed by ultrasonication. The injection sample volume was 10 μL, the run time was 9 min in isocratic mode (1 mL/min), and the optimal mobile phase ratio was A (40%), B (55%) and C (5%). The column temperature was maintained constant at 30°C during the chromatographic operation with UV-Vis detection for VEM (λ = 252 nm). The quantitative and qualitative analysis of VEM was based on its retention time and peak areas, respectively. The LabSolutionDB software 6.106SP1 was used for peak integration.

The calibration curve for VEM was obtained using the following procedure: stock solutions of VEM were prepared by dissolving 0.002 g of pure drug using acetonitrile in a 10 mL volumetric flask, from which we made serial dilutions, in the 100-0.78125 mg/L concentration range (r^2 = 0.9999). We found that VEM has a retention time of 6 min. To quantify the drug from the loaded matrices, we weighed the hydrogel samples, and after dissolution in 50/50 (*v/v %)* methanol/acetonitrile mixture using 2 mL vials, we centrifuged them, filtered the supernatants through 0.22 μm filter, and then 10 μL of each sample was injected and analyzed using the HPLC method described above. We also determined the limit of detection, LOD = 10.7982 μg/mL and the limit of quantification, LOQ = 32.7216 μg/mL. The amount of loaded VEM was calculated using Equation (2) derived from the calibration curve:

$$y = 45988x + 11405;\ r^2 = 0.9999 \quad \textbf{(2)}$$

The drug entrapment efficiency (DEE) represents the quantity of loaded drug (%) that is encapsulated in the formulation. The DEE of VEM from the hydrogel was calculated using Equation (3).

$$\textbf{DEE} = \frac{\text{Actual drug (VEM) content loaded in the matrix}}{\text{Theoretical drug content (VEM)}} \times 100 \quad \textbf{(3)}$$

RESULTS AND DISCUSSION

Given that the aim of the study was to optimize a treatment option for cutaneous melanoma for topical application and that semisolid forms are the most commonly used formulations for the transport of active substances through skin (18), we chose the option of incorporating an antitumor drug (VEM) in a hydrogel. The advantages are multiple, first of all the reduced risk of side effects, an enhanced patient compliance to treatment given the non-irritant properties of the hydrogels and their ability not to disrupt skin functions.

We selected the hydrogels' preparation method using PVA, a physical cross-linking agent with high biocompatibility and high mechanical strength, due to the simplicity of their synthesis process. We chose the typical preparation method by three freeze-thaw (F-T) cycles. As it is known, the number of cycles is correlated with their stability, so including a higher amount of PVA should be advantageous in the gelation process.

Structure Identification of Hydrogels

FTIR Spectra. The FTIR spectra of CS, β-CD, CS/β-CD/PVA and of the loaded hydrogels are presented in figure 2. We can observe in the graph the following representative aspects for β-CD: broad transmittance peak at 3303 cm^{-1} due to -OH stretching vibrations, asymmetric stretching vibrations of -CH were represented at 2928 cm^{-1} whereas asymmetric stretching vibrations of C-O were represented at 1633 cm^{-1}. A band at 1021 cm^{-1} was due to coupling vibrations of C-O and C-C. A characteristic band observed at 897 cm^{-1} was due to the vibration of α-(1-4) glucopyranose ring of β-CD, indicating the success of complexing β-CD to the polymeric backbone of CS. As far as the characteristic peaks of CS are concerned, from the graph we can deduce the following aspects: characteristic peaks at 2921-2887 cm^{-1} ($-CH_3$, $-CH_2$), 1636 cm^{-1} (C=O stretch vibration), 1548 cm^{-1} (secondary amide), 1655 cm^{-1} (amide band I) and 1068-1020 cm^{-1} (C-O stretching of saccharide moiety). The IR spectra of VEM should exhibit distinct absorption bands at 1734 cm^{-1} (C=O), 1325 and 1147 cm^{-1} (SO_2), and 1635 cm^{-1} (C=N), 817 cm^{-1} (C-Cl), not all very clearly observed because of the small amount that we have used for drug loading, but consistent with other studies (19). The intermolecular hydrogen bonding formed in between the drug and β-CD were evidenced by the widening of OH groups. Moreover, the hydrogel's formation is based also on hydrogen bonding, evidenced within the spectra as well.

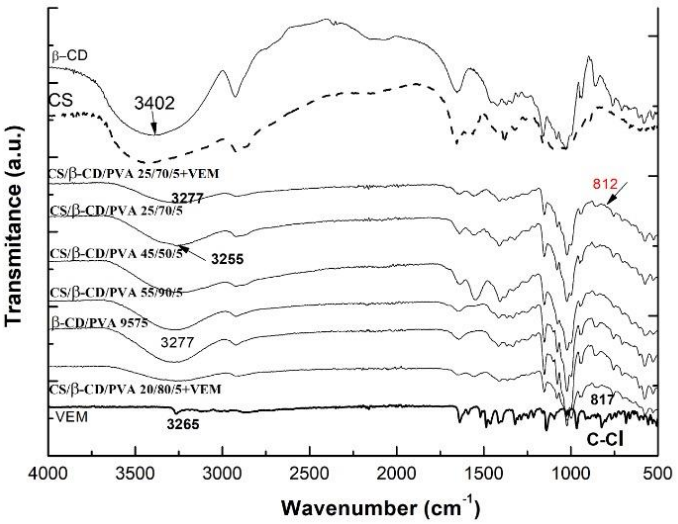
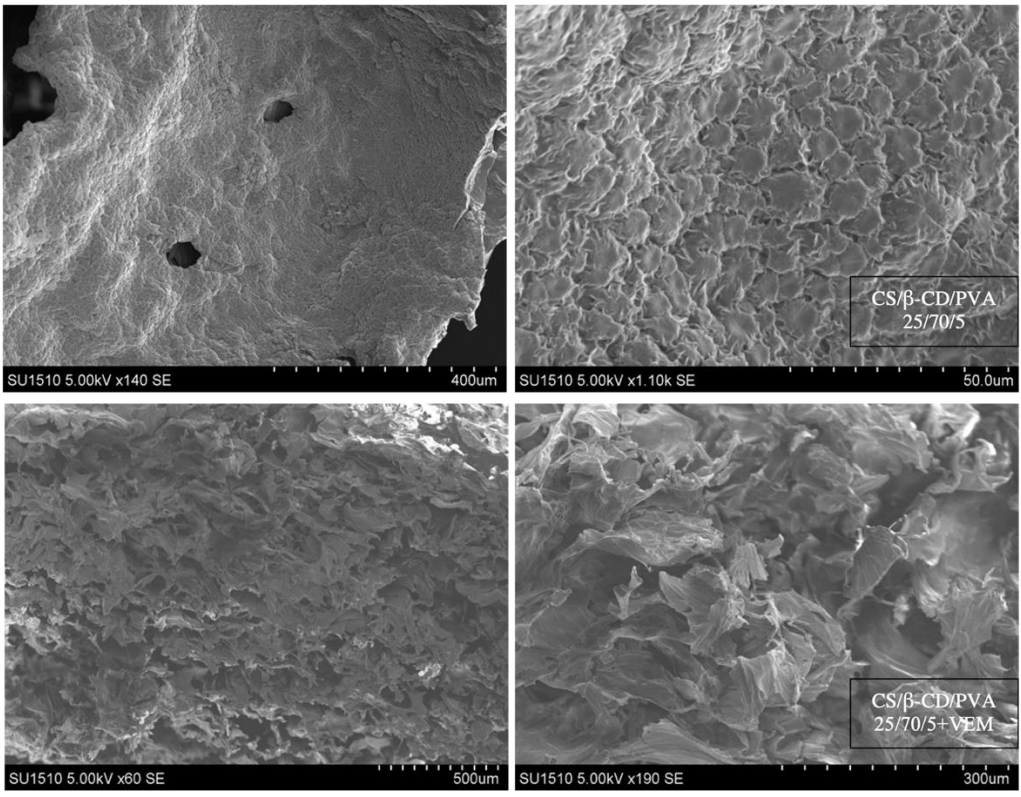


Fig. 2. ATR-FTIR spectra of the freeze-thawed hydrogels based on pure CS, β-CD, PVA, VEM and copolymers CS/β-CD/PVA loaded and unloaded with VEM

As shown within Figure 2, some characteristic vibration bands of β-CD were shifted in the inclusion complex spectra. The O-H stretching vibration from 3400 cm^{-1} of β-CD was shifted to 3250-3270 cm^{-1} within the spectra of the complexes. The shift of lower vibration bands indicated the formation of H-bonds between the OH groups of the biopolymers and PVA and thus proving the physical crosslinking.

The morphology of the prepared Hydrogels. For morphological analysis of the prepared matrices, we used Scanning Electron Microscopy (SEM) at various magnifications, as shown in figure 3.

As it is shown in the third figure, the hydrogels possess a continuous and stable network structure. Furthermore, all hydrogel samples exhibited large amounts of relatively loose and small pores, with irregular shapes and sizes providing great storage space for suitable active substances. The white dots with regular shape, that appeared in the loaded hydrogels, confirmed the presence of VEM. The roughness of the porous network that appeared on the microstructure surface of hydrogels exhibited in that figure may be attributed to the preparation and crosslinking process. The data was consistent with other SEM images found for that type of copolymer (20). The hydrogel based solely on β-CD and PVA was not taken into consideration for morphological analysis and further studies, because its very powdery texture after lyophilization was not adequate.



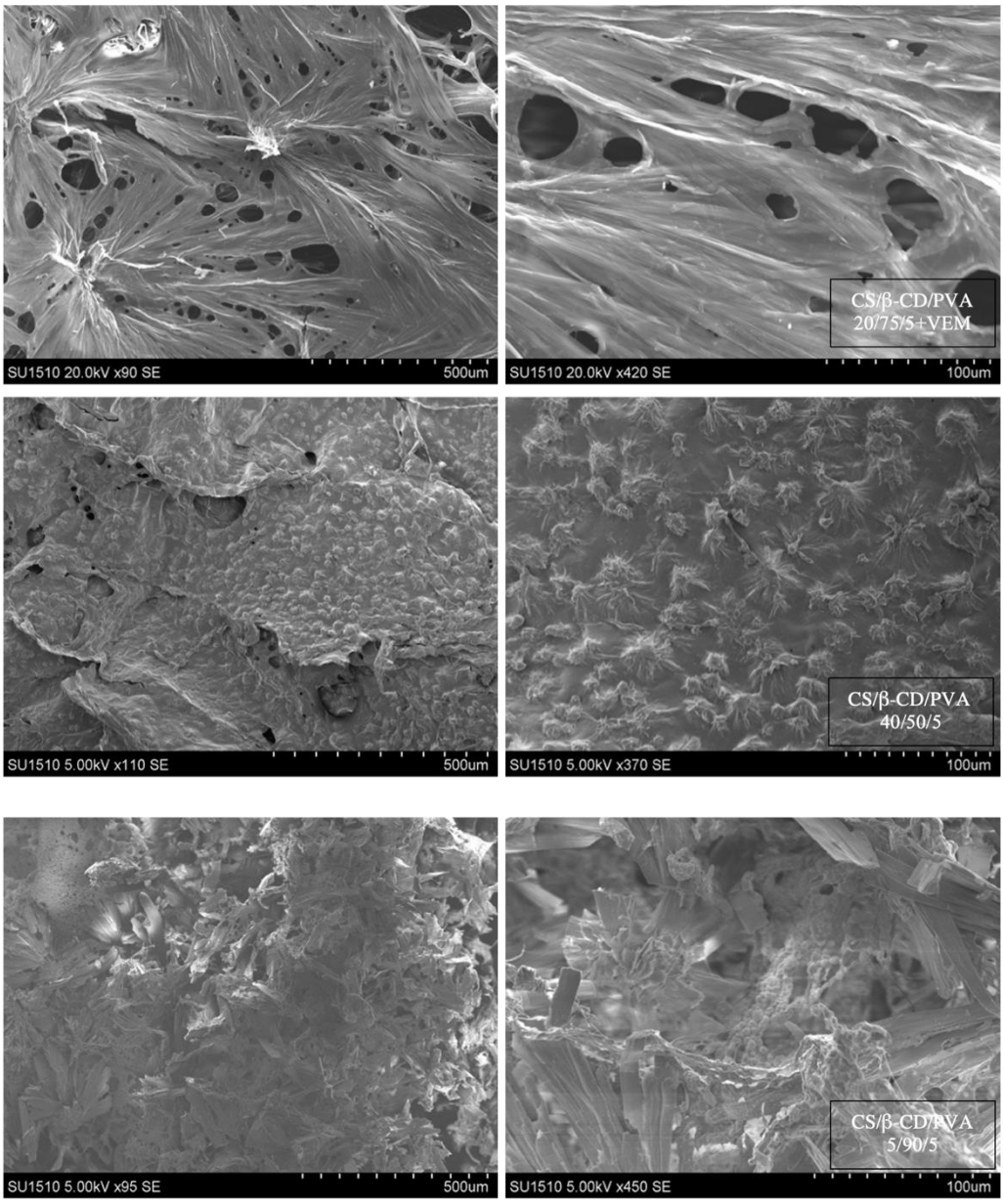


Fig. 3. SEM images for the prepared hydrogels at various magnifications

The porous aspect of the hydrogels was formed during the lyophilization process, due to the phenomenon of water sublimation from the matrix. CS/β-CD/PVA 25/70/5+VEM showed a more defined porous structure with well-interconnected pores. The CS/β-CD/PVA 20/75/5+VEM hydrogel also had a porous structure, but the diameter of the pores was larger and the walls were thicker.

The surface of the CS/β-CD/PVA 5/90/5 hydrogels was condensed with few observable pores. CS/β-CD/PVA 40/50/5 revealed spherical shapes on a smooth surface. Uncharged hydrogels exhibited a homogeneous, sponge-like morphology with well-interconnected pores.

Swelling Ability of CS/β-CD/PVA. The prepared matrices were designed to exhibit dual responsive behavior depending on various temperature and pH values at which we tested their swelling ability. The behavior of both control and drug loaded matrices were examined at various pH and temperature values as their properties may alter due to such physical changes (21).

Considering that the proposed matrices were aimed to release the active substance from a semi-solid form, the optimal pH was set at 5.5, since skin's pH is around 5.

From the graphs we observed the maximum swelling for acidic pH (being the value around which we are most interested in, considering the acidic microenvironment of tumors (22)) was around 800% due to the protonation of the free -NH_2 groups of CS, which demonstrated that optimal formulation for drug loading was CS/β-CD/PVA 25/70/5 or CS/β-CD/PVA 20/75/5 (their behavior was similar throughout the study). Similar pH-dependent swelling behavior for CS based matrices were reported in previous studies (17, 23).

Figures 4, 5 and 6 emphasize the dual responsiveness to the two stimuli, temperature and pH, for both the VEM-loaded and the control matrix. It can be observed that the loaded matrix showed a slightly lower swelling maximum, which demonstrated the incorporation of the active substance, the formation of electrostatic bonds between VEM and the polymer, and the prevention of a rapid burst release, which is not desirable, its aim being to achieve sustained drug release.

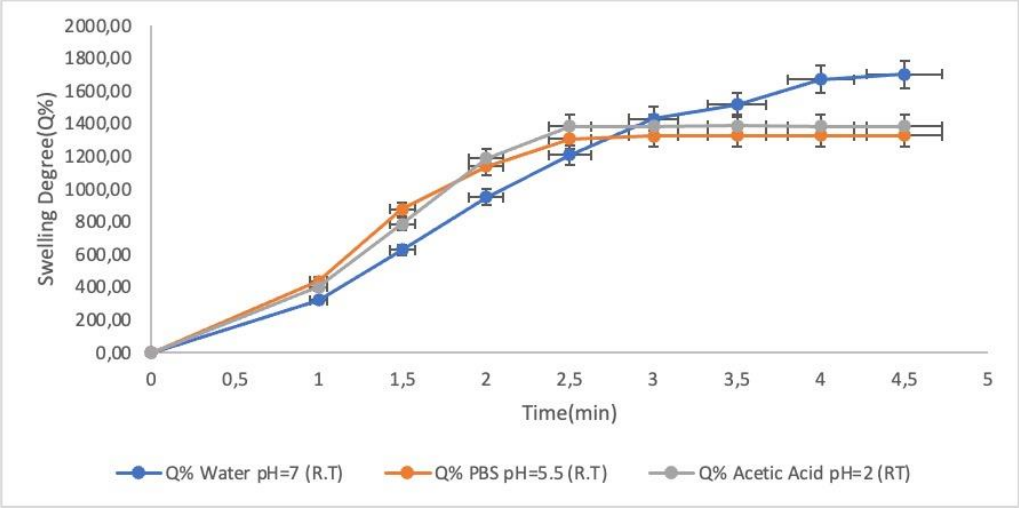


Fig. 4. Swelling behavior of the unloaded CS/β-CD/PVA 25/70/5 hydrogel at room temperature and various pH values

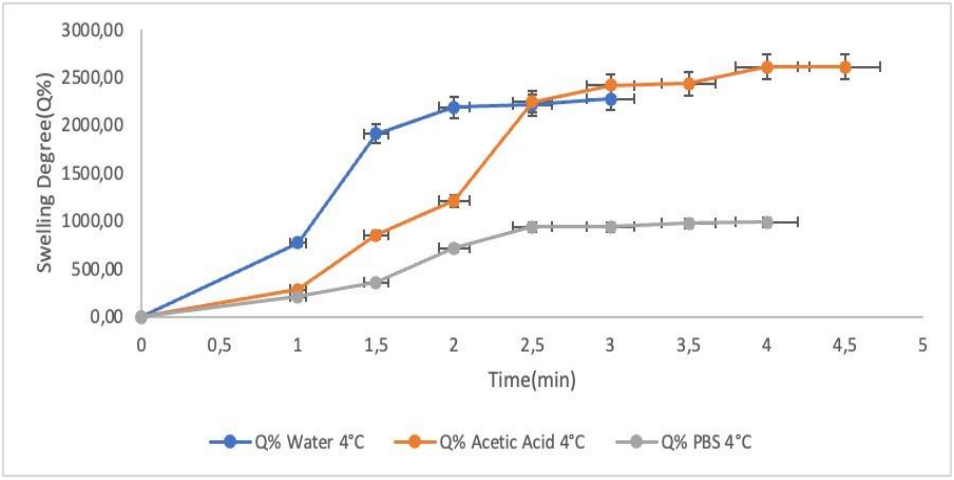


Fig. 5. Swelling behavior of the unloaded CS/β-CD/PVA 25/70/5 hydrogel at room temperature and various pH values

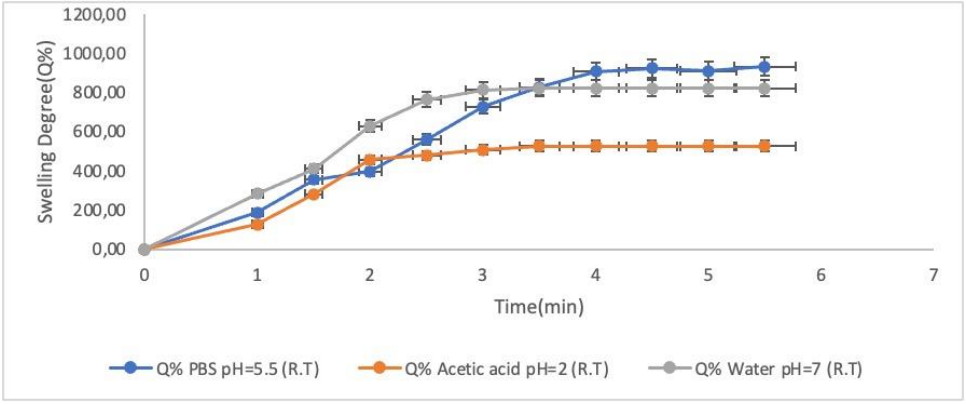


Fig. 6. Swelling behavior of the CS/β-CD/PVA 25/70/5 hydrogel loaded with VEM at room temperature and various pH values

Hydrogels are considered smart drug delivery systems precisely because of their ability to encapsulate drug substances that pose solubility problems, such as VEM. We have analyzed the drug loading capacity (DL) of our hydrogels, and we have found that for both loaded hydrogels DL was around 5.7 μg/mg, which led to 65-70% drug entrapment efficiency (DEE%), confirming a good capacity of drug encapsulation. The exact amounts and loading efficiencies are presented in table II.

TABLE II.
Hydrogels Loading VEM

Sample code	VEM loaded Hydrogel, μg/mg	VEM loading Efficiency, %
CS/β-CD/PVA 25/70/5 + VEM	5.7146	65.63%
CS/β-CD/PVA 20/75/5 + VEM	5.7672	70.93%

CONCLUSIONS

When it comes to achieving high levels of adherence to cancer treatment and drug selectivity towards cancer cells with little toxicity to healthy tissues and few unfavorable side effects, finding new smart drug delivery systems is the pillar of future therapeutic developments. The exploitation of local pH and the chemical potential of other stimuli responsive drug delivery vehicles combined with the physicochemical properties of therapeutic agents may lead to the discovery of new treatments to keep pace with the challenges brought by the increasingly aggressive forms of cancer that mankind is currently facing.

Among the various combinations of hydrogels we have experimented with, those obtained from combining chitosan, β-cyclodextrin and polyvinyl alcohol in two combination ratios (25/70/5 and 20/75/5) demonstrated excellent loading efficiency (65.63% and 70.93%, respectively).

Our proposed hydrogels have the potential to be used for that purpose, but future studies need to be performed to analyze *in vitro* and *ex vivo* release, as well as toxicity studies on melanoma cell lines.

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CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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JOB SATISFACTION AMONG ROMANIAN DENTISTS

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JOB SATISFACTION AMONG ROMANIAN DENTISTS (Abstract). **Aim:** To analyze the determinants of career satisfaction among a group of dental practitioners. **Material and methods:** Cross-sectional survey conducted in 2016 in a group of 165 dentists working both in private dental practices and in the public sector in Iasi County, Romania. A 16-item questionnaire developed by us was sent via email, the response rate being 87.3%. Data were analyzed with the SPSS 18.0 system for Windows. **Results:** Seventy-six percent of the dentists consider themselves to be satisfied with their work, whereas 65% stated they are satisfied with their career. The aspects the dentists consider most important in their career are the income level (55%) and financial security (49%), while social insurance (6.6%) and continuous medical education (12.1%) were the less important. Fifty-five percent of the surveyed dentists believe that fatigue is the most important negative factor causing lack of job satisfaction, 62.9% are unhappy with their current wages and only 40.7% say that they have a monthly income over 1,000 euros; 52.1% of dentists would choose dentistry again as a career. **Conclusions**: The statistical analysis shows that the best predictor of job satisfaction is the income level, followed by working in a private dental office, level of autonomy and the lack of work-related fatigue. **Keywords**: JOB SATISFACTION, IASI, DENTISTS.

Dentistry in not only an attractive and interesting but also a difficult profession. Without forgetting the satisfaction and benefits brought by this profession, we must also mention the risk factors which may affect dentists' quality of life, like nervousness and depression (1). As in any other career, job satisfaction and motivation do not come only from the financial profit, but should be corroborate with other factors: stress reduction, a pleasant and calm atmosphere in the dental office, collaboration and motivation of the dental team which may result in increased performance (2).

That is why understanding career satisfaction/dissatisfaction of dental practitioners is of special interest both for the political decision makers to implement the best solutions for the oral health system, as well as for the dental students who should be informed about these issues. Numerous surveys on this topic have been published in the literature (3-12).

The aim of our study was to evaluate the aspects related to dentists' satisfaction with their professional career by considering the following factors: the legal status of

the dental practice, monthly income, and satisfaction with the performed medical activities, working relationships with the team members, with the patients and personal relationships.

MATERIAL AND METHODS

The cross-sectional survey was carried out in 2016 in Iasi County, Romania, using our own questionnaire. The questionnaire contained 16 open and closed-ended questions. Questions referred to the following aspects: overall satisfaction, delivery of care, income level, working environment and psychological factors (doctor-patient relationship, relationships with the staff, personal and professional time).

The study group included 165 dentists aged 26 to 54 years, mean age 36.02 ±8.033 years. They were divided into 2 categories: junior dentists aged 26-35 years, 95 (57%), and experienced dentists aged 36-54 years, 70 (43%); by gender 92 (55%) were women and 73 (45%) men. Of the 165 subjects, 102 (61.8%) were private practice dentists and 63 (38.2%) worked in the public sector as interns or specialists. Data were analyzed using SPSS 18.0 for Windows (SPSS Inc. Chicago, IL, SUA). Statistical significance of the bivariate analysis was assessed by the Pearson chi-square test, at the 0.05 level. The multiple logistic regression analysis was performed to determine the predictors of job satisfaction.

RESULTS

1. Overall satisfaction, delivery of care and earned income (tab. I)

TABLE I

Overall satisfaction, delivery of care and income value

Items	Participants		Chi-squre test p
	No.	%	
Overall professional satisfaction			
satisfied and very satisfied	125	76	p=0.001*
dissatisfied and very dissatisfied	40	24	
Work fatigue			
satisfied and very satisfied	75	45	p=0.122
dissatisfied and very dissatisfied	90	55	
Current place of work satisfaction			
satisfied and very satisfied	107	65	p=0.003*
dissatisfied and very dissatisfied	58	35	
First place job satisfaction			
income	90	55	p=0.001*
opportunity to develop professionally	46	27	
promotion opportunities	19	12	
social security	10	6	
Important aspects in professional career			
financial security	81	49	p=0.001*
social insurances	11	6.6	
continuous medical education	20	12.1	
professional training	53	32.3	

Items	Participants		Chi-squre test p
	No.	%	
Choosing the same professional career again			
yes	86	52.1	
no	18	10.9	p=0.001*
undecided	61	37	
Attitude towards the income obtained			
paid according performances	61	37.1	p=0.002*
need to earn more	104	62.9	
Salary			
average income (€600)	28	17.3	
€600 - €1,000	70	42	p=0.001*
> €1,000	67	40.7	

* statistically significant at p<0.05

2. Satisfaction with work environmental and psychological factors. Questions 9-12 assess the satisfaction with the activities in the dental office. Table II shows that significant statistical differences were recorded for: the evaluation of personal work performance, 90.9% of respondents considering it a key element for motivation in career, (p=0.001); the pleasant atmosphere in the dental office represents a satisfaction factor for 61.2% of respondents (p=0.021); the level of autonomy is appreciated by 77% of dentists (p=0.002). Insignificant statistical differences were recorded only for the appreciation of promotion opportunities (p=0.551). Another aspect that may generate satisfaction or dissatisfaction in the dental profession is represented by workplace relationships (tab. II). Significant statistic differences were obtained for answers to questions 13-15 (p<0.05): 71% of dentists were satisfied with their relationship with patients (p=0.002); 75% were happy to collaborate with their colleagues (p=0.001); 85% had good relationships with staff members (p=0.001). The answer to question 16 (Do you have enough free time to meet your personal needs?) had no statistical significance, p=0.331.

TABLE II

Satisfaction with work environmental and pshychological factors

Items	Satisfied and very satisfied		Dissatisfied and very dissatisfied		Chi-square test
	No.	%	No.	%	p
Professional apreciación	150	90.9	16	9.1	0.001*
Pleasant atmosphere	101	61.2	64	38.8	0.021*
Autonomy	127	77	38	23	0.002*
Promotion opportunities	85	51	80	49	0.551
Patient relations	117	71	48	29	0.002*
Relationships with colleagues	123	75	42	25	0.001*
Coworker relationships	140	85	25	15	0.001*
Personal time	79	47.8	86	52.2	0.331

* statistically significant at p<0.05

3. Career satisfaction regarding independent variables (tab. III). The results of multiple logistic regression analysis show that the best predictors of job satisfaction are represented by a monthly income higher than 1,000 euros (OR=6.32, CI=3.11-35.8), employment in a private practice (OR= 3.21,CI=4.23-23.5), professional autonomy (OR=2.23, CI=1.45-17.6), lack of fatigue (OR=2.01, CI=1.09-3.56) and good dentist-patient relationships (OR=1.56**,** CI=1.23-1.97).

TABLE III
Multiple logistic regression analysis: career satisfaction regarding independent variables

Independent variables	Odds ratio (95% CI)	p
Gender		
female	0.25 (1.08- 3.54)	0.123
male		
Age (years)		
26-35	0.22 (0.70-3.84)	0.322
36-54		
Employment		
private office	3.21 (4.23-23.5)	0.001
public service		
Place of work		
capital: Iasi	0.23 (0.45-1.09)	0.112
county		
Income value		
€600 - €1,000		
> €1,000	6.32 (3.11-35.8)	0.001
Fatigue		
satisfied and very satisfied		
dissatisfied and very dissatisfied	2.01 (1.09-3.56)	0.003
Promotion opportunities		
satisfied and very satisfied	0.87 (0.11-1.02)	0.441
dissatisfied and very dissatisfied		
Personal time		
satisfied and very satisfied	0.09 (0.77-3.89)	0.234
dissatisfied and very dissatisfied		
Professional autonomy		
satisfied and very satisfied	2.23 (1.45-17.6)	0.008
dissatisfied and very dissatisfied		
Patient relationship		
satisfied and very satisfied	1.56 (1.23-1.97)	0.005
dissatisfied and very dissatisfied		

p – significance level, OR – odds ratio, CI – confidence interval

DISCUSSION

The purpose of this survey was to understand if in today's Romania the dentist still has the *motivation* and *satisfaction* necessary to continue practicing dentistry, if there is still passion and wish to treat patients, as well as the related sacrifice. Other factors should not be omitted from

this equation, such as: the low socioeconomic status of some communities, a degree of rigidity of the education systems, and an inadequate fiscal and budgetary policy (13).

At the same time, the option to practice dentistry in other European countries, where the net salary is much higher, represents for the young graduate a fantastic opportunity for career development (14).

In this survey, we found that 76% of dentists consider themselves satisfied and very satisfied with their carrier, and 65% with their current workplace**.** Only 52.1% of the total 165 dentists would choose the same career again, 10.9% say that they consider a carrier change, with the percentage of the undecided ones being surprisingly high, namely 37%. This last variant may be interpreted as a lack of motivation towards their current career. One possible explanation might be that many dentists who participated in this survey are still at the beginning of their career and the problems they face in terms of low income and lack of opportunities may determine them to consider a career change. Satisfaction with the dentist career also involves analyzing the elements that young graduates consider essential at the beginning of their professional activity, of which the earned income was ranked first by 55% of the surveyed dentists. Unfortunately, post-graduate training, which is a key factor in career development, was appreciated by only 32.2% of the study participants. On the contrary, this motivation is considered a priority for dentists in India and Lithuania (3, 8). Another element considered to be less important for a successful career is the continuous medical education appreciated by only 12.1 % of the respondents. In India, Nayak (14) shows that a little less than half of the practitioners attended professional courses once in 6 months, 32.7% attended at least once in a year, and 22.1% attended rarely or never. It is known that the level of professional satisfaction among physicians in the light of earned income is dependent on the country level of economic development. In our survey, we found out that the best predictor for job satisfaction is a monthly income of more than 1,000 euro (OR=6.32)

In the literature, there are surveys showing that a low income is the most import factor of job dissatisfaction.

Dental professionals are exposed to many risk factors that may affect the quality of life, including physical exhaustion and stress with multiple effects on general health, behavioral disturbances and psychological symptoms. In our study, 55% of the surveyed dentists consider fatigue as a factor of dissatisfaction and from the logistic regression analysis we have found that lack of fatigue and stress at work can be a good predictor of career satisfaction. (OR = 2.01). If other specific factors, such as the stressful conditions, fatigue, lack of recognition and respect for the importance of the activity they carry out are added to these elements related to the poor funding of the public health system, migration or professional reorientation become a fully motivated action.

CONCLUSIONS

In our study, we found that financial security is the most important determinant of job satisfaction and a monthly income of more than 1,000 euro is the best predictor for the motivation to practice dentistry. Monthly income is considered the main cause of satisfaction/dissatisfaction, with 62.9% of dentists believing they should earn more. Other predictors of career satis-

faction include: degree of autonomy, private dental practice, lack of overwork and stress, as well as positive relationships with patients. A negative aspect was related to the lack of motivation for continuing medical education and ongoing professional training, aspects that dentists consider to be of little importance for career success.

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MEASUREMENT OF DYNAMIC SOLICITED AREA, A PROPOSAL OF OBJECTIVE MEASURE FOR COMFORT EVALUATION IN TRANSTIBIAL PROSTHETICS

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MEASUREMENT OF DYNAMIC SOLICITED AREA, A PROPOSAL OF OBJECTIVE MEASURE FOR COMFORT EVALUATION IN TRANSTIBIAL PROSTHETICS (Abstract): Probably the most important factor in evaluating a patient's prosthesis is quality of life. This paper proposes an objective method for assessing patient's comfort by optimizing stump-sleeve-cup assembly, a correlation between the effort supported by skin areas upon intense stress during the movement cycle and the state of comfort**. Material and methods:** The changed work proposed as an objective measure was evaluated by simulation with processing of the image resulted from simulation with a finite element. We assume that the person wearing a prosthesis feels better (higher comfort and satisfaction) if prosthetic areas are less stressed (similarly to a shoe that we assume it is comfortable if it does not cause damage to the skin or if it fits properly. **Results:** The stump-prosthesis assembly is scanned and then, using contour detection routines, we determined the cluster set of points for each element (stump, tibia, fibula, skin, sleeve, and cup). We suggest a new objective measure for evaluating patient's comfort, a measure based on mathematical calculations. **Conclusions:** Validation of results is possible with a single method, the questionnaire method. In fact, almost all alternative comfort optimization and measurement methods lead to implementable solutions, but a medical validation is ultimately linked to the questionnaire, which is subjective. Some numerical simulations have been presented to illustrate theoretical analysis. These results are important and can provide a direction for future research. **Keywords:** FINITE ELEMENT, TRANSTIBIAL PROSTHESIS, GEOMETRIC ALGORITHMS, MECHANICAL WORK

Basic sensations from the neuroreceptors in the upper and lower limbs include pain, touch (pressure and vibration), temperature and muscle and tendon tensions (1). Generally, the term of discomfort refers to the problems related to matching the cup or sleeve with the patient's stump, therefore, it is not a basic feeling of the human body. Discomfort perception is basically a combination of two or more sensations that lead to a negative value of the patient's condition. It is worth noting that the feeling of discomfort is not always associated with pain, but pain will always make the patient feel uncomfortable. Age and geographical area and of course pros-

thetic materials influence the patient's comfort. The feeling of comfort or discomfort is usually assessed based on questionnaires (2, 3) that are mechanically validated, but inherently subject to medical validation both by those developing the questionnaire and deciding the questions and numerical scale (usually numbered from 0 to 10) (3), but also by the subject, who has a personal sensory perception, with subjective aspects in their answers to questions. Therefore, the assessment of discomfort is subjective and there are several methods that deal with this subject using measurement scales: categorical scale, interval-based scale and Borg's categorical rating scale. We have considered various scales, among which the most commonly used in hospitals and clinics is VAS (visual analogue scale), but there are reports stating that this scale is not sensitive to verbal descriptors separating pain intensity from a painful unpleasant feeling. An objective method of analyzing prosthesis quality can be a measure of deviation from the normal movement calculated by a method that considers the movement cycle and kinematic values of certain points on the human body (and prosthesis) that are qualitative characteristics of movement. Methods using kinematic indices of movement mainly seek to assess a (possibly pathological) situation compared to a normal situation (given by a reference group or person). There are many papers dealing with quantification of kinetic deviation from normal trends, but - to the author's best knowledge - there is only one paper that discusses the applicability of summaries on measuring the movement cycle of transtibial amputations (unilateral, a single limb) (4). The most used indexes are: Gillette (Gillette Gait index - GGI), movement profile score (Gait Profile Score - GPS), and movement analyze profile (Movement Analyze Profile - MAP). A summary of medical measurements, including techniques based on questionnaires and assessment of quality of life in patients wearing prostheses is presented in (5). In most cases, authors record and calculate kinematics using the Plug-in-Gait model (Vicon, Oxford Metrics) and placing of markers in compliance with the requirements for Helen Hayes set of markers (6). Markers are devices that generate light or reflect light on a dark background, making it possible to determine the trajectory of the bright sport using video image processing.

MATERIAL AND METHODS

A suggestion of objective measuring of comfort uses a principle based on energy minimization of patient effort, which reduces in a causal order the sensation of effort→ discomfort→ and increases the sensation of comfort. It assumes that the level of discomfort is given by the measure of work on the surface considered, which depends on the surface and force acting on it. Stump surface and skin-prosthesis interaction parameters are considered constant (coefficient of friction, moisture, temperature etc.). *C* is a closed curve (which does not intersect itself) *D* is the domain bounded by this curve. According to Green's theorem, the area bounded by *C* curve C is given by a double surface integral, difficult to apply for the pressure contour located on the stump, which has a shape curve very difficult to express in parametric form. The curve is approximated with a polygon (convex or concave), with a finite number of vertices, number given by the precision desired to approximate the area of the closed curve. For polygon area calculation, the topographic method described in sec-

tion (7) is suggested. We considered a polygon with n vertices in a plane (2D), which is covered in normal direction (counterclockwise). We note the vertices of the polygon with (x_0, y_0), (x_1, y_1),…., (x_{n-1}, y_{n-1}). Polygon area is given by the sum of determinants (7).

Basically, in case of images, the best possible approximation is at pixel level, while polygon vertices approximating the curve are in the center of the pixels on the contour (fig. 1).

$$(1) A = \frac{1}{2}\left\{\begin{vmatrix} x_0 & x_1 \\ y_0 & y_1 \end{vmatrix} + \begin{vmatrix} x_1 & x_2 \\ y_1 & y_2 \end{vmatrix} + \ldots + \begin{vmatrix} x_{n-2} & x_{n-1} \\ y_{n-2} & y_{n-1} \end{vmatrix} + \begin{vmatrix} x_{n-1} & x_0 \\ y_{n-1} & y_0 \end{vmatrix}\right\}$$

$$(2) A = \frac{1}{2}\left((x_0 y_1 - x_1 y_0) + (x_1 y_2 - x_2 y_1) + \ldots (x_{n-2} y_{n-1} - x_{n-1} y_{n-2}) + (x_{n-1} y_0 - x_0 y_{n-1})\right)$$

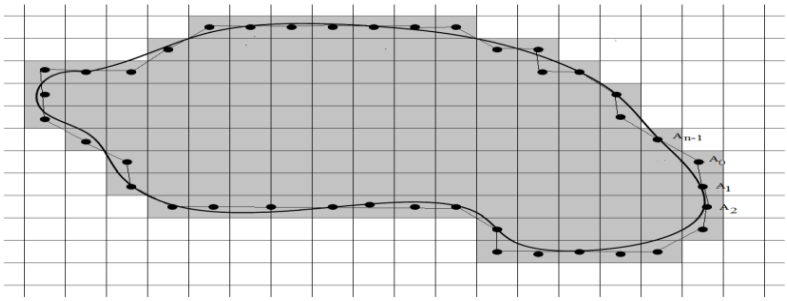

Fig. 1. Polygon area given by an extracted surface in pixels

Measure of the area depends on resolution, side of a rectangular pixel corresponds to *k* mm in real dimensions, therefore we have a A_t= k*A* scaling. On the other hand, in the 3D simulation image, if we approximate the residual limb with a truncated cone (common approximation in prostheses of lower limbs), what we measure is a projection of an area from the truncated cone on a flat surface that is the frontal plane viewed (fig. 2).

Developing a formula involves many calculations and is not justified for an algorithm with efficient computational effort, as this approach is a matter of future refining of the proposal in this paper (8).

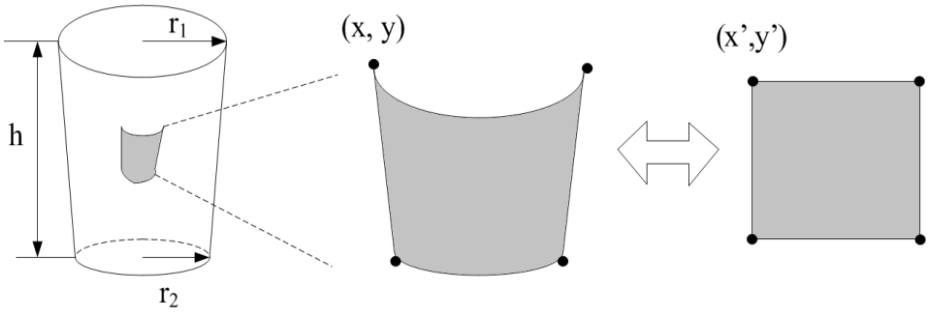

Fig. 2. A curvilinear rectangular surface on the truncated cone seen as distorted image

RESULTS

The stump-prosthesis assembly is scanned and then, using contour detection routines, we determined the cluster set of points for each element (stump, tibia, fibula, skin, sleeve, cup) and used NURBS curves for spatial reconstruction Non-uniform rational Basis spline – Non-uniform rational Basis spline - mathematical model for curves and surfaces, implemented in the CAD / CAE SolidWorks 2012 package. Elements are re-assembled into a 3D spatial reconstruction (fig. 3).

Bones and soft tissue, and skin and soft tissue are modelled as connected bodies (friction coefficient 1.0). The skin friction coefficient depends on the material used for the sleeve and cup, and varies generally between 0.4 and 0.8. A common modelling of the friction coefficient, where skin and soft tissues are considered homogeneous, isotropic bodies of 0.5 sleeve-skin and 0.7 sleeve-cup.

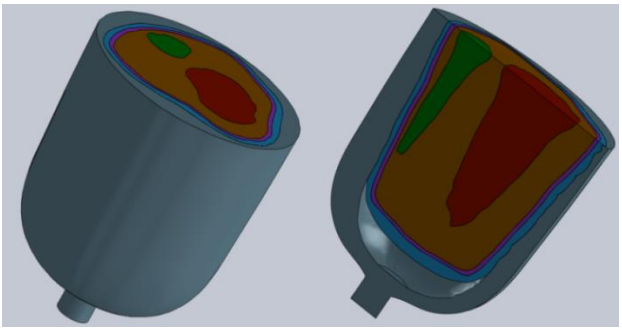

Fig. 3. Spatial reconstruction (3D) of the cup-prosthesis-stump ensemble and a section in this ensemble.

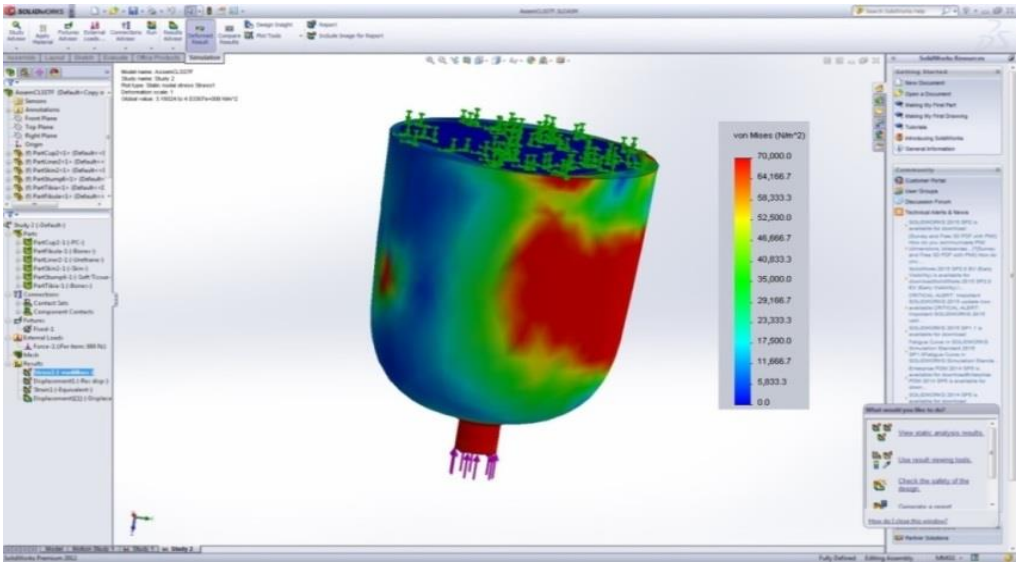

Fig. 4. Cup-prosthesis-stump ensemble with maximum pre-load and ground reaction force, F_{zmax} = 870 N, von Mises forces

The main stress at stump level in the stump-sleeve-cup interface is of a mechanical nature. The presence of two bone residues (tibia and fibula) and knee presence in leg amputation requires an approach and results different than trans femoral amputation, one bone residue and lack of the knee from previous doctoral thesis. Areas subject to intense stress are mainly those who raise medical issues, of structural integrity of the stump and ultimately essentially contribute to the patient's feeling of comfort/discomfort (fig. 4).

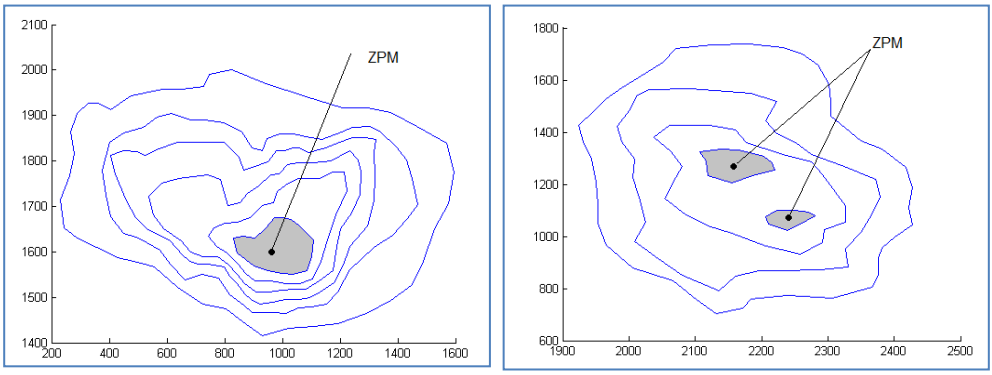

Fig. 5. Front transtibial location, transtibial amputation and local ZPM lateral position (maximum pressure area). Values on the horizontal and vertical axes are in pixels.

Note that in figure 5 (left-side) in all cases of simulation, there is only one island of maximum pressure in a large area, while in figure 5 (right-side) there are two islands (simulations also contained a situation where there were 3 islands) separated by a very small passage area. Basically, in this case we can consider that the maximum pressure per area is formed from a single zone, as in Figure 5 (left-side) and per **sum of areas** of two zones in figure 5 (right-side). Motivation is primarily of a medical nature, the larger the surface on which a maximum pressure is applied, the greater the likelihood that the surface in question develops skin lesions / deep wounds and therefore these areas should be identified and numerically evaluated.

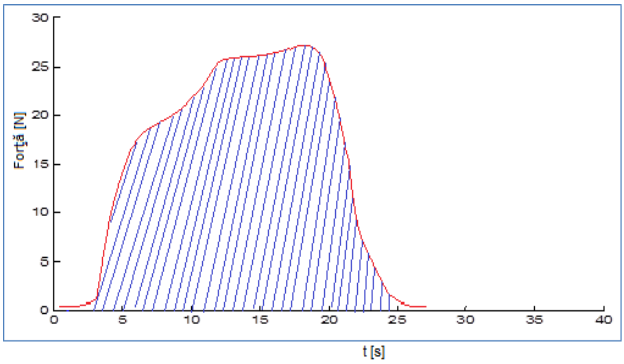

Fig. 6. Calculation $L_{FT.}$

We suggest a new objective measure for evaluating patient's comfort, a measure based on mathematical calculations and which is not modified by subjective assessments of the patient L_{FT} (fig 4).

$$(3) L_F = K\int_{x_0}^{x_f} \vec{F}\cdot d\vec{x} = K\int_{x_0}^{x_f} F\cos\alpha \cdot dx$$

$$(4) L_{FT} = K\int_{t_0}^{t_f} \vec{F}\cdot dt = K\int_{t_0}^{t_f} F\cos\alpha \cdot dt$$

In the above formulas, the force acts perpendicularly on the stump, therefore α =1, and the movement speed is approximately constant. K is a constant associated with the area and a first approximation is K=A·*v*, where *v* is the speed. In our case, speed is constant and we approximate with a *v* = *b* constant. This is explained by the fact that work on a surface is greater in the broader sense of patient comfort issue is all the greater as the surface is larger.

$$(5) L_{FT} = K\cdot b\cdot\int_{t_0}^{t_f} F(t)\cdot dt$$

$$(6) L_{FT} = K\cdot b\cdot\int_{t_0}^{t_f} |F(t)|\cdot dt$$

In the above formulas L_{FT} is the measure inspired from work, α- the angle of the acting force, *v*- speed (equal to constant *b*), *t* – time, *A* area covered by the sensor as a circular disc, *K* a constant and F the force as absolute value. We considered a movement (on the treadmill) at constant acceleration *a* = *dv*/*dt*) with a complete movement cycle over approximately 2.4 m. Current data were collected from a group of two patients with a set of 32 repetitions of a complete movement cycle (8 normal gait cycles, 8 cycles down the stairs, 8 cycles up the stairs and 8 rest cycles). We will assign a greater state of comfort as L_{FT} is lower. The results showed that for patient A the optimum is given by L_{FTmin} = 875.244 for the soft Pelite-PP/PE sleeve-cup combination, and for patient B, the optimum is given by L_{FTmin} = 915.244 for the soft Pelite-Polypropylene sleeve-cup combination. Corresponding values are 4 and 5 (maximum) on the comfort scale given by the questionnaire method commonly used to assess the comfort status of a person wearing a prosthesis, which creates the prerequisites for a possible effective evaluation method of comfort. Of course, all results must be medically validated, but in this area there are known cumbersome procedures which require time, and the gap between research and practical applications is sometimes very large. The results were consistent with the theory (and the results published in the literature by other authors) and, in our opinion, better than the questionnaire method. This assumption is based on two and four series of short tests applied to the person who presented a difference between the value of comfort level on PEQ scale and the objective scale. Two cases were with A combination and two with B combination. The patient was informed on the tests with combinations of material A - 3 tests and B - 1 test (the patient was informed for each test). Surprisingly, for the B combination, the questionnaire method in a test was 4 and in the other it was 5, suggesting that the proposed method is more consistent.

CONCLUSIONS

In this article measuring of comfort uses a principle based on energy minimization of patient effort and It assumes that the level of discomfort is given by the measure of work on the surface considered. The

stump-prosthesis assembly is scanned and then, using contour detection routines, we determined the cluster set of points for each element. Elements are re-assembled into a 3D spatial reconstruction and we suggest a new objective measure for evaluating patient's comfort, a measure based on mathematical calculations.

In this paper, we can consider that the maximum pressure per area is formed from a single zone, and per sum of areas of two zones and the results were consistent with the theory and, in our opinion, better than the questionnaire method.

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NEWS

SYNTHESIS OF HIERARCHICAL CuO NANOSTRUCTURES: BIOCOMPATIBLE ANTIBACTERIAL AGENTS FOR GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Copper Oxide is one of the important materials among other antimicrobial agents such as silver, zinc oxide, or titanium dioxide. CuO nanostructures have stimulated great interest in the field of environmental remediation because of their important characteristics which include: high stability, structural properties, antimicrobial activity. The novelty of this study is the biocompatibiliy determination of nanostructured CuO. The findings of the present work proved that the prepared CuO nanostructures are highly stable in aqueous solution and biocompatible in blood. They also exhibited pronounced bactericidal activity towards both Gram positive and Gram negative bacteria (Sonia S, Jayasudha R, Jayram ND, et al. Synthesis of hierarchical CuO nanostructures: Biocompatible antibacterial agents for Gram-positive and Gram-negative bacteria. *Current Applied Physics* 2016, 16: 914-921).

Oana Dragostin

CHANGES IN SOME DIVALENT CATIONS CONCENTRATIONS IN AN EXPERIMENTAL RAT MODEL OF GENTAMICIN-INDUCED ACUTE RENAL FAILURE

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CHANGES IN SOME DIVALENT CATION CONCENTRATION IN AN EXPERIMENTAL RAT MODEL OF GENTAMICIN-INDUCED ACUTE RENAL FAILURE (Abstract) **Aim:** The changes in divalent cations concentration were assessed in and experimentally gentamicin-induced renal failure in white Wistar rats. **Material and methods**: The white male Wistar rats were distributed into 4 groups of 7 animals each and were treated intraperitoneally as follows: Group I (Control): distilled water in a volume of 0.5ml/100g rat/day for10 days; Group II (Ge): gentamicin 80 mg/kbw/day for 7 days; Group III (Ge+Zn): gentamicin 80 mg/kbw/day for 7 days and ZnCl2 5 mg/kbw/day for 10 days prior to administration of Ge and then another 7 days simultaneously with Ge administration; Group IV (Zn): ZnCl2 5 mg/kbw/day for 17 days. Before starting the experiment (I_0) and at 3, 7 and 10 days after the first Ge administration, magnesium, copper and zinc plasma concentrations and urinary magnesium levels were determined. **Results**: Zn administration significantly decreased ($p<0.001$) plasma Mg concentrations in Ge+Zn group compared to Ge group after 7 days in the experiment, and induced a lower urinary elimination of Mg in Ge+Zn group ($p<0.05$) than in Ge group ($p<0.01$). Also, Zn induced a slight augmentation of Cu concentration in Ge+Zn group ($p<0.05$) compared to Ge group after 7 and 10 days. **Conclusions**: The variation in divalent cation concentrations in the context of renal diseases may be helpful for an early diagnosis and effective alternative therapeutic measures. **Keywords**: DIVALENT CATIONS, RAT MODEL, ACUTE RENAL FAILURE, GENTAMICIN.

The kidney plays an important role in the homeostasis of divalent cations (1). This adjustment is processed at different levels of the nephron involving the participation of ion channels and some transporters.

Zinc (Zn) is the second most abundant metal in the body, after iron, and is the only metal that can be found in all types of enzymes. Zn, being found in all tissues of the body, plays a role in immune system function, tissue repair processes, protein production, DNA synthesis and cell division. Severe Zn deficiency can induce immune system disturbances, alteration of macrophage and neutrophil functions, apoptosis and impairment of complement activity (2, 3).

Magnesium (Mg), the second most common cation in the extracellular space after potassium (K), plays an essential role in physiological cellular processes due to its ability to form complexes with the important intracellular anionic ligands (especially with adenosine triphosphate – ATP), and its capability to compete with calcium for membrane sites. It is known that over 300 enzymatic reactions are dependent on the presence of Mg, those involved in ATP generation and utilization (4).

Copper (Cu) is an essential trace element, being stored primarily in the liver, with small amounts also present in the brain, heart, kidney and muscles. Cu is involved in the functioning of some essential enzymes. Cu maintains the resistance of the skin tissue, blood vessels, epithelial and connective tissues. It also has a major role in the synthesis of hemoglobin, myelin, melanin, and in maintaining of the thyroid gland normal functions (5).

In the present study, we aimed at evaluating the changes in divalent cation concentration, in experimentally gentamicin-induced renal failure in white Wistar rats.

MATERIAL AND METHODS

The following substances were used in the experiment: gentamicin solution for injection 80mg/2ml (Krka, Slovenia), zinc chloride (ZnCl2) ≥98% (Sigma-Aldrich Corporation), distilled water (Sicomed Romania).

Male white Wistar rats (weighting 210-320g), housed in rooms with controlled temperature (22-24°C) and constant 12 hours' light-dark cycle, and kept in metabolic cages with free access to water and standard food, were monitored for 24-hour water intake and urine output.

The animals were distributed into 4 groups of 7 animals each, treated intraperitoneally (i.p.) as follows: Group I (Control): distilled water in a volume of 0.5ml/100g rat/day for10 days; Group II (Ge): gentamicin 80mg/kbw/day for 7 days; Group III (Ge+Zn): gentamicin 80 mg/kbw/day for 7 days and ZnCl2 5 mg/kbw/day for 10 days prior to administration of Ge and then another 7 days simultaneously with Ge administration; Group IV (Zn): ZnCl2 5 mg/kbw/day for 17 days.

Blood samples were collected from retro-orbital plexus of anaesthetized animals, before starting the experiment (I_0) and at 3, 7 and 10 days after the first administration of the nephrotoxic agent to determine the magnesium, copper and zinc plasma concentrations. Urine samples were collected at the same intervals as the blood samples, to measure the calcium and magnesium levels.

Cu and Zn plasma concentrations were determined by atomic absorption spectrophotometry using an Analyst 600 Atomic Absorption Spectrophotometer (Perkin Elmer), a AS-800 Graphite Furnace Autosampler, and a copper or zinc HCL (Hallow Cathode Lamp). A Randox kit and a Xylidyl blue colorimetric method were used to measure serum and urinary magnesium levels.

All data were presented as mean ± standard deviation (SD) of mean for 7 animals in a group. P-values less than 0.05 were considered statistically significant. Data were processed using one-way ANOVA method and Tukey's test.

The experimental protocol was implemented in compliance with the instructions of the "Grigore T. Popa" University of Medicine and Pharmacy Iaşi Committee for Research and Ethical Issues, per the Re-

search Law 206/27.05.2004 regarding the conduction of scientific research, technological development and innovation and the actual European legislation.

RESULTS

The biochemical analysis revealed a statistically significant increase in serum Mg levels at 7 days compared with I_0 in Ge group ($p<0.01$) and Ge+Zn group ($p<0.05$). A reduction in Mg levels was recorded at 7 days (after 17 days of Zn administration), statistically significant compared with I_0 in Zn group ($p<0.01$). The administration of Zn significantly diminished ($p<0.01$) the plasma Mg concentrations in Ge+Zn group compared to Ge group at 7 days in the experiment (tab. I).

TABLE I

Variation in plasma Mg concentration (mg/dl) in Ge, Zn and Ge+Zn groups

Lot	initially (I0)	3 days	7 days	10days
Ge	2,35±0,26	2,31±0,07	*4,31±0,29	#2,30±0,06
Ge+Zn	2,36±0,17	2,44±0,14	*;^2,97±0,18	#2,45±0,18
Zn	2,46±0,20	2,52±0,41	*1,86±0,25	2,36±0,15
Control	2,39±0,08	2,43±0,18	2,40±0,14	2,45±0,13

* $p<0.01$ vs. I_0 and Control; #$p<0.05$ vs. 7 days; ^ $p<0.01$ vs. Ge

Significant increases ($p<0.01$) in serum Cu levels were recorded in Ge+Zn group, but not in Ge group after 3, 7 and 10 days, compared to the initial moment I_0.

The laboratory determinations highlighted a progressive decrease in plasma Cu values, statistically significant ($p<0.01$) at 7 days, compared with I_0 in Zn group (after 17 days of $ZnCl_2$ administration). The treatment with Zn induced a significant augmentation of Cu concentration in Ge+Zn group ($p<0.01$), compared to Ge group at 7 and 10 days in rats with experimentally gentamicin-induced acute renal failure (tab. II).

TABLE II

Variation in plasma Cu concentration (µg/dl) in Ge, Zn and Ge+Zn groups

Lot	I0	3 days	7 days	10 days
Ge	139,30±5,93	148,59±5,88	144,37±6,10	145,7±4,61
Ge+Zn	127,78±8,09	*146,94±11,4	*;^175,37±9,03	*;^165,62±6,68
Zn	125,28±13,89	119,38±10,33	*98,86±13,13	128,58±13,04
Control	148,06±6,75	145,95±4,67	148,23±2,45	143,60±5,59

*$p<0.01$ vs. I_0; ^$p<0.01$ vs. Ge

Our investigation showed a statistically significant elevation ($p<0.01$) of serum Zn levels at 3, 7 and 10 days compared to I_0 in Ge and Ge+Zn groups. The treatment with Zn was associated with a significant increase of plasma Zn levels in Ge+Zn group compared with Ge group at 3, 7 and 10 days in the experiment (tab. III).

The biochemical analysis detected an elevation of Mg urinary excretion, statistically significant at 7 and 10 days compared to I_0 in Ge group ($p<0.01$). In Ge+Zn group

it was also registered an increased excretion of urinary Mg at 7 days compared to I_0 ($p<0.01$). The administration of Zn resulted in a lower urinary elimination of Mg in Ge+Zn group than in Ge group ($p<0.01$) at 10 days (fig.1).

TABLE III
Variation in plasma Zn concentration (µg/dl) in Ge, Zn and Ge+Zn groups

Lot	I0	3 days	7 days	10 days
Ge	126,45±5,91	*146,03±6,66	*158,74±8,26	*144,17±8,02
Ge+Zn	157,41±8,14	^168,12±13,45	*;^189,49±16,26	*;^196,78±11,96
Zn	168,04±12,91	*183,67±16,58	*198,64±12,22	*204,27±10,72
Control	122,50±7,07	124,32±10,90	121,22±15,14	124,94±10,56

*p<0.01 vs. I_0; ^p<0.05 vs. Ge

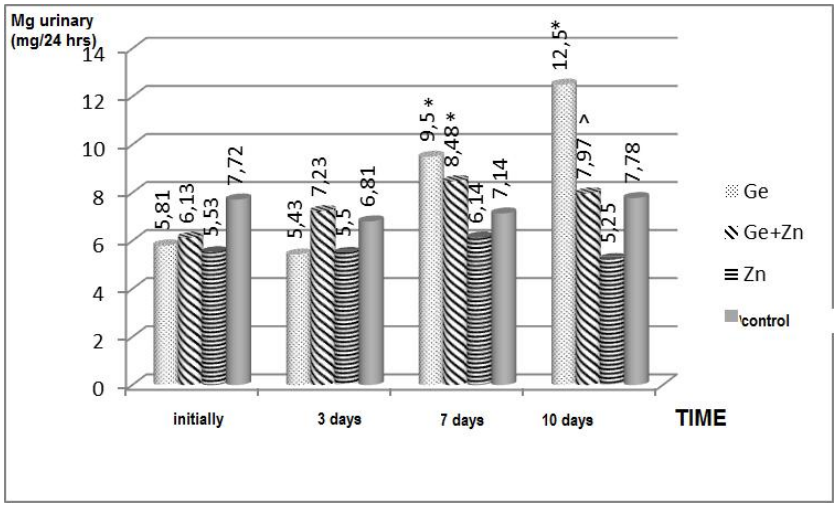

Fig. 1. Urinary Mg concentration (mg/24hrs) in Ge, Zn and Ge+Zn groups
*p<0.01 vs. I_0; ^p<0.01 vs. Ge

DISCUSSION

Zn is considered an important element necessary for the physiological function of cell signaling, and influences the redox status, enzyme activity, gene transcription, energy metabolism, cell cycle, apoptosis and cell proliferation. Zn deficiency increases the levels of nitric oxide and superoxide radicals by inhibiting the N-methyl-D -aspartate receptor (6, 7).

Literature data suggest that, besides the action of highly reactive radicals, another mechanism of Ge nephrotoxicity is represented by the changes of trace elements in the kidney (8). In our study, Zn loading resulted in a significantly lower increase of serum Mg in Ge+Zn group compared to Ge group (2.97 ± 0.18 mg/dl *vs* 4.31 ± 0.29 mg/dl). Similarly, after Ge administration, on day 7 we recorded elevated serum Mg levels as compared with I_0. However, Zn administration did not significantly affect

serum Ca concentration in Ge+Zn group compared to Ge group.

The values showed no significant change in plasma Cu following Ge administration, but Zn treatment generated increased Cu concentration in Ge+Zn group compared to Ge group (175.37 ± 9.03 μg/dl *vs.*144.37 ± 6.1 μg/dl at 7 days). Plasma Cu values showed an increase in the group treated with Ge compared to I_0, and Zn charge resulted in higher plasma Zn levels in Ge+ Zn group compared to Ge group (189.49±16.26 μg/dl *vs.* 158.74±8.26 μg/dl at 7 days).

Mg modulates the contractility and electrical activity of myocardial cells, and the conduction system that influences the transport of ions (sodium, potassium, calcium) in the sarcolemma. Mg can also influence vascular smooth muscle tone. The exact physiological mechanism that regulates plasma Mg concentration is not completely elucidated. It seems that serum Mg is regulated through a dynamic mechanism and interaction between the transport in the kidney, intestine and bones (9).

Approximately 95% of ultra-filtered Mg is reabsorbed in the kidney: 25-30% in the proximal tubules, about 60-65% is reabsorbed in the ascending loop of Henle, and 5% in the distal segment. Mg excretion is controlled primarily by serum Mg concentration (10).

Hypermagnesemia is associated with an increase in Mg excretion which can be up to 100% of the filtered amount. Literature data confirm that Ge can generate renal Mg loss through a furosemide-like mechanism, by inhibiting the paracellular transport in the ascending loop of Henle (11).

Copper can act both as an antioxidant and as a pro-oxidant. As an antioxidant, it removes or neutralizes free radicals and reduces the damage caused by these agents, which can promote pathological processes through interaction with genetic material. When acting as a pro-oxidant, Cu can induce the generation of free radicals and contribute to the development of such diseases as Alzheimer's disease. Normal Cu homeostasis, as well as other mineral balance (Zn, Mg), is important for maintaining health (12).

Some studies investigated the Cu/Zn imbalance in Cu deficiency in rats. In case of Cu deficiency, decreased activity of superoxide dismutase and increased malondialdehyde levels compared to normal values were recorded (13).

CONCLUSIONS

Divalent cations homeostasis is essential for the proper functioning of the entire organism. Our study suggested the importance of taking in consideration the variation in concentrations of these cations in the context of renal diseases, being possibly helpful for early diagnosis and effective alternative therapeutic measures.

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NEWS

CALCIUM SUPPLEMENTS MAY AFFECT THE CORONARY ARTERIES

This is the conclusion of a recent article published in Journal of American Heart Association. This result represents part of a bigger study conducted in six research American universities, the Multi-Ethnic Study of Atherosclerosis. The researchers used dietary questionnaires and CT scans spanning 10 years apart to asses the daily calcium intake (either as supplements or food) and the calcium score. The results showed that supplement users have a 22% higher risk of developing coronary artery disease (defined as a rise of the calcium score over the 10 years) than the subjects with a high dietary intake of calcium. It is still unclear why taking the same amount of calcium as supplement is more harmful, but the ipothesis takes into account the form of calcium that can be differently absorbed (calcium salt in supplements) and the inability of the body of processing a large dose of calcium all at once (John J.B. Anderson, PhD; Bridget Kruszka, MPH et al. Calcium Intake From Diet and Supplements and the Risk of Coronary Artery Calcification and its Progression Among Older Adults: 10-Year Follow-up of the Multi-Ethnic Study of Atherosclerosis (MESA). *J Am Heart Assoc*. 2016; 5:e003751).

Maria-Madalina Bostan

INSIGHTS ON THE SOCIAL PERCEPTION OF MENTALLY DISABLED CHILDREN

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INSIGHTS ON THE SOCIAL PERCEPTION OF MENTALLY DISABLED CHILDREN (Abstract): **The aim** of the research is to analyze the social perception of mentally disabled people and of attitudes toward them among teachers, according to certain variables such as subjects' age, gender, type of education and degree of interaction with mentally disabled people, understood as work experience with mentally disabled children. **Material and methods**: A number of 120 teachers (44 men and 76 women) answered to a 26 items of *Scale of perceptions and attitudes regarding mentally disabled people* investigating the following dimensions: the concept of mental disability, the image of mentally disabled people, integration into society, education. Several variables were taken into consideration. Result analysis and interpretation has been performed by means of the specialized psychological statistics software *SPSS 23* for Windows. **Results**: female teachers (M=17.00) have a significantly more favorable attitude - t (118)=-3.75, p=.001 than men (M=12.25) who work as teachers and younger teachers and those with no experience with children with mental disability have a more favorable attitude toward mentally disabled people than their colleagues. **Conclusions**: Age, experience with mental disabled children and gender of teachers are influencing the perception and the attitudes regarding people with disability. **Keywords**: CHILDREN, MENTAL DISABILITY, SOCIAL PERCEPTION, TEACHER

Working with children with mental disability is a demanding task and people who are involved in special education have to prove not only emotional balance, empathy, intellectual capability, pedagogical tasks or good training in working with a special category of children, but they have to deal also with their personal limits. Teachers' attitudes are crucial to the success of inclusion programs for children with special needs (1) and the consequences of the disability on child's life and family are extremely important (2). Family involvement is necessary (3) and in majority of cases parents are becoming home teachers too, so a good collaboration between teachers and caregivers are assuring a better development for the children in need (4,5,6).

Both education and contact were revealed to have had positive effects on reducing stigma for adults and adolescents with a mental illness and to assure a better

social integration (7). Some studies pointed that teachers are concerned about integrating children with special needs in their classrooms (8) even if they had positive feelings about them.

The aim of our research is to emphasize the social perception of mentally disabled people and of attitudes toward them among teachers, according to certain variables such as subjects' age, gender, type of education and degree of interaction with mentally disabled people, understood as work experience with mentally disabled children.

MATERIAL AND METHODS

In order to achieve the aim, we have built (starting from the results of the qualitative analysis obtained following a questionnaire with open answers) the *Scale of perceptions and attitudes regarding mentally disabled people*. The open answer questionnaire on which the instrument of our research has been built has included the investigation of the following dimensions: the concept of mental disability (cognitive vs. global perspective), the image of mentally disabled people (characteristics, capacities, disabilities), integration into society (social perception, support measures, support institutions, vocational options), education (types of schooling, adaptation problems: relational, behavioral, educational and perspectives after graduation).

After pretesting and eliminating items with an internal consistency coefficient lower than 0.60, 26 items remained, corresponding to four subscales: *capacity for social integration* - 6 items with Cronbach's alpha=0.91), *positive characteristics* - 3 items with Cronbach's alpha=0.73), *negative characteristics* - 9 items with Cronbach's alpha=0.75), *attitudes toward mentally disabled people* - 8 items with Cronbach's alpha=0.71. The score for each scale has been obtained by adding up the values assigned to the items, values which can be assessed on a scale of 1 to 5. The items ranked in reverse are: 7, 14, 15, 16, 17, 25.

The target population of the study, from which the batch of 120 subjects has been extracted by random sampling, consists of teachers with ages ranging 23-35, 36-50 and over 50 years old; subject samples have been small and independent.

Thus, by the gender variable, the batch includes 44 men and 76 women. By the age variable, the batch includes 26 subjects included in the category 23-35, 65 subjects with ages 36-50 and 29 subjects with ages over 50. By variable “level of interaction with mentally disabled people”, the batch includes 70 teachers who have not interacted with such people, 31 subjects who have under 10 years' experience working with mentally disabled children and 19 subjects with over 10 years' experience. 70 subjects work in primary education and 50, in secondary education.

The research has been carried out in two regular schools (where mentally disabled children are also integrated) and in one special school. The questionnaire has been submitted individually, and subjects' anonymity has been ensured.

Result analysis and interpretation has been performed by means of the specialized psychological statistics software *SPSS 23* for Windows, trial version, downloaded from the official IBM website. The following statistical analyses have been performed:

- the Student's t-test for independent samples, determining the significance of the difference between means according to variables “gender” and “type of education”.

▪ the one-way ANOVA (single factor) for comparing means according to variables "age" and "level of interaction".

RESULTS

The influence of subjects' gender on their social perception of mentally disabled people

Applying the t-test for independent samples, we have analyzed the results obtained by male and female subjects. According to the data obtained, we notice no statistically significant differences between male and female subjects in variable "capacity for social integration", but there are statistically significant differences between the mean obtained by women (MF=10.4) and that obtained by men (MM=8.9) in variables "positive characteristics" (t (118)=-3.12, p=.002 < 0.05) and "negative characteristics" (t (118)=3.06, p=.003 < 0.05; MF=26.7, MM=31.2), in that the mean obtained by women is significantly lower than that of men concerning negative characteristics and significantly higher concerning positive characteristics. Moreover, in terms of the attitude to mentally disabled people, female teachers (M=17.00) have a significantly more favorable attitude (t (118)=-3.75, p=.001 < 0.05) than men (M=12.25) who work as teachers. Results are presented in Table I.

TABLE I

The influence of subjects' gender on their social perception of mentally disabled people

Dimensions	Gender	N	Mean	Standard deviation	t	p
capacity for social integration	M	44	15.4	4.8	-.092	.927
	F	76	15.5	4.5		
positive characteristics	M	44	8.9	2.6	-3.12	**.002**
	F	76	10.4	2.4		
negative characteristics	M	44	31.2	7.8	3.06	**.003**
	F	76	26.7	7.5		
attitude to mentally disabled people	M	44	12.25	5.8	-3.75	**.001**
	F	76	17.00	7.9		

The influence of the type of educational system that subjects are involved in (primary and secondary) on their social perception of mentally disabled people

Applying the t-test for independent samples, we have analyzed the results obtained by subjects involved in primary and secondary education (tab. II). There are statistically significant differences between subjects according to variable "type of education" (t (118) =3.082, p=.003 < 0.05), in that teachers working in primary education (M=17.00) have a significantly more favorable attitude toward mentally disabled people, compared to those working in secondary education (M=12.82). In addition, teachers working in primary education have a social perception with more positive characteristics, obtaining significantly higher means in subscales "capacity for integration" (t (118)=4.674, p=.000; MP=17.02, MG=13.32) and "positive characteristics" (t (118)=5,577, p=.000; MP=10.84, MG=8.48) and significantly lower means in subscale "negative characteristics" (t (118)=-5.103, p=.000; MP=25.55, MG=32.36), compared to their colleagues working in secondary education.

TABLE II

The influence of the type of education that subjects are involved in (primary and secondary) on their social perception of mentally disabled people

Dimensions		N	Mean	Standard deviation	t	p
Capacity for social integration	Primary school	70	17.02	4.4	4.674	**.000**
	Secondary school	50	13.32	4.1		
Positive characteristics	Primary school	70	10.84	2.2	5.577	**.000**
	Secondary school	50	8,48	2.3		
Negative characteristics	Primary school	70	25.55	6.6	-5.103	**.000**
	Secondary school	50	32.36	7.8		
Attitude to mentally disabled people	Primary school	70	17.00	7.2	3.082	**.003**
	Secondary school	50	12.82	7.3		

The influence of teachers' age on their social perception of mentally disabled people and their attitude to the latter

Applying the one-way ANOVA, we have analyzed the effect of the age variable on the social perception of mentally disabled people and on the attitude to them.

According to the data obtained, we notice that age has a statistically significant effect only on assessing the capacity for integration (F=11.644, p=.000) and the positive characteristics of mentally disabled people (F=7.643, p=.001). When it comes to assigning negative characteristics and to subjects' attitude toward these people, age has no significant influence.

After carrying out the Bonferroni test, we highlighted that there are statistically significant differences on the assessment of mentally disabled people's capacity for integration between subjects aged between 23-35, those aged between 36-50 and those over 50 years old. Thus, teachers aged 23-35 years old consider that mentally disabled people have a higher capacity for integration than subjects from the other two age groups (M (23-35)–M (36-50) =4.21, p=.000<.05; M (23-35)–M (over 50) =5.10, p=.000 < .05).

There are no statistically significant differences between age groups 36-50 and over 50 concerning the assessment of mentally disabled people's capacity for integration (tab. III).

Regarding the assignment of positive characteristics, we notice statistically significant differences between the 23-35 age group, compared to the other two groups. Thus, teachers aged between 23 and 35 assign more positive characteristics to mentally disabled people compared to their colleagues in the other two age groups (M (23-35)–M (36-50) =1.53, p=.022 < .05; M (23-35)–M (over 50) =2.54, p=.001 < .05).

There are no statistically significant differences between the 36-50 and over 50 age groups, when it comes to assigning positive characteristics to mentally disabled people.

The influence of variable "level of interaction" on teachers' social perception of mentally disabled people and their attitude to the latter

Applying the one-way ANOVA, we have analyzed the effect of variable "level of interaction" (experience working with

mentally disabled children) on the social perception of mentally disabled people and on the attitude to them. According to the results obtained (tab. IV), the level of interaction also has a significant effect on the social perception of mentally disabled people and on the attitude toward them.

Thus: capacity for integration: F(2,117) =19.266, p=.000<.05; positive characteristics: F(2,117)=19.403, p=.000<.05; negative characteristics: F(2,117)=17.129, p=.000<.05; attitude toward mentally disabled people: F(2,117)=12.091, p=.000< .05.

After carrying out the Bonferroni test, we may note the following differences between groups in table V.

TABLE III

The influence of teachers' age variable on their social perception of mentally disabled people and their attitude to the latter

Dependent Variable	(I) age	(J) age	Mean Difference (I-J)	Std. Error	Sig
Capacity for social integration	23-35	36-50	4.21538*	.99280	**.000**
		>50	5.10345*	1.15553	**.000**
	36-50	23-35	-4.21538*	.99280	**.000**
		>50	.88806	.95541	1.000
	> 50	23-35	-5.10345*	1.15553	**.000**
		36-50	-.88806	.95541	1.000
Positive characteristics	23-35	36-50	1.53846*	.56368	**.022**
		>50	2.54907*	.65607	**.001**
	36-50	23-35	-1.53846*	.56368	**.022**
		>50	1.01061	.54245	.195
	> 50	23-35	-2.54907*	.65607	**.001**
		36-50	-1.01061	.54245	.195
Negative characteristics	23-35	36-50	-3.40769	1.82651	.194
		>50	-2.73607	2.12588	.602
	36-50	23-35	3.40769	1.82651	.194
		>50	.67162	1.75773	1.000
	>50	23-35	2.73607	2.12588	.602
		36-50	-.67162	1.75773	1.000
Attitude to mentally disabled people	23-35	36-50	3.06154	1.74645	.247
		> 50	3.52785	2.03270	.256
	36-50	23-35	-3.06154	1.74645	.247
		> 50	.46631	1.68068	1.000
	> 50	23-35	-3.52785	2.03270	.256
		36-50	-.46631	1.68068	1.000

TABLE IV

Results regarding the influence of level of interaction with people with disability on social perception

		Sum of Squares	df	Mean Square	F	Sig.
Capacity for integration	between groups	636.198	2	318.099	19.266	**.000**
	within groups	1931.769	117	16.511		
	total	2567.967	119			
Positive characteristics	between groups	194.417	2	97.209	19.403	**.000**
	within groups	586.174	117	5.010		
	total	780.592	119			
Negative characteristics	between groups	1691.088	2	845.544	17.129	**.000**
	within groups	5775.503	117	49.363		
	total	7466.592	119			
Attitude toward mentally disabled people	between groups	1171.751	2	585.875	12.091	**.000**
	within groups	5669.241	117	48.455		
	total	6840.992	119			

TABLE V

The influence of variable "level of interaction" (experience working with mentally disabled children) on teachers' social perception

Dependent Variable	(I) level of interaction	(J) level of interaction	Mean Difference (I-J)	Std. Error	Sig.
Capacity for integration	none	<10 years	3.85530*	.87663	.000
		> 10 years	5.63459*	1.05112	**.000**
	<10 years	none	-3.85530*	.87663	**.000**
		> 10 years	1.77929	1.18389	.407
	> 10 years	none	-5.63459*	1.05112	**.000**
		<10 years	-1.77929	1.18389	.407
Positive characteristics	none	<10 years	2.41244*	.48289	**.000**
		> 10 years	2.82331*	.57901	**.000**
	<10 years	none	-2.41244*	.48289	**.000**
		> 10 years	.41087	.65215	1.000
	> 10 years	none	-2.82331*	.57901	**.000**
		<10 years	-.41087	.65215	1.000
Negative characteristics	none	<10 years	-6.66406*	1.51577	**.000**
		> 10 years	-8.83383*	1.81749	**.000**
	<10 years	none	6.66406*	1.51577	**.000**
		> 10 years	-2.16978	2.04705	.874
	> 10 years	none	8.83383*	1.81749	**.000**
		<10 years	2.16978	2.04705	.874
Attitude toward mentally disabled people	none	<10 years	5.42350*	1.50176	**.001**
		> 10 years	7.47444*	1.80069	**.000**
	<10 years	none	-5.42350*	1.50176	**.001**
		> 10 years	2.05093	2.02814	.942
	> 10 years	none	-7.47444*	1.80069	**.000**
		<10 years	-2.05093	2.02814	.942

There are statistically significant differences in what concerns the assessment of the capacity for integration of mentally disabled people between subjects who have never worked with mentally disabled children and those who have worked with them for under 10 years or over 10 years. Thus, teachers who have not interacted with mentally disabled children consider that mentally disabled people have a higher capacity for integration than assessed by their colleagues with experience working with these children (M (none)–M (under 10 years) =3.85, p=.000 < .05 / M (none)–M (over 10 years) =5.63, p=.000 < .05). There are no statistically significant differences between teachers with less than 10 years of experience working with mentally disabled children and those with over 10 years of experience, in what concerns the assessment of the capacity for integration of mentally disabled people.

Regarding the assignment of positive characteristics, we noticed statistically significant differences between teachers who have never interacted with mentally disabled children, compared to the other two groups. Thus, teachers who have never interacted with these children assign more positive characteristics to mentally disabled people than their colleagues in the other two groups (M (never)–M (under 10 years) =2.41, p=.000 <.05 / M (never)–M (over 10 years) =2.82, p=.000 < .05).

There are no statistically significant differences between colleagues with under 10 years of experience working with mentally disabled children and those with over 10 years of experience, concerning the assignment of positive characteristics to mentally disabled people.

In terms of assigning negative characteristics, there are statistically significant differences between teachers who have never interacted with mentally disabled children, compared to the other two groups. Thus, teachers who have never interacted with these children assign fewer negative characteristics to mentally disabled people than their colleagues in the other two groups (M (never)–M (under 10 years) =-6.66, p=.000 < .05/ M (never)–M (over 10 years) =- 8.83, p= .000 < .05).

There are no statistically significant differences between teachers with under 10 years of experience working with mentally disabled children and those with over 10 years of experience, concerning the assignment of positive characteristics to mentally disabled people.

In what concerns the assignment of negative characteristics, there are statistically significant differences between teachers who have never interacted with mentally disabled children, compared to the other two groups. Thus, teachers who have never interacted with these children assign less negative characteristics to mentally disabled people compared to their colleagues in the other two groups (M (never)–M (under 10 years) =-6.66, p=.000 < .05; M (never)–M (over 10 years) =- 8.83, p=.000 < .05).

There are no statistically significant differences between teachers with under 10 years of experience working with mentally disabled children and those with over 10 years of experience concerning the assignment of negative characteristics to mentally disabled people.

When it comes to the attitude toward mentally disabled people, we notice from the results obtained that there are statistically significant differences between teachers who have never interacted with mentally disabled children, compared to the other two groups. Thus, teachers who have never

interacted with these children have a more favorable attitude toward mentally disabled people than their colleagues in the other two groups (M (never)–M (under 10 years) =5.42, p=.001 <.05 / M (never)–M (over 10 years) =7.47, p=.000 < .05).

There are no statistically significant differences between teachers with under 10 years of experience working with mentally disabled children and those with over 10 years of experience, concerning the attitude toward mentally disabled people.

DISCUSSION

Working with children with disabilities demand a lot of determination. It is not about the intellectual disability because studies proved that there are a lot of differences between children with or without mental disability, not only the mental aspects: social problems, family problems, attention problems and aggressiveness (10). The influence of the environmental on the quality of life of children with such problems is proved and together with family members, medical team, friends, policy makers, school members must assure a protective (safe) and adapted (good integration, also in public schools) place for them (11). The issue regarding including children with disabilities in special or normal schools has been generated a lot of disputes, shaping both positive and negative effects on children's development. The most powerful source of facilitating their inclusion is the human environment (12). By consequence, teachers-student with disability seems to be the key for a developing social behavioral skills of children (13, 14) meaning that the attitude of teachers regarding children with disability will assure a better support for adapted intervention programs.

The results of our study prove the fact that there is a significant difference in the characterization of those suffering from mental disability in that women have a more positive attitude than men. This may be due to the fact that women have more developed communication skills and better social empathy. Women are more willing to characterize a person with disabilities positively. Male subjects are not more pessimistic than women regarding the capacity for social integration, but have a more negative attitude than women when characterizing mentally disabled people.

Teachers working in primary education witness more spectacular developments in children during their first four years of primary school. Perhaps for this reason, results show that they have a deeper maternal attitude toward their pupils than teachers working in secondary education. Thus, the former is more prone to an optimistic approach regarding the development of mentally disabled people and to assessing them positively.

Young teachers show a higher level of optimism regarding the social insertion of mentally disabled people, an optimism also expressed by their positive characterization. On the other hand, older teachers are more reserved regarding social insertion and less generous with the positive characteristics used to portray the mentally disabled person.

Our research has also been aimed at emphasizing the differences between teachers with experience working with mentally disabled children and those without experience. The results have shown that young and less experienced teachers are more optimistic regarding integration; they use more positive characteristics and have a more favorable attitude toward mentally disabled

children. As with age-related differences, we consider that we have found here the cautious optimism of teachers who work or have worked with mentally disabled people. They know the problems the mentally disabled person really faces and are consequently more moderate both in their trust in the person's social insertion and in the person's characterization. We consider that the less favorable attitude which surprised us in experienced teachers may be explained by the fact that working with children with this type of disabilities is difficult, as they have a different behavior from a normal child - a behavior which causes problems in the classroom. The way to relate and communicate with these children is not at all easy and teachers must be trained to have skills from different areas: psychology, medicine, sociology, pedagogy (9).

CONCLUSIONS

Our research emphasizes that age, experience with mental disabled children and gender of teachers as independent variables are influencing the perception and the attitudes regarding people with disability.

Results are important for university curricula in order to train professionals working with mentally disabled people to have an objective opinion and to proper evaluate them.

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VITAMIN D AND TISSULAR EXPRESSION OF VITAMIN D RECEPTOR IN OBESITY

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VITAMIN D AND TISSULAR EXPRESSION OF VITAMIN D RECEPTOR IN OBESITY (Abstract): Vitamin D (VitD), a lipid-soluble hormone, is able to regulate the transcription of many genes through vitamin D receptor (vitD receptor - VDR). It has been shown that VitD deficiency is associated with obesity, characterized by a low degree inflammatory state, which contribute to the pathogeny of metabolic syndrome and type 2 diabetes mellitus. VitD deficiency is a public health problem, at the same time the global prevalence of obesity and cardiovascular diseases is continuously growing. Evidence from recent studies on animal models suggest that VitD or VDR deficiency promotes cardiomyocyte hypertrophy, which can be one of the mechanisms for increasing cardiovascular risk. The heart is one of the target organs of action for VitD, because VDR is expressed in cardiomyocytes. Also, previous in vitro studies have shown that VitD is able to inhibit the production of monocyte chemotactic factors (MCP-1) and other pro-inflammatory mediators in human preadipocytes and mature adipocytes. Inflammation is an important factor in the pathogenesis of atherosclerosis. In obesity there are not known data about correlations between plasma levels of VitD and VDR expression in the subcutaneous fat tissue, epicardial visceral adipose tissue, and in particular in myocardium. Also, there are still no studies to test VDR expression in myocardial cells and to investigate the results of dietary VitD supplementation on the expression of VDR in the epicardial adipose tissue and myocardium. **Keywords**: OBESITY, HEART, INFLAMMATION, VITAMIN D RECEPTOR.

VITAMIN D – MORE THAN A CALCIUM REGULATOR

It has been established in time the vital role of vitamin D (VitD) in the regulation of calcium and phosphate homeostasis, a key nutrient for maintaining the health of the musculoskeletal system (1). VitD deficiency is a public health problem. Humans obtain vitD either from exposure to sunlight or dietary intake, both whole food and supplementation (2).

The circulating form of Vit D is 25-hydroxycholecalciferol (25OHD) and the most active form is 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D) or calcitriol. The enzymes responsible for the activation to 1α,25(OH)2D are vitamin D-25-hydroxylase (25-OHase) and 25-

hydroxyvitamin D-1α-hydroxylase (1α-OHase or CYP27B1). Calcitriol is further degraded by 24-hydroxylase CYP24A1.

The worldwide prevalence of obesity has nearly doubled between 1980 and 2008. According to World Health Organization, in 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 kg/m2), compared with 5% for men and 8% for women in 1980 , and the prevalence is rapidly increasing. As a group, obese individuals have decreased levels of circulating 25(OH)D, and, according to a recently published meta-analyses, are at a 35 % higher risk for vitD deficiency across all age ranges (1*).*While actually recommended daily dietary supplementation of vitD are at least 600 IU for healthy adults aged 19–70 (2), and 800 IU/d for those over 70 years old, these doses reflect adequate intake for maximal bone health and muscle function, but for obese populations may be necessary at least two to three times more vitD (1,3*).*

Recent experimental and human studies demonstrated an inverse correlation between adiposity and the level of VitD. VitD deficiency has been showed to be closely associated with obesity, which is a chronic, low-grade inflammatory state, and contribute to the pathogenesis of insulin resistance, metabolic syndrome, and type 2 diabetes mellitus (1*).*

VitD, a fat-soluble hormone, is able to regulate the transcription of many genes through vitD receptor (VDR) (4). The VDR functions in cells such as osteoblasts, skin keratinocytes, macrophages, and epithelial cells and modulates the action of 1,25-$(OH)_2D$, and in turn controls transcription of several genes (1*).* Adipose tissue is recognized as a target for VitD actions. In particular, adipocytes may be directly involved in the local synthesis and degradation of calcitriol, which is able to affect adipocyte biology by modulating preadipocyte differentiation, lipid accumulation and mobilization and also adipokine production (4). There are contradictory data in the literature regarding the expression of VDR in subcutaneous or visceral adipose tissues.

Muscle fat infiltration can be the result of aberrant trans differentiation of myogenic precursor cells into adipocytes, resulting in the formation of fat within the intermuscular space. Recent studies demonstrated that myogenic precursor cells have the potential to transdifferentiate towards the adipogenic lineage (5) and VitD has potent effects on both adipogenesis and myogenesis (6). Muscle fat infiltration has been shown to have a direct consequence on muscle strength and functionality, but it is also a key independent risk factor for metabolic diseases, such as insulin resistance, obesity and diabetes mellitus (3).

In rats, adipose tissue has been shown to sequester more vitamin D for significantly longer periods of time than other major tissues or organs. In humans, central adiposity in obese states is a key marker of metabolic syndrome, which, along with type 2 diabetes mellitus, has been identified to be associated with vitD deficiency. VitD deficiency may also be associated with lipid synthesis via action of the VDR. VitD and VDR also regulate de novo adipogenesis since VDR has been shown to be expressed in preadipocytes (1*).*

Recent studies reported that calcitriol induces genomic effects, leading to the synthesis of new proteins that affect muscle cell contractility, proliferation and differen-

tiation (7). Furthermore, mice lacking the vitamin D receptor showed a skeletal muscle phenotype with smaller and variable muscle fibers and persistence of immature muscle gene expression during adult life, suggesting a role of VitD in muscle development (8).

VitD deficiency can lead to myopathy, characterized by muscle hypotonia, weakness and atrophy of skeletal muscle. Muscle biopsies from VitD-deficient adults demonstrated enlarged interfibrillar spaces, fibrosis and loss of type II fiber complement (7). There was also found an increase in fat infiltration within the muscles (9), similar effects being observed in elderly individuals, where progressive loss in muscle mass and strength are seen at the onset of sarcopenia, associated with an increase in fat deposition within the tissue. Sarcopenia consists of a deterioration in muscles quantity and quality, a gradual slowing of movement, a decline in strength and power, and an increased risk of falls and fall-related injuries (10).

VITAMIN D, CARDIOVASCULAR SYSTEM AND INFLAMMATION

Cardiovascular diseases (CVD) are the most common causes of morbidity and remain the most important cause of death. Recent observational and prospective studies have shown an association between VitD deficiency and hypertension, diabetes mellitus, metabolic syndrome, coronary and peripheral arterial disease (PAD), and heart failure. Vit D has been shown to exert many biological activities, including reduction in blood pressure through down regulation of renine-angiotensin system (RAS), enhancement in insulin secretion and insulin sensitivity (11), protection against angiogenesis and regulation of cellular differentiation and proliferation through locally-formed calcitriol in tissues (12).

VitD deficiency has been also shown to impair endothelial function, which may be a supplementary contributing factor to increased CVD risk (1*)*. Recent studies suggested that serum 25(OH)D levels were inversely associated with coronary lesion severity established by coronary angiography, but not with arterial stiffness or peripheral arterial disease (12).

Epicardial adipose tissue (EAT) (epicardial fat) is a metabolically active visceral adipose tissue, which may locally interact with myocardium and coronary arteries through secretion of various adipokines and cytokines. Recent reports indicated that EAT accumulation may be a risk factor for coronary artery disease (CAD) and alterations in EAT biology, such as increased thickness, elevated inflammatory infiltrate and cytokine production, have been observed in CAD patients (6). The secretion of various epicardial inflammatory adipokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), adiponectin, and monocyte chemoattractant protein-1 (MCP-1), contribute to an inflammatory milieu and play a significant role in the development and progression of atherosclerosis. Subjects with coronary artery disease (CAD) had elevated inflammatory infiltrates in EAT (13).

Evidence from studies on animal models suggest that vitD or vitD receptor (VDR) deficiency promotes cardiac hypertrophy, which might be also one of the mechanisms for increased cardiovascular risk (13). Thus, a recent experimental study revealed that vitD deficiency was associated with cardiomyocyte hypertrophy in

hypercholesterolemic miniswine and significantly decreased expression of VDR was found in ventricular cardiomyocytes of vitD-deficient swine (13). The heart is believed to be one of the target organ of vitD, because VDR is expressed in cardiomyocytes (14).

In addition to its well-known role as a regulator of calcium homeostasis and bone metabolism, vitamin D may exert other different functions. In particular, due to its ability to modulate T-lymphocyte proliferation and function, vitamin D exert immune-regulatory effects and suppress the production of inflammatory cytokines, thus being recognized as a potential anti-inflammatory molecule. Previous in vitro studies showed that vitD is able to down-regulate the expression of pro-inflammatory mediators in human monocytes stimulated with interferon and to inhibit the production of MCP-1 and other pro-inflammatory mediators in human preadipocytes and mature adipocytes (6).

In human adipose tissue, there are few studies which investigated the potential anti-inflammatory role of vitamin D. One study demonstrated that, as 25OHD level decreases, local EAT expression of both VDR and proinflammatory cytokines increases (13). In condition of plasma 25OHD deficiency, local vitamin D metabolism seemed to be suppressed and associated to an increased production of inflammatory mediators. It was also observed an increased expression of VDR associated to reduced plasma 25OHD level, as well as to increased EAT inflammation (15).

In conclusion, besides the vital role of vitamin D in maintaining the health of the musculoskeletal system, studies showed that VitD deficiency has been closely associated with obesity, induced an increase in fat infiltration within the muscles and cardiomyocyte hypertrophy, and significantly increased proinflammatory cytokines. It is not yet known which are the correlations between the plasmatic level of VitD and expression of VDR in subcutaneous adipose tissue, visceral epicardial adipose tissue and especially in myocardium in obesity. There are no studies which tested the expression of VDR in the myocardial cells and to investigate and correlate the results of diet supplementation with VitD on VDR expression in the visceral epicardial adipose tissue and myocardium.

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THERMOPLASTIC MATERIALS APPLICATIONS IN RADIATION THERAPY

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THERMOPLASTIC MATERIALS APPLICATIONS IN RADIATION THERAPY (Abstract) This is an example of the use of thermoplastic materials in a high-tech medicine field, oncology radiation therapy, in order to produce the rigid masks for positioning and immobilization of the patient during simulation of the treatment procedure, the imaging verification of position and administration of the indicated radiation dose. Implementation of modern techniques of radiation therapy is possible only if provided with performant equipment (CT simulators, linear accelerators of high energy particles provided with multilamellar collimators and imaging verification systems) and accessories that increase the precision of the treatment (special supports for head-neck, thorax, pelvis, head-neck and thorax immobilization masks, compensating materials like bolus type material). The paper illustrates the main steps in modern radiation therapy service and argues the role of thermoplastics in reducing daily patient positioning errors during treatment. As part of quality assurance of irradiation procedure, using a rigid mask is mandatory when applying 3D conformal radiation therapy techniques, radiation therapy with intensity modulated radiation or rotational techninques.Key words: IMMOBILIZATION, THERMOPLASTIC MASKS, RADIOTHERAPHY.

This article presents one of the uses of thermoplastic materials in high-energy radiation treatment of malignant neoplasms, produced by linear particles accelerators (radiation).

Modern techniques of external radiation therapy allow high doses of X-rays or electrons in target tumor volumes defined after adapted localization and tumor extension criteria, reducing radiation doses to organs at risk in the immediate vicinity of the tumor.

These complex treatments involve accessories to immobilize the patient, high performance imaging methods: computer tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) for the precise localization of the tumor volume, algorithms that also include tissue inhomogeneities, high performance accelerators with multilamellar collimators, 3D conformal treatment, treatment with intensity modulated radiation therapy (IMRT), rotational therapy and imaging system for checking the treatment position of the patient [1].

MATERIAL AND METHOD:

Steps to follow in radiation therapy

1. CT Simulation. Carrying out an ac-

curate CT simulation by proper preparing and positioning the patient and through use of means of immobilization that allow further daily reproducibility of the position during the 5-7 weeks of treatment, is one of the most important steps for a proper treatment [2].

Depending on the anatomical region where the tumor that will be subjected to radiation is, the patient is comfortably positioned on a special support placed on the table of the simulator, afterwards the patient is immobilized in this position with the use of masks obtained by soaking for a few minutes (the time depends on the composition and thickness of the material) at 75^0C thermoplastic plates, of adequate size for the immobilized area. On the external surface of the formed mask are placed radiopaque markers corresponding to the cutaneous projection of the origin point for the CT scanning. After completion of the simulation, on the mask are noted patient identification and accelerator data that will be used for treatment and the mask will be handed to the assistent from the accelerator (fig. 1).

In case that during treatment the patient undergoes significant changes regarding the immobilized area (weight loss, reduction of initially voluminous adenopathy or local edema), leading to a looser mask or, conversely, to a tighter mask, the patient undergoes a process of resimulation and immobilized with a mask molded according the new aspect of the area of interest [3].

For preventing local changes in the pretreatment phase it is recommended that the time from the CT simulation to onset of treatment does not exceed two weeks.

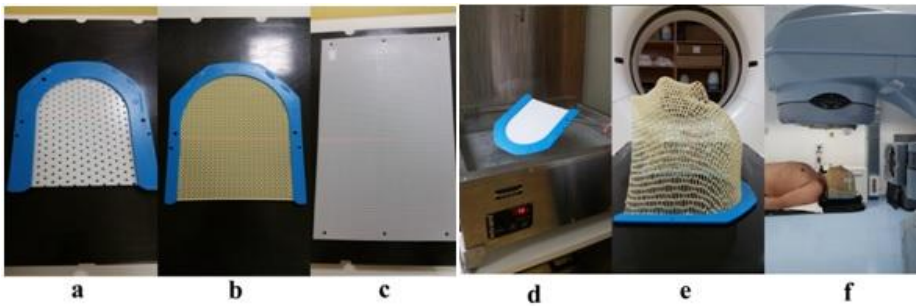

Fig. 1. Examples of the thermoplastic material prior to molding (1a, 1b, 1c), modeling in tank at 75^0C (1d) and after molding (1e, 1f).

2. The treatment plan. The developement of the treatment plan is based on shaping the target volumes and organs at risk on CT images obtained from simulation, followed by the prescription of the dose to the target volume and dose constraints for organs at risk.

The following volumes are identified: tumor macroscopic volume - GTV (gross tumor volume), the volume that estimates the microscopic extensions, the area of tumoral risk - CTV (clinical target volume) and target volume planned, which takes into account movement and positioning errors - PTV (planning target volume). The edges that are added to PTV depend on the irradiated zone, availability and quality of immobilizers used, the frequency of imaging verifications of the treatment position (daily, weekly), the average errors recorded in each radiation laboratory.

Completion of the treatment plan (fig.

2) involves the use of algorithms that simulate the effect of passing through of the beams of radiation through the target volume and generates the characteristic izodoses as well as dose-volume histograms [4].

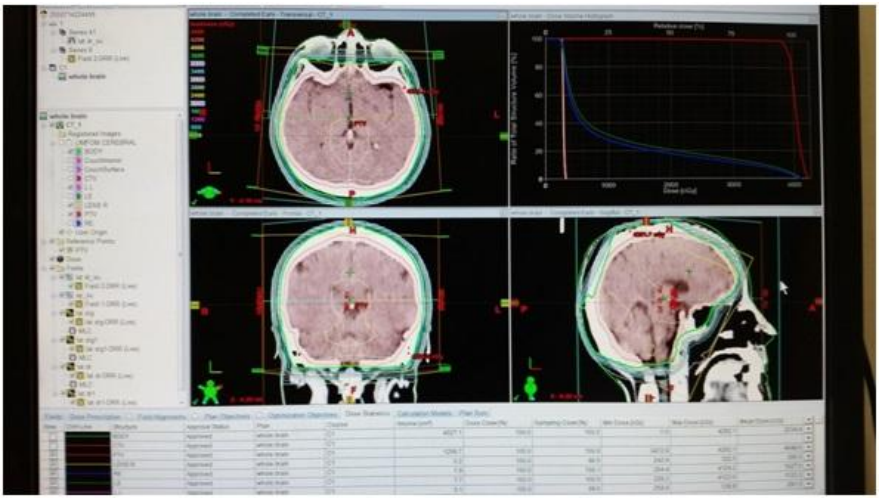

Fig. 2. The treatment plan for a patient with primitive brain lymphoma

3. Controls and implementation of treatment. The administration of high total radiation doses (50-70Gy) in tumor volume is done fractionated, with daily doses of 1,8-2Gy during 5-7 weeks, which involves repeating the verification procedures daily.

Positioning errors are verified through imaging by overlapping anatomic landmarks contoured in the images from the simulation with landmarks from the control images on the accelerator table (fig. 3) and are corrected before each treatment session [5].

Fig. 3. Imaging treatment position control, achieved at the accelerator

DISCUSSIONS

The use of thermoplastic masks for patients treated in the Radiation Laboratory of Regional Oncology Institute, Iasi has become a common practice, especially for irradiation of primitive or secondary brain tumors and also head and neck tumors. Availability of immobilization systems has reduced positioning errors (average errors 1-2mm corrected prior to administration of radiation dose) and enables advanced radiation techniques (IMRT, RapidArc).

CONCLUSIONS

This is a model of practice for accurate irradiation of tumor target volume, while protecting adjacent critical structures, which demonstrates the importance of patient immobilization for treatment reproducibility and safety, widely recognized and which continuesly raises concerns to optimize immobilizers [6].

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α,β -DIBROMOCHALCONE DERIVATIVES – SYNTHESIS AND ANTIMICROBIAL ACTIVITY

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α,β-DIBROMOCHALCONE DERIVATIVES – SYNTHESIS AND ANTIMICROBIAL ACTIVITY (Abstract): **Aim:** To obtain some chalcones and their dibrominated analogues and to evaluate their antimicrobial potential. **Material and methods:** Eight chalcones were synthesized using the Claisen-Schmidt condensation of acetophenone/4-bromoacetophenone and different benzaldehyde derivatives. These chalcones were further brominated using two different bromination agents: molecular bromine and pyridinium tribromide. The antimicrobial activity was tested using the disk diffusion method. **Results and discussions**: The classical bromination technique was compared to the eco-friendly one using pyridinium tribromide. Pyridinium tribromide bromination did not improve the reaction yields (except for one compound), but it had the advantage of being a stable, non-corrosive and non-toxic salt. The results of the antimicrobial assessment indicated that the bromination of the double bond slightly increased the antimicrobial potential in some cases, but the results obtained during the antimicrobial evaluation were modest, some of the derivatives being active especially on *Sarcina lutea* ATCC 9341 and *Bacillus cereus* ATCC 14579. **Conclusions:** In this study, eight chalcones and their dibrominated analogues were synthesized, four of the α,β-dibromochalcones being reported for the first time. Pyridinium tribromide was used as an alternative for liquid bromine, the main advantage of this method being related to the reduced toxicity of the reagents. The synthesized compounds did not exhibit a very good antimicrobial potential. **Keywords:** α,β-DIBROMOCHALCONE, BROMINATION, ANTIMICROBIAL.

Chalcones are an important class of compounds with a wide range of biological activities: antimicrobial, antiviral, antimalarial, antitubercular, anti-protozoal, cytotoxic, anti-inflammatory, immunosuppressive, antioxidant and analgesic (1, 2, 3). These substances, with relatively simple structures, which are generally synthesized by base catalyzed Claisen-Schmidt condensation of an aldehyde and the appropriate ketone in a polar solvent (ethanol), have a great synthetic potential, chalcones and

α,β-dibromochalcones being potential intermediates for the synthesis of various heterocyclic compounds (flavones, benzofurans, quinolones, pyrazoles, isoxazoles) (4, 5, 6). Different bromination agents can be used to obtain dibromochalcones: molecular bromine in different organic solvents, tetrabutylammonium tribromide, phenyltrimethylammonium bromine, boric acid with potassium bromide (7, 8). Due to the environmental issues caused by using molecular bromine and aggressive solvents in conventional bromination, there is a constant concern to develop new eco-friendly bromination techniques (8, 9, 10). One of these techniques involves the use of stable crystalline quaternary ammonium tribromides. Pyridinium tribromide ($PyrBr_3$) is an example of a stable, non-corrosive and non-toxic salt, easy to use for bromination under mild conditions. In solution, $PyrBr_3$ is in equilibrium with pyridinium bromide and Br_2, both acting as bromination agents (11).

The purpose of this study was to investigate the bromination of some chalcones using $PyrBr_3$, in comparison to standard bromination conditions.

MATERIAL AND METHODS

All the reagents and solvents employed were of the best quality available and were used without further purification. Melting points were determined using an electrothermal apparatus and were uncorrected. Elemental analyses were performed on an Elemental Exeter Analytical CE 440 Analyzer. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DRX 400 MHz Spectrometer (Bruker, Rheinstetten, Germany) equipped with a 5mm QNP (quadruple nucleus probe) direct detection probe and z-gradients. Spectra were recorded in deuterated chloroform ($CDCl_3$) at room temperature. The chemical shifts are reported as δ values (ppm) relative to the solvent residual peak (7.26 ppm for ^{1}H-NMR and 77.01 ppm for ^{13}C-NMR spectra). The atom labelling for the NMR assignments is that from fig. 1. The assignments of all the signals in the 1D NMR spectra were done using 2D NMR experiments such as H,H-COSY, H,C-HMQC and H,C-HMBC.

Chalcones synthesis – general procedure: Equimolecular quantities of acetophenone/4-bromo-acetophenone and the appropriate aromatic aldehyde were dissolved in ethanol 96° and cooled to 5-10°C in an ice bath. A solution of sodium hydroxide 15M was added dropwise and the mixture was stirred at room temperature for 90 minutes. The final products were purified by recrystallization from ethanol:water (1:1).

α,β-dibromochalcones – general procedures: Standard bromination with molecular bromine: A solution of bromine (5 mmoles) in chloroform was added dropwise while stirring over 5 mmoles chalcone previously dissolved/suspended in chloroform. The reaction was performed on an ice-bath. The bromination products were separated by filtration.

Bromination with pyridinium tribromide: 3 mmoles chalcone were dissolved in 6 ml acetic acid and 3.6 mmoles pyridinium tribromide were added. The mixture was heated under reflux for 30 minutes and, after cooling, the precipitate was filtrated.

The synthesis of the compounds is described in fig. 1:

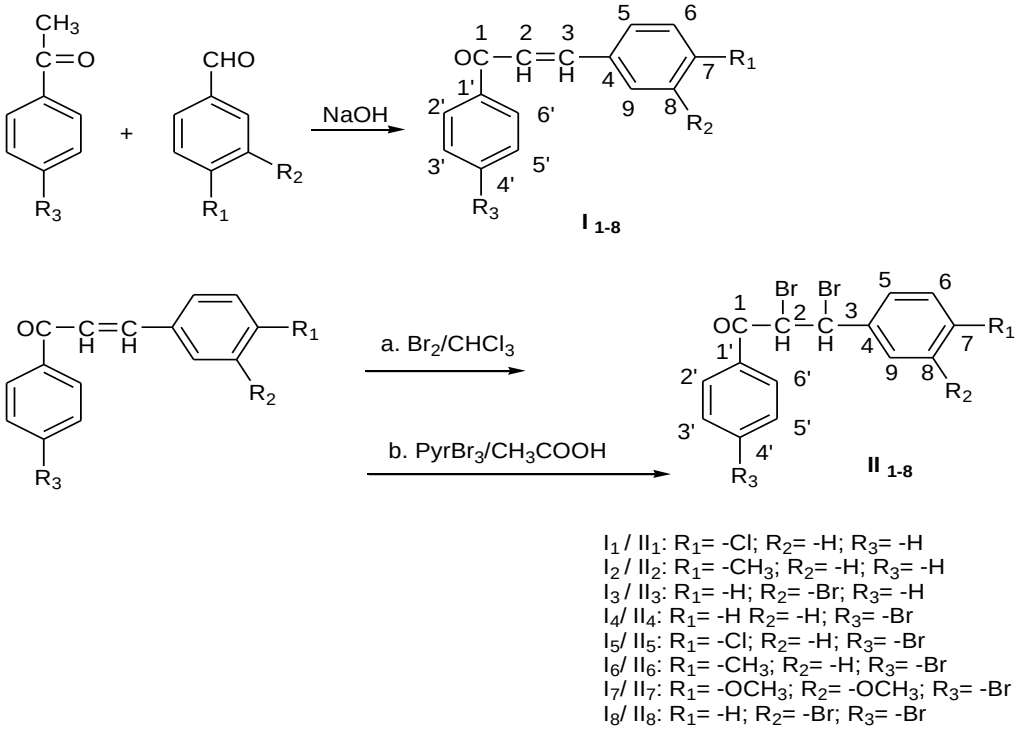

Fig. 1. Synthesis of chalcones and α,β-dibromochalcones

3-(4'-clorofenil)-1-fenil-2-propen-1-one (I_1) yellow crystals, m.p.= 113-115 ºC. Anal. calc. for $C_{15}H_{11}OCl$ (242.5): C 74.22, H 4.53, O 6.59; found: C 74.16, H 4.51, O 6.51. IR (KBr, cm^{-1}): 3055 (C-H arom.), 1670 (C=O), 1620 (C=C). ^{1}H-RMN ($CDCl_3$, δ ppm): 8.01 (d, J=8.8Hz, 2H, $H_{2'}$, $H_{6'}$), 7.75 (d, J=16Hz, 1H, H_3), 7.61-7.48 (m, H_2, H_5, H_9, $H_{3'}$, $H_{4'}$, $H_{5'}$ overlapped peaks), 7.39 (d, J=8.8Hz, 2H, H_6, H_8). ^{13}C-RMN ($CDCl_3$, ppm): 190.22 (C_1), 143.29 (C_3), 138.04 ($C_{1'}$), 136.43 (C_7), 133.39 (C_4), 132.92 ($C_{4'}$), 129.57 (C_6, C_8), 129.24 (C_5, C_9), 128.66 ($C_{3'}$, $C_{5'}$), 128.49 ($C_{2'}$, $C_{6'}$).

3-(4'-metilfenil)-1-fenil-2-propen-1-one (I_2) yellow crystals, m.p.= 93-94 ºC. Anal. calc. for $C_{16}H_{14}O$ (222): C 86.48, H 6.30, O 7.20; found: C 86.43, H 6.29, O 7.18. IR (KBr, cm^{-1}): 3026 (C-H arom.), 1658 (C=O), 1532 (C=C).

3-(3'-bromofenil)-1-fenil-2-propen-1-one (I_3) yellow crystals, m.p.= 81-83 ºC. Anal. calc. for $C_{15}H_{11}OBr$ (287): C 62.71, H 3.83, O 5.57; found: C 62.69, H 3.80, O 5.52. IR (KBr, cm^{-1}): 2923 (C-H arom.), 1654 (C=O), 1581 (C=C).

3-fenil-1-(4'-bromofenil)-2-propen-1-one (I_4) yellow crystals, m.p.= 95-96 ºC. Anal. calc. for $C_{15}H_{11}OBr$ (287): C 62.71, H 3.83, O 5.57; found: C 62.67, H 3.81, O 5.55. IR (KBr, cm^{-1}): 3059 (C-H arom.), 1657 (C=O), 1603 (C=C).

3-(4'-clorofenil)-1-(4'-bromofenil)-2-propen-1-one (I_5) yellow crystals, m.p.= 166-168 ºC. Anal. calc. for $C_{15}H_{10}OClBr$ (321.5): C 55.98, H 3.11, O 4.97; found: C

55.95, H 3.07, O 4.96. IR (KBr, cm^{-1}): 3084 (C-H arom.), 1656 (C=O), 1602 (C=C). ^{1}H-RMN ($CDCl_3$, δ ppm): 7.87 (d, J=8.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.75 (d, J= 15.6Hz, 1H, H_3), 7.64 (d, J=8.4Hz, 2H, $H_{3'}$, $H_{6'}$), 7.56 (d, J=8.4Hz, 2H, H_5, H_9), 7.44 (d, J=15.6Hz, 1H, H_2), 7.39 (d, J=8.8Hz, 2H, H_6, H_8). ^{13}C-RMN ($CDCl_3$, ppm): 189.01 (C_1), 143.83 (C_3), 136.72 ($C_{1'}$), 136.66 (C_7), 133.16 (C_4), 131.97 ($C_{3'}$, $C_{5'}$), 129.99 ($C_{2'}$, $C_{6'}$), 129.63 (C_5, C_9), 129.29 (C_6, C_8), 128.06 ($C_{4'}$), 121.83 (C_2).

3-(4'-metilfenil)-1-(4'-bromofenil)-2-propen-1-one (I_6) yellow crystals, m.p. = 164-166 ºC. Anal. calc. for $C_{16}H_{13}OBr$ (301): C 63.78, H 4.31, O 5.31; found: C 63.74, H 4.28, O 5.30. IR (KBr, cm^{-1}): 3022 (C-H arom.), 1657 (C=O), 1598 (C=C). ^{1}H-RMN ($CDCl_3$, δ ppm): 7.88 (d, J=8.4Hz, 2H, $H_{2'}$, $H_{6'}$), 7.79 (d, J=16Hz, 1H, H_3), 7.63 (d, J=8.4Hz, 2H, $H_{3'}$, $H_{5'}$), 7.54 (d, J=8.0Hz, 2H, H_5, H_9), 7.43 (d, J=15.6Hz, 1H, H_2), 7.23 (d, J=8.0Hz, 2H, H_6, H_8), 2.39 (s, 3H, CH_3 attached to C_7). ^{13}C-RMN ($CDCl_3$, ppm): 189.45 (C_1), 145.51 (C_3), 141.36 (C_7), 137.08 ($C_{1'}$), 131.96 (C_4), 131.88 ($C_{3'}$, $C_{5'}$), 129.99 ($C_{2'}$, $C_{6'}$), 129.75 (C_6, C_8), 128.55 (C_5, C_9), 127.73 ($C_{4'}$), 120.48 (C_2), 21.55 (CH_3 attached to C_7).

3-(3',4'-dimetoxifenil)-1-(4'-bromofenil)-2-propen-1-one (I_7) yellow crystals, m.p.= 118-119 ºC. Anal. calc. for $C_{17}H_{15}O_3Br$ (347): C 58.78, H 4.32, O 13.83; found: C 58.70, H 4.28, O 13.80. IR (KBr, cm^{-1}): 3075 (C-H arom.), 1659 (C=O), 1594 (C=C). ^{1}H-RMN ($CDCl_3$, δ ppm): 7.88 (d, J=8.4Hz, 2H, $H_{2'}$, $H_{6'}$), 7.76 (d, J=15.6Hz, 1H, H_3), 7.64 (d, J=8.4Hz, 2H, $H_{3'}$, $H_{5'}$), 7.33 (d, J=15.6Hz, 1H, H_2), 7.23 (d, J=8.4Hz, 1H, H_5), 7.15 (s, 1H, H_9), 6.90 (d, J=8.4Hz, 1H, H_6), 3.95 (s, 3H, CH_3 attached to C8), 3.94 (s, 3H, CH_3 attached to C7). ^{13}C-RMN ($CDCl_3$, ppm): 189.41 (C_1), 151.63 (C_7), 149.28 (C_8), 145.58 (C_3), 137.18 ($C_{1'}$), 131.83 ($C_{3'}$, $C_{5'}$), 129.95 ($C_{2'}$, $C_{6'}$), 127.65, 127.59 (C_4, $C_{4'}$), 123.32 (C_5), 119.41 (C_2), 111.14 (C_6), 110.14 (C_9), 56.00, 55.97 (CH_3 attached to C_7 and CH_3 attached to C_6).

3-(3'-bromofenil)-1-(4'-bromofenil)-2-propen-1-one (I_8) yellow crystals, m.p.= 204-206 ºC. Anal. calc. for $C_{15}H_{10}OBr_2$ (366): C 49.18, H 2.73, O 4.37; found: C 49.17, H 2.70, O 4.31. IR (KBr, cm^{-1}): 2924 (C-H arom.), 1655 (C=O), 1582 (C=C).

2,3-dibromo-3-(4-clorofenil)-1-fenilpropan-1-one (II_1) white crystals, m.p.= 180-181ºC. Anal. calc. for $C_{15}H_{11}OClBr_2$ (402.5): C 44.76, H 2.75, O 3.97; found: C 44.72, H 2.71, O 3.96. IR (KBr, cm^{-1}): 3012 (C-H arom.), 1684 (C=O), 575 (C-Br). ^{1}H-NMR ($CDCl_3$, 400.13 MHz, δ, ppm): 8.09 (d; J=7.2 Hz; 2H; $H_{2'}$, $H_{6'}$), 7.67 (t, J=7.4 Hz; 1H; $H_{4'}$), 7.55 (t, J=7.6 Hz; 2H; $H_{3'}$, $H_{5'}$), 7.47 (d, J=8.4 Hz; 2H; H_5, H_9), 7.40 (d, J=8.4 Hz; 2H; H_6, H_8), 5.77 (d, J=11.2 Hz; 1H; H_2), 5.61 (d, J=11.6 Hz; 1H; H_3). ^{13}C-NMR ($CDCl_3$, 100.16 MHz, δ, ppm): 190.92 (C_1), 136.82 (C_4), 135.13 (C_7), 134.28 ($C_{1'}$, $C_{4'}$), 129.71 (C_5, C_9), 129.13 (C_6, C_8), 129.05 ($C_{3'}$, $C_{5'}$), 128.90 ($C_{2'}$, $C_{6'}$), 48.68 (C_3), 46.62 (C_2).

2,3-dibromo-1-fenil-3-p-tolilpropan-1-one (II_2) white crystals, m.p. = 166-167 ºC. Anal. calc. for $C_{16}H_{14}OBr_2$ (382): C 50.29, H 3.69, O 4.19; found: C 50.27, H 3.61, O 4.15. IR (KBr, cm^{-1}): 3055 (C-H arom.), 1683 (C=O), 578 (C-Br). ^{1}H-NMR ($CDCl_3$, 400.13 MHz, δ, ppm): 8.11 (d; J=7.6 Hz; 2H; $H_{2'}$, $H_{6'}$), 7.67 (t, J=7.2 Hz; 1H; $H_{4'}$), 7.55 (t, J=7.4 Hz; 2H; $H_{3'}$, $H_{5'}$), 7.42 (d, J=7.2 Hz; 2H; H_5, H_9), 7.26-7.23 (m, H_6, H_8, overlapped with solvent residual peak), 5.84 (d, J=11.2 Hz; 1H; H_2), 5.64 (d, J=11.2 Hz; 1H; H_3), 2.39 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, 100.16 MHz, δ, ppm): 191.27 (C_1),

139.41 (C_7), 135.30 (C_4), 134.51 ($C_{1'}$), 134.14 ($C_{4'}$), 129.60 (C_6, C_8), 129.00 ($C_{3'}$, $C_{5'}$), 128.91 ($C_{2'}$, $C_{6'}$), 128.21 (C_5, C_9), 49.97 (C_3), 46.92 (C_2), 21.33 (-CH_3)

2,3-dibromo-3-(3-bromofenil)-1-fenilpropan-1-one (II_3) white crystals, m.p = 154-155 ºC. Anal. calc. for $C_{15}H_{11}OBr_3$ (447): C 40.31, H 2.48, O 3.58; found: C 40.27, H 2.42, O 3.53. IR (KBr, cm^{-1}): 3027 (C-H arom.), 1687 (C=O), 576 (C-Br). ^{1}H-NMR ($CDCl_3$, 400.13 MHz, δ, ppm): 8.14 (d; J=7.6 Hz; 2H; $H_{2'}$, $H_{6'}$), 7.73-7.70 (m, 2H; $H_{4'}$, H_9), 7.60 (t, J=7.8 Hz; 2H; $H_{3'}$, $H_{5'}$), 7.56 (d, J=8.0 Hz; 1H; H_7), 7.49 (d, J=7.6 Hz, H_5), 7.43 (t, J=7.8 Hz, 1H, H_6), 5.80 (d, J=11.2 Hz; 1H; H_2), 5.62 (d, J=11.2 Hz; 1H; H_3). ^{13}C-NMR ($CDCl_3$, 100.16 MHz, δ, ppm): 190.81 (C_1), 140.46 (C_4), 134.30-134.25 ($C_{1'}$, $C_{4'}$), 132.39 (C_7), 131.35 (C_9), 130.33 (C_6), 129.05 ($C_{3'}$, $C_{5'}$), 128.92 ($C_{2'}$, $C_{6'}$), 127.14 (C_5), 122.75 (C_8), 48.34 (C_3), 46.48 (C_2).

2,3-dibromo-1-(4-bromofenil)-3-fenilpropan-1-one (II_4) white crystals, m.p. = 188-189 ºC. Anal. calc. for $C_{15}H_{11}OBr_3$ (447): C 40.31, H 2.48, O 3.58; found: C 40.25, H 2.44, O 3.53. IR (KBr, cm^{-1}): 3030 (C-H arom.), 1687 (C=O), 582 (C-Br).

2,3-dibromo-1-(4-bromofenil)-3-(4-clorofenil) propan-1-one (II_5) white crystals, m.p. = 150-152 ºC. Anal. calc. for $C_{15}H_{10}OClBr_3$ (481.5): C 37.42, H 2.09, O 3.32; found: C 37.39, H 2.05, O 3.30. IR (KBr, cm^{-1}): 3032 (C-H arom.), 1689 (C=O), 569 (C-Br).

2,3-dibromo-1-(4-bromofenil)-3-p-tolilpropan-1-one (II_6) white crystals, m.p.= 143-145 ºC Anal. calc. for $C_{16}H_{13}OBr_3$ (461): C 41.69, H 2.84, O 3.47; found: C 41.67, H 2.79, O 3.43. IR (KBr, cm^{-1}): 3028 (C-H arom.), 1685 (C=O), 570 (C-Br). ^{1}H-RMN ($CDCl_3$, δ ppm): 7.96 (d, J=8.8Hz, 2H, $H_{2'}$, $H_{6'}$), 7.69 (d, J=8.8Hz, 2H, $H_{3'}$, $H_{5'}$), 7.40 (d, J=8.0Hz, 2H, H_5, H_9), 7.23 (d, J=8.0Hz, 2H, H_6, H_8), 5.76 (d, J=11.2Hz, 1H, H_2), 5.61 (d, J=11.2Hz, 1H, H_3), 2.39 (s, 3H, CH_3 attached to C7). ^{13}C-RMN ($CDCl_3$, ppm): 190.33 (C_1), 139.50 (C_7), 135.06 (C_4), 133.20 ($C_{1'}$), 132.37 ($C_{3'}$, $C_{5'}$), 130.32 ($C_{2'}$, $C_{6'}$), 129.62 (C_6, C_8), 129.56 ($C_{4'}$), 128.18 (C_5, C_9), 49.83 (C_3), 46.80 (C_2), 21.33 (CH_3 attached to C_7).

2,3-dibromo-1-(4-bromofenil)-3-(3,4-dimetoxifenil) propan-1-one (II_7) white crystals, m.p.= 91-93 ºC. Anal. calc. for $C_{17}H_{15}O_3Br_3$ (507): C 40.27, H 2.98, O 9.47; found: C 40.25, H 2.94, O 9.43. IR (KBr, cm^{-1}): 3057 (C-H arom.), 1687 (C=O), 576 (C-Br).

2,3-dibromo-3-(3-bromofenil)-1-(4-bromofenil) propan-1-one (II_8) white crystals, m.p.= 150-152 ºC. Anal. calc. for $C_{15}H_{10}OBr_4$ (526): C 34.26, H 1.92, O 3.04; found: C 34.24, H 1.91, O 3.06. IR (KBr, cm^{-1}): 3029 (C-H arom.), 1683 (C=O), 575 (C-Br). ^{1}H-NMR ($CDCl_3$, 400.13 MHz, δ, ppm): 7.95 (d, J=8.8 Hz; 2H; $H_{2'}$, $H_{6'}$), 7.70 (d, J=8.4 Hz; 2H; $H_{3'}$,$H_{5'}$) 7.67 (s, 1H; H_9), 7.52 (d, J=8 Hz; 1H; H_7), 7.43 (d, J=7.6 Hz; 1H; H_5), 7.30 (t, J=7.8 Hz; 1H; H_6), 5.68 (d, J=11.2 Hz; 1H; H_2), 5.55 (d, J=11.6 Hz; 1H; H_3). ^{13}C-NMR ($CDCl_3$, 100.16 MHz, δ, ppm): 189.90 (C_1), 140.22 (C_4), 132.92 ($C_{1'}$), 132.48 (C_7), 132.43 ($C_{3'}$, $C_{5'}$), 131.32 (C_9), 130.36 (C_6), 130.34 ($C_{2'}$, $C_{6'}$), 129.77 ($C_{4'}$), 127.13 (C_5), 122.79 (C_8), 48.19 (C_3), 46.35 (C_2).

The antimicrobial activity of the compounds was evaluated using the disk diffusion method (12). Agar diffusion tests are often used as qualitative methods to determine whether bacteria are resistant, intermediately resistant or susceptible to different antimicrobial agents. Mueller Hinton (Oxoid) medium for bacteria and Sabouraud agar for fungal were used. The test microorganisms were *Staphylococcus aureus* ATCC

25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. The bacterial and fungal strains were incubated over night at 30 ^{0}C and suspensions were prepared from each microbial culture with the same density as the McFarland 0.5 turbidity standard. The suspensions of microorganisms were incorporated in the growth medium (melted and cooled afterwards at 50 ^{0}C) in a 1/10 ratio. After homogenization, 25 mL of this mixture were placed in Petri dishes (with a diameter of 9 cm). On the surface of each plate, after solidification, absorbent disks impregnated with 10 µL from the DMSO solutions (10 mg/mL) of the tested compounds were placed (the quantity of active substance/ disk = 100 µg). Positive control disks of Ampicillin 25 µg, Chloramphenicol 30 µg (for bacteria) and Nystatin 100 µg (for *Candida albicans*) were also used. After incubation (24 hrs at 37°C for bacteria and 48 hrs at 24°C for *Candida albicans*), the plates were observed (13).

RESULTS AND DISCUSSION

Eight chalcones and their dibrominated analogues were synthesized. Of the eight α,β-dibromochalcones, four had been previously reported – II_1, II_2, II_4 and II_7 (7, 8, 14). Due to the inconveniences caused by using liquid bromine in standard bromination, $PyrBr_3$ was used as an alternative. The main advantages of using $PyrBr_3$ were the reduced toxicity of the reagents and the fact that the brominating agent was gradually generated "in situ". $PyrBr_3$ is a solid substance and was easily manipulated in comparison with bromine – a corrosive liquid, capable of producing serious skin burns and of affecting the respiratory system. Standard bromination usually involves the use of chlorinated solvents (15), very aggressive for the environment and also suspected to have a carcinogen effect; $PyrBr_3$ bromination was performed in acetic acid, which was less toxic.

A comparison was made between the yields obtained by the two methods (fig. 2). Yields were not optimized. $PyrBr_3$ bromination did not improve the reaction yields (except for compound II_3), so the advantages of using $PyrBr_3$ were related to the safety of the executant and environment.

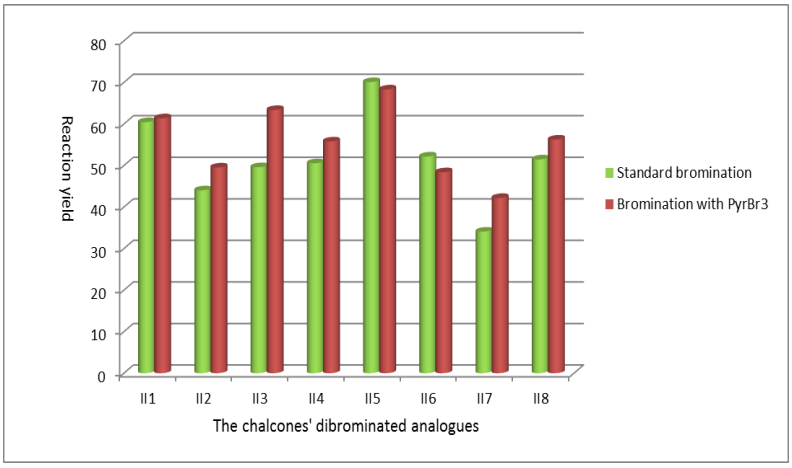

Fig. 2. Comparison of standard bromination and $PyrBr_3$ bromination yields

Antimicrobial screening: The presence of antibacterial or antifungal activity was indicated by the presence of an inhibition zone surrounding the absorbent disk impregnated with the tested compound. The zone of inhibition was measured and expressed in millimeters (tab. I).

The antimicrobial activity of the investigated substances was expressed in terms of the diameter of the inhibition zones: <9 mm – inactive; 9-12 mm - partially active; 13-18 mm - active and >18 mm - very active (16, 17).

Despite a great number of previous studies indicated a good antimicrobial activity for similar compounds (1, 2, 18), all the investigated chalcones (I_{1-8}) were inactive against all the tested bacterial and fungal strains (tab. I). Similar results have also been reported by other research teams (19). The bromination of the double bond moderately increased the antimicrobial potential in some cases (II_1, II_4, II_5, II_7), although it was previously observed that the antibacterial activity was due to the α,β-unsaturated carbonyl group, the hydrogenation of the double bond destroying the activity partially or completely (4). The α,β-unsaturated carbonyl group was considered a pharmacopeic group and the bactericidal effect of chalcones was related to the ability of the α,β-unsaturated ketone to undergo a conjugated addition to a nucleophilic group (ex. thiol) in an essential protein (1).

TABLE I

Antimicrobial activity - The diameter (mm) of inhibition zones for the tested microorganisms

Compound	*S. aureus* ATCC 25923	*Sarcina lutea* ATCC 9341	*B.cereus* ATCC 14579	*B. subtilis*	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
I_1	0	0	0	0	0	0
I_2	0	0	0	0	0	0
I_3	0	0	0	0	0	0
I_4	0	0	0	0	0	0
I_5	0	0	0	0	0	0
I_6	0	0	0	0	0	0
I_7	0	0	0	0	0	0
I_8	0	0	0	0	0	0
II_1	12	25	20	0	11	17
II_2	0	0	0	0	0	0
II_3	0	0	0	0	0	0
II_4	12	21	20	0	12	16
II_5	12	25	18	0	12	12
II_6	0	0	0	0	0	0
II_7	9	20	17	0	12	12
II_8	0	0	0	0	0	0
Ampicillin 25 µg	39	35	16	30	16	-
Chloramphenicol 30µg	35	35	26	35	26	-
Nystatin 100 µg	-	-	-	-	-	26

Sarcina lutea ATCC 9341 and *Bacillus cereus* ATCC 14579 were the most sensitive bacterial strains. Methoxy and chlorine radicals had a favorable influence, these results being in agreement with other studies (1, 20). Due to the modest results of the qualitative evaluation of antimicrobial activity, the quantitative determination (the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations) was not justified.

CONCLUSIONS

In conclusion, we synthesized eight chalcones and their dibrominated analogues, four of the α,β-dibromochalcones being reported for the first time. The compounds were characterized by elemental and spectral analysis. In the context of finding eco-friendly synthesis techniques, we used pyridinium tribromide as an alternative for liquid bromine. The main advantage of this method was the reduced toxicity of the reagents. The synthesized compounds did not exhibit a very good antimicrobial potential, some of the derivatives being active especially on *Sarcina lutea* ATCC 9341 and *Bacillus cereus* ATCC 14579.

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NEWS

ANTI-INFLAMMATORY EFFECT OF *STAPHYLOCOCCUS AUREUS* ON AIRWAY EPITHELIAL CELLS

A study by Chekabab *et al* examined the way bacterial cells modulate inflammatory responses in the setting of polymicrobial infections. Two major respiratory pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* can simultaneously colonize the airways of patients with chronic obstructive diseases (e.g. cystic fibrosis). The mechanism by which airway epithelial cells integrate bacterial stimuli in the case of polymicrobial colonization or infection is unknown. Since epithelial cell signaling is crucial for the activation of innate immune responses, the study examined the inflammatory responses to *P. aeruginosa* and *S. aureus* co-stimulations. Immortalized airway epithelial cells exposed to bacteria-free filtrates from *P. aeruginosa* induced a high synthesis of IL-8, while bacteria-free filtrates from *S. aureus* showed a minimal effect. However, in the case of co-stimulation with bacteria-free filtrates from *P. aeruginosa* and *S. aureus*, *S. aureus* filtrates inhibited IL-8 production induced by *P. aeruginosa*, showing potent anti-inflammatory effects. It also decreased IL-8 production induced by the TLR1/TLR2 ligand Pam3CysSK4 and dampened TLR1/TLR2-mediated activation of the NF-κB pathway. These results demonstrate that *S. aureus* modulates inflammatory responses in the setting of polymicrobial infections (Chekabab SM, Silverman RJ, Lafayette SL, Luo Y, Rousseau S, Nguyen D. *Staphylococcus aureus* Inhibits IL-8 Responses Induced by *Pseudomonas aeruginosa* in Airway Epithelial Cells. *PLoS One*. 2015;10(9):e0137753. doi: 10.1371/journal.pone.0137753. eCollection 2015).

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COLORECTAL CANCER - EPIDEMIOLOGICAL PERSPECTIVES IN THE ONCOGENETIC CONTEXT

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COLORECTAL CANCER - EPIDEMIOLOGICAL PERSPECTIVES IN THE ONCOGENETIC CONTEXT (Abstract): The neoplastic pathology is one of the challenges of modern medicine, both in terms of epidemiology (morbidity, mortality and risk factors), as well as in terms of prevention, diagnosis or treatment. The progress of knowledge regarding risk factors and the identification of new diagnosis or therapy methods in the management of patients with colorectal cancer have led to a series of approach changes which create new perspectives not only for those affected by the disease, but also for their families. Risk factors for CRC include: modifiable factors (endogenous and exogenous factors) and non-modifiable factors (genetic factors). The risk of an individual belonging to the general population to develop CRC at a certain point in life is of 5%, while the risk of a subject with Lynch syndrome may reach 70-80%. The genes which potentially increase the risk of CRC include: MMR, MLH1, MSH2, MSH6 (high-penetrance genes) and APC, BRAF, CTNNB1 / beta-catenin, FBXW7, KRAS, PIK3CA, SRC and P53 (low-penetrance genes). The most used methods of screening for the general population are: the HEMOCCULT test, the colonoscopy and the flexible sigmoidoscopy, while for the groups at risk (with one or more risk factors) these include: the oncogenetic investigation (including the assessment of family history of familial adenomatous polyposis (or FAP), attenuated FAP (AFAP), hereditary non-polyposis CRC (HNPCC), personal history of colorectal cancer or adenoma, chronic ulcerative colitis and Crohn's disease and molecular testing of individuals with risk factors). **Keywords**: COLORECTAL CANCER, EPIDEMIOLOGY, INCIDENCE, SURVIVAL, RISK FACTORS.

Neoplastic pathology is one of the challenges of modern medicine, both in terms of epidemiology (morbidity, mortality and risk factors), and in terms of prevention, diagnosis or treatment. The estimated incidence per year has an upward trend from 10 million new cases in 2000 to 15 million in 2020 (1), cancer mortality being ranked 2nd after cardiovascular diseases.

Colorectal cancer (CRC) is a real public health issue both in Romania and worldwide, which is accounted for by the mor-

bidity and mortality recorded. The evolution of progress regarding the knowledge related to risk factors and the identification of new diagnosis or therapy methods in the management of patients with colorectal cancer have led to a series of approach changes which create new perspectives not only for those affected by the disease, but also for their families (2, 3).

CRC is the third oncological pathology in the world in terms of incidence (with approximately 1-2 million new cases each year); it is the second pathology in terms of prevalence and the fourth cause of death in the world (with 600,000 deaths annually) (4). At a worldwide level, there is a wide variation in incidence, but the geographical pattern is similar for women and men. Colorectal neoplasia is the 3rd most common cancer type in men (10% of all cancers) and the 2nd most common cancer in women (9.2% of the total), 55% of cases being identified in more developed geographical areas. Regarding the global mortality rate, the variability is low, with the highest percentage recorded in Central and Eastern Europe (20.3 deaths / 100,000 men and 11.7 deaths / 100,000 women). Most deaths (52%) were recorded in less developed countries, where the survival rate is lower. In Romania, there is also an upward trend in the CRC incidence in both sexes – the male / female ratio is of 1/3 and is rapidly growing; for every 10 years of life after the age of 45 the numbers double (4).

RISK FACTORS

CRC risk factors include: age (over 40% of cases involve patients aged over 75); gender (more common in men); diet (high in red meat or processed meat and animal fats); obesity and overweight; alcohol consumption; smoking; occupational exposure to carcinogens (asbestos); ionizing radiation; presence of infection history; various medical conditions (adenomatous polyps, inflammatory bowel disease-Crohn's disease, ulcerative hemorrhagic rectocolitis, biliary lithiasis, diabetes, metabolic syndrome); personal history of cancer (colorectal, oesophageal, laryngeal, lung, prostate, endometrial and breast cancer, as well as chronic lymphocytic leukaemia or melanoma); family history and genetic syndromes (familial adenomatous polyposis; hereditary nonpolyposis colorectal cancer; BRCA1 mutations) (5). Risk factors for cancer are multiple, presenting a low positive predictive value, except hereditary risk factors. Genetic predisposition for the development of malignancies due to genetic modifications is 70-80%, which is the only risk factor that reaches the positive predictive value, justifying medical and oncogenetic monitoring.

The risk of an individual belonging to the general population to develop CRC at a certain point in life is 1 out of 20 (5%), while the risk of a subject with Lynch syndrome may reach 70-80% (6).

Hereditary CRCs are classified into two main groups: polyposis forms, which include *Familial Adenomatous Polyposis* (FAP), *attenuated FAP* (a subtype of FAP in which the subject has less than 100 polyps and colorectal cancer that occurs at a young age), *Gardner syndrome* (a subtype of FAP which is accompanied by benign tumors of the skin, soft tissue and bones) and nonpolyposis forms, comprising: *Lynch syndrome* (LS) / *hereditary nonpolyposis CRC* (Hereditary Nonpoly-posis Colon Cancer) (HNPCC), *Turcot Syndrome* or *Peutz-Jeghers Syndrome* (7, 8).

The genes that modify the risk of CRC include: genes with major contribution

(high-penetrance genes): MMR, MLH1, MSH2, MSH6 and genes with variable contribution (low-penetrance genes), for which the most common somatic mutations of CRC described by Roy and Majumdar in 2012 appeared with genes such as APC, BRAF, CTNNB1 / beta-catenin, FBXW7, KRAS, PIK3CA, SRC and P53 (9). Genetic instability (10) in hereditary CRC is due to germinal mutations in the APC gene for cases diagnosed with FAP / attenuated FAP (9) and to DNA repair gene mutations (MMR) for cases of Lynch Syndrome (LS) (11, 12). This genetic instability in the case of FAP has its origins in the germ-line mutations of the APC gene on chromosome 5q21-22, but it may also exist in the MYH gene. Lynch syndrome is caused by mutations in DNA repair genes, namely *MLH1, MSH2, MSH6, PMS2* (13). Among these, *MLH1 and MSH2* genes, which cause a high level of instability, are responsible for the appearance of more than 90% of the cases of LS (14), in contrast to the 10% of cases attributed to mutations in the MSH6 gene, which causes a partial deficiency of MMR functions (13). The presence of hereditary mutations in *MLH1 vs. MSH2* is associated with an increased risk of developing extra-colonic malignancies (15, 16).

SCREENING (EARLY DETECTION)

Screening methods for the early detection of colorectal cancer, evaluated by numerous studies, include tests for the detection of occult blood in the feces, colonoscopy, flexible sigmoidoscopy and double contrast iriography. Recently, some modern and non-invasive methods have been introduced – virtual colonoscopy and genetic testing of feces for neoplastic DNA (17).

Currently, there are different types of faecal occult blood tests: *Faecal Occult Blood Testing (FOBT)*, *Hemoccult SENSA* (a guaiac test, but more sensitive) *Heme-Select* (a test to detect human hemoglobin by immunochemical methods) or *Hemo-Quant* (for quantitative detection of faecal occult blood by means of fluorescent light) (18), but the most used is the *Hemoccult test*, which involves the detection of fecal occult blood by using an indicator substance (guaiac) impregnated on a piece of paper. The test is not specific to CRC, as there are also other non-cancerous lesions that may be tested by using this method. False positive results (about 2-6%) may occur in case of colorectal polyps, diverticulitis or anorectal lesions (hemorrhoids, fissures, rectitis). A big disadvantage of the *Hemoccult test* is represented by the false negative results (40% of cases), which may be due to: the presence of vitamin C in the diet, failure to follow a high-residue diet, use of expired tests, degradation of hemoglobin by bacteria in the colon or a cancerous lesion that is not bleeding when the faeces sample is collected. Other disadvantages include: the need to collect 3-6 faeces samples (due to the intermittent bleeding) and the high specificity only for the lesions of the left colon. In its non-hydrated form, the *Hemoccult test* has a sensitivity of 72-78% and specificity of 98%; after rehydration, the sensitivity of the test increases to 88-92%, but the specificity drops to 90-92% (17, 19). Another test used in the detection of feces occult blood is the *Fecal Immune Test* (FIT), which, although it has proved to be a more reliable screening method as it is based on a "Ag-Ab" reaction, does not replace the common use of the *Hemoccult test* because it is more expensive (20).

A modern approach to the early detection of CRC involves the use of DNA

markers, RNA and protein as diagnosis strategy (*Faecal DNA* and *RNA Test*). These molecular tests are non-invasive and are based on the fact that the neoplastic cells (CRC) have a high mitotic index and a low adhesion to the basal membrane, which facilitates their ***continuous*** exfoliation into the lumen of the colon (as opposed to the ***intermittent*** loss of blood detected by FOBT) used for molecular analysis (17).

Colonoscopy is considered a method of maximum accuracy for the diagnosis of CRC and of colorectal polyps ("golden standard"), which allows multiple and targeted biopsy of lesions detected in the lumen of the colon as well as polyp resection. The literature shows that colonoscopy can allow visualization of the *caecum* in 98.6% of cases. The sensitivity of the method is of 96.7% for the detection of cancerous lesions of and of 85% for the detection of large polyps, but it falls to 78.5% for the diagnosis of polyps sized <5 mm; its specificity is of 98% (21).

Following the results of numerous epidemiological studies which support the idea that the risk of developing colorectal cancer decreases when precancerous lesions are removed (adenomatous polyps), *flexible sigmoidoscopy (FS)* was recommended to be used as a screening method. Sigmoidoscopy has the advantage that, in comparison with colonoscopy, it requires less preparation time and is usually performed without sedation, but has the great disadvantage that it only detects distal colon cancers. *FS* (of 60 cm) has replaced rigid rectosigmoidoscopy because it can help to explore a larger segment of the colon and is better tolerated by patients. Compared to the *Hemoccult test*, FS has the advantage that it can help to directly visualize the colon or to identify and take targeted biopsy samples from the detected lesions (17).

Although *computed tomographic colonography* (CT colonography or CTC) or *virtual colonoscopy* is better accepted by the investigated subject compared to colonoscopy, in the case of symptomatic patients it is still little explored for screening (22). Sedation is not required for this technique, yet preparation is necessary in conventional colonoscopy. Meta-analysis studies reveal that sensitivity and specificity in CT colonography are high for identification of polyps > 10 mm (82%), but not for smaller polyps (56% for polyps <5 mm and 63% for lesions between 6 and 10 mm) (17). Although its use as a first option for CRC screening is unclear, the American Gastroenterological Association recommends CT colonography every five years (21).

Other Exploratory Techniques. There are many new endoscopic techniques (wide-angle colonoscopy, capsule endoscopy, narrow band imaging, auto-fluorescence imaging system, etc.) that are still in the evaluation stage but appear to be promising for CRC screening, as they will help to increase the CRC rate detection, especially for proximal colon and they will improve the population acceptability (17). The effectiveness of these methods will be evaluated after a period of time of use (23, 24).

The logical approach in the management of the case of hereditary CRC where a mutation of the genes involved in increasing the risk for cancer (MMR, APC) was identified, early detection of subjects at high risk of developing this neoplasia (*oncogenetic screening*) and adequate monitoring through personalized oncogenetic supervision of these subjects, of patients operated for colorectal cancer, and of their families represent a modern approach to be

included in current strategies for colorectal cancer prevention.

The consequence of detecting a mutation is the placement of the patient under oncological supervision and the application of primary, secondary or tertiary prevention measures such as: adaptation / modification of medical or surgical therapeutic measures, differentiated use of screening and early diagnosis measures (onset at younger age, high frequency, special tests) or individualized application of preventive measures: removal of polyps, etc. (25, 26). Subjects who present mutations in the APC gene are monitored regularly by repeating the colonoscopy every 2-3 years since adolescence. The frequency of testing is adjusted according to the number, size and character of the polyps. Besides monitoring, risk reduction strategies such as colectomy or procto-colectomy can be proposed given that the risk of cancer onset reaches 100% at the age of 50 and that the peak incidence of occurrence is during the 3rd decade of life (26).

CONCLUSIONS

A review of risk factors, of main early detection methods and of other methods of prevention for colorectal cancer reveals the evolution recorded in the management of patients with this pathology. The main risk factors include: age; gender (more common in men); diet (high in red meat or processed meat and animal fats); obesity and overweight; alcohol consumption; smoking; occupational exposure to carcinogens (asbestos); ionizing radiation; presence of infection history; various medical conditions (adenomatous polyps, inflammatory bowel disease – Crohn's disease, ulcerative hemorrhagic rectocolitis, biliary lithiasis, diabetes, metabolic syndrome); personal history of cancer (colorectal, esophageal, laryngeal, lung, prostate, endometrial and breast cancer, as well as chronic lymphocytic leukemia or melanoma); family history and genetic syndromes (familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer; BRCA1 mutations). The most used methods of screening for the general population are: test HEMOCCULT, colonoscopy and flexible sigmoidoscopy, while the risk groups (with one or more risk factors) these include: the oncogenetic investigation (including the assessment of family history of familial adenomatous polyposis (or FAP), attenuated FAP (AFAP), hereditary nonpolyposis CRC (HNPCC), personal history of colorectal cancer or adenoma, chronic ulcerative colitis and Crohn's disease and molecular testing of individuals with risk factors). Among the preventive measures, genetic screening in high-risk groups and individualized oncogenetic monitoring stand out as modern approaches. Oncogenetics will have an essential contribution in the early detection of hereditary risk cases as well as in the effective management of patients diagnosed with this condition, which, given the epidemiological evolution of the phenomenon, has proved to be one of the major public health issues at present and in the near future.

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NEWS

HORMONAL CONTRACEPTIVES, GENITAL TRACT INFECTIONS AND HIV RISK

The hormonal contraceptives and genital tract infections are associated with altered cervical immunity. A recent study that evaluated changes in cervical immunity after taking hormonal contraceptives showed that the women who used injectable depot medroxyprogesterone acetate and those who use estrogen-progesterone oral contraceptives had increased levels of proteins that attract HIV host cells. Also, the effect of hormonal contraceptives was exacerbated in presence of disturbed vaginal microbiota or genital tract infections such as gonorrhea, chlamydia, genital herpes, trichomoniasis and candidiasis. The levels and the type of immune mediators, infection status and hormonal contraceptives increase HIV risk by their cumulative action (Fichorova RN, Chen P-L, Morrison CS, Doncel GF, Mendonca K, Kwok C, Chipato T, Salata R, Mauck C. 2015. The contribution of cervicovaginal infections to the immunomodulatory effects of hormonal contraception. mBio 6(5):e00221-15. doi:10.1128/mBio.00221-15).

Cătălina Luncă

ELECTRONIC CIGARETTE – A WAY OF SMOKING CESSATION?

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ELECTRONIC CIGARETTE–A WAY OF SMOKING CESSATION? (Abstract): Specialists have the ethical obligation to promote smoking cessation using evidence-based therapeutic strategies. In the context of the growing popularity of the electronic cigarettes (ECs), the smokers asking us about it and we must be ready to provide expert advice. With the evidence available to date we must be cautious in recommending of the smokers to use ECs for smoking cessation because these have not proven superiority over drugs used for smoking cessation approved by the Food and Drug Administration (FDA), ECs are not FDA approved, short-term safety data shows that they cause airway reactivity and health risk of exposure to ECs in the long term is unknown. Before the ECs to occupy a place in the standard clinical approaches for the treatment of tobacco addiction longer needed more data about their safety clinical and regulatory of their use. **Keywords:** ELECTRONIC CIGARETTE, SMOKING CESSATION, EXPERT ADVICE.

Complete tobacco cessation is the best outcome for smokers. Electronic cigarettes (ECs) are the newest and most promising products for Tobacco Harm Reduction (THR) (1). ECs are electrically-devices: a lithium battery, attached to a heating element (atomizer) that vaporizes a humectant (propylene glycol, glycerol, distilled water, vegetable glycerin and/or polyethylene glycol), flavorings and liquid nicotine. Consumers (namely " vapors") may choose from several nicotine strengths, non-nicotine liquids, and a more than 7500 unique flavors of e-juice were available in January 2014 (2); this selection is a characteristic feature of ECs from any other THR products. Since they appeared in 2003, there has been constant development of more efficient and appealing ECs (2).

E-cigarettes are attractive because they provide behavioral aspects of smoking (hand-to-mouth movement, vapor that simulates tobacco smoke, flavorings that simulate cigarette taste), address to the sensory stimulation (3), psychopharmacologic (i.e., nicotine) (4) and social aspects. A range of reasons are reported for using ECs, mainly to quit or reduce smoking, to use a product

apparently healthier than tobacco, to overcome smoking restrictions, and to reduce costs related to tobacco dependence (5). All of this explains why ECs is believe to be effective in reducing tobacco smoking (6, 7) and are efficient as long-term substitutes of traditional cigarettes (3).

The use of ECs is apparently booming. It is estimated that in 2014 there were 466 brands (2). Sales are forecasted to increase by a factor of 17 by 2030 (8). Data from the United States, United Kingdom, Canada and Australia suggest that more than three-quarters of current and former smokers consider ECs less harmful than traditional tobacco. In the same study ECs use is higher among non-daily smokers and heavier smokers than among daily-smokers and smokers of fewer cigarettes (9). The number of nonsmoking youth that use ECs is rising and associated with increased intentions to smoke cigarettes (10). Adolescent ECs use it seems to be related much more with adolescent sensation seeking (11), rather to tobacco reduction or cessation behavior. It should be emphasized that this increase in ECs use has emerged despite the concerns raised by public health authorities about the safety and appropriateness of using them as alternatives to smoking (12, 13).

We can divide the studies targeted on safety/risk profile of ECs use into chemical, toxicological and clinical studies (14).

Clinical studies are the most informative, but the relatively recent entry of ECs into the market and the lengthy lag time for onset of many diseases of interest (i.e. cancer), conclusive evidence about the association of ECs use with such diseases will not be available for years or even decades. Accordingly, most research is now focused on in vitro effects, with clinical studies limited into evaluation of short-term use or pathophysiological mechanisms of smoking-related diseases (14, 15, 16). At present, ECs are highly variable (differing design, materials, utilization, combustion voltage, and liquid cartridge concentration), because the manufacturing and distribution of these products, and also cartridge manufacturing and production is unregulated. This is another barrier to effectively studying the potential adverse and beneficial effects of ECs (17) and adding to the complexity of trying to counsel patients regarding their safety (16).

Chemical studies have two mainly problems: one is fails to address unknown, potentially toxic contaminants because usually focused on the known specific chemicals (investigated concentrations of several substances known to be toxic/carcinogenic in conventional cigarette) (18), and the other one arise from unknown effects when these substances are heated and inhaled, although these chemicals in flavors are approved for use in the food industry (14, 18). Thus makes difficult to appreciate in vivo effects of such substances. Therefore, we would be cautious in conclusions, because this kind of studies does not offered any objective information about effects of use of these substances (14). Many studies found that the product labels did not show the concentrations of solvents and flavorings (18). Much more, some studies showed that nicotine-free products were found to contain nicotine, sometimes in high concentrations (20, 21). In a recent study (22), cancers causing agents, formaldehyde and other toxicants like acrolein have been found into some ECs brands to

be as high as in the smoke produced by some cigarettes.

At present, there are only few **toxicological studies**, mostly on established cell lines and approach of cytotoxic effects has its deficiency. Patterns of ECs toxicity may fluctuate due to design features in the device, type and source of ingredients, and the manufacturing (including quality control measures) (19). Again, we cannot directly applied the results in vivo situation (14, 19), because a range of results is to be expected from different or the same cell lines and exists the risk of over or under estimating the toxic effects interpretation (23, 24, 25, 26). For example, in a recent study (26), it was found that at least four of their samples were not refills but concentrated flavors. It must be note that, the levels of cinnamaldehyde found to be cytotoxic were about 400 times lower than those currently approved for use (27). Another study of the ECs use cytotoxicity on embryonic and adult cells showed a decreased cellular survival due to the flavoring agents (23), so that maybe these flavors, not the nicotine, are the potentially harmful ingredients in e-juice. It should be done studies that comparing the potential cytotoxicity effects of ECs vapor with those resulting from the exposure of tobacco smoke should be mandatory, but unfortunately standards for vapor production and exposure protocols have not been clearly defined (14).

Data from clinical trial have found no significant differences in adverse events between ECs use and nicotine patches (6). Supporting this, other clinical studies have announced clinically minor adverse events with ECs use (mouth irritation, cough, and nausea) (28) or lower rates of adverse events than tobacco use (7). On the other hand, the Food and Drug Administration (FDA) has received reports related to the ECs use associated of both minor adverse events (headache, chest pain, nausea, cough) and major adverse events (hospitalizations for pneumonia, congestive heart failure, seizure, rapid heart rate, and burns related to routine use) (29). It must be note that, the longest duration of a clinical trial has been 24 months (30). Accordingly, these clinical studies evaluated the adverse effects of short-term EC use. Despite on evidences from these clinical trials of harmful effects of vamping (cardiovascular and respiratory functional outcomes), these are considered milder compared with smoking traditional cigarettes. It is required data from longer-term clinical studies before any clearly conclusions are made because it is difficult to assess the prognostic implications from few and short existing studies (14, 19).

Regarding chemical, toxicological and clinical studies, a recent review about ECs use health effects (18) appreciated that many studies found harmful substances: fine/ultrafine particles, cytotoxicity, metals, carcinogenic tobacco-specific nitrosamines and carbonyls, some in most samples, others in few, some in high concentration, as high as in conventional cigarettes or higher, but mostly slow or trace levels. In November 2014, the FDA reported that has detected levels of carcinogens and toxins, such as diethylene glycol (a harmful ingredient found in antifreeze) in laboratory analyses of 18 flavors and different cartridge types of ECs (31).

CONCLUSIONS

At present, on the base of studies per-

formed until now (many methodological problems, few and small studies, inconsistencies and contradictions in results, lack of long-term results), no definite conclusions can be established on the safety of ECs use (16, 18, 19).

It must be note that ECs use can also have adverse health effects on non-users (16). During pregnancy due to the perception that ECs are safer than traditional tobacco, may increase their use (32). A toxically study revealed the cytotoxic effects associated with ECs flavoring agents on embryonic cells and this could leading to embryonic loss or developmental defects (23). The risks associated with embryonic exposure to the chemical constituents in ECs in not completely understood. E-cigarettes may be a potential source of third hand exposure to nicotine (33). As ECs use has proliferated, the number of calls to poison centers involving unintentional exposure to ECs and e-juice, ingestion, inhalation, or skin absorption by young children, has markedly increased (34). Importantly, the lethal dose of nicotine to a previously unexposed person is around 0.5 to 1 g (35), and bottles of nicotine solution can be obtained via Internet (home mixing) (16). Some lithium batteries are poorly designed (low-quality materials, manufacturing defects) and improper use can causes fires or explosions (in house, in car, skin burns) (36).

ECs are nicotine-delivery products. As a consequence, the ECs users will always risk associated with nicotine use – the potential disease and related side effects (increased blood pressure, heart rate, micro vascular injury and dependence) (17). All medically approved treatments for smoking, have concentrated on total abstinence from nicotine (37). It must be note that, almost all the health risks come from various substances (tar, chemicals) found in the smoke, not from nicotine (38). Nicotine is considered as harmless by some health professionals, but others do not agree this point of view (39). The existing evidence shows that ECs aerosol is not just "water vapour" as is often claimed in the marketing for these products (15). Much more, ECs use represented serious threats to adolescents (10, 11) and fetuses (23). Additionally, it increases exposure of non-smokers to nicotine and a number of toxicants (15, 16, 33, 35).

Despite the fact that direct claims about cessation efficacy are prohibited by law, the ECs manufacturers make indirect claims about cessation, through product user testimonials (16). For many years ECs have been used as a healthier alternative to smoking or as an aid to smoking cessation (40, 41, 42, 43). The only existing randomized smoking cessation study has showed that ECs were not significantly more effective than nicotine patch therapy (6). Some prospective studies were very promising about ECs' effect as a smoking or as an aid to cut down or quit (7, 44) and a recent 'real-life' study showed that ECs increased cessation rates more than no aid/nicotine replacement treatment (45). A meta-analysis found that ECs users were significantly less likely than non-users to have cessation smoking (46) and a study in cancer patients showed that EC-users were twice as likely to be smoking at the time of follow-up as non-users (47, 48).

Specialists have the ethical obligation to promote smoking cessation using evidence-based therapeutic strategies. In the context of the growing popularity of the ECs, the

smokers asking us about it and we must be ready to provide expert advice. With the evidence available to date we must be cautious in recommending of the smokers to use ECs for smoking cessation because these have not proven superiority over drugs used for smoking cessation approved by the FDA, ECs are not FDA approved, short-term safety data shows that they cause airway reactivity and health risk of exposure to e-cigarettes in the long term is unknown. Before the ECs to occupy a place in the standard clinical approaches for the treatment of tobacco addiction longer needed more data about their safety clinical and regulatory of their use.

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FROM PUNISHMENT TO EDUCATION – JUVENILE DELINQUENCY IN ROMANIAN CRIMINAL LAW

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FROM PUNISHMENT TO EDUCATION – JUVENILE DELINQUENCY IN ROMANIAN CRIMINAL LAW (Abstract): For centuries children were considered "mini-adults". Together with expressing the need to educate children and putting a stop to their integration in the work field from the earliest years the 19th century also displayed a new image of the child, which clearly separates him from the adults. In this paper the authors analyze the Romanian legislation addressing juvenile delinquency in criminal temporal evolution. On the one hand the minority age limits are sought and modulation of legislative provisions according to these, and on the other hand, types of penalties for minors are discussed. The authors conclude that the approach to juvenile delinquency in the current Romanian Criminal Code is the result of a long process of reflection of the legislators on adopting a different system of sanctions for juvenile offenders and on creating special regulations concerning the prosecution, trial and enforcement of the decisions regarding them. **Keywords:** MINORITY, DELINQUENCY, ROMANIAN CRIMINAL LAW.

A minor is a person before their coming of age, set at 18 in most countries, which legally confines the child's status of adulthood.

For centuries children were considered "mini-adults". Together with expressing the need to educate children and putting a stop to their integration in the work field from the earliest years the 19th century also displayed a new image of the child, which clearly separates him from the adult.

The idea of juvenile crime emerged in history from a sociological perspective, first being developed conceptually in the 19th century in Great Britain, and became a well defined social phenomenon, with clear outline of specific criminal behavior in children (1).

Nowadays it is recognized that juvenile delinquency is a multi-factorial phenomenon that is imposed on a particular, fully evolving and changing, psychosomatic field. From a sociological perspective, the juvenile antisocial behavior may represent a normal part of growing up but it can also be a warning sign that predicts a pattern of criminal behavior, persistently evolving over time.

In the developing of the underage person, childhood is succeeded by adoles-

cence, with no clear chronological boundaries in between though. As a statistically-based convention, adolescence includes the age of puberty, ranging between 11 and 12 years old and 14 and 15 years old and adolescence itself, until the age of 18. By the age of 18 all the psycho-physiological and biological processes are produced, as well as the events that mark the transition to adulthood.

During adolescence there is acceleration of both the physical development from a somatic and morphological point of view and of the changes in social and psycho-emotional sphere. These changes are produced in an uneven manner, with individual variations that create new models of proper functioning.

The process of moral development, whose disturbances are directly involved in the dynamics of juvenile delinquency, was dividing into three levels, starting with preconvention and going through convention, in order to reach the post-conventional level. At a preconvention level, specific to children under 9 and to some teenagers, social norms are external to the self, so the individual appears to not understand or support the rules of society. The conventional level is characteristic to most teenagers and it is aimed at rational individuals that integrate social norms and act to achieve acceptance and admiration of others. The post-conventional level is reserved to a small number of individuals who distinguish the "Self" from the social rules and define their values in terms of principles (2).

Antisocial acts committed by juveniles show the outline of a model of deviant behavior compared to the accepted model within society. The deviant model is actually an expression of personality structures organized in disharmony, with predominance of certain traits such as aggressiveness as a form of reactivity, intolerance to frustration, social and emotional immaturity. Due to such features, the minor cannot adapt to social life and he deviates from the accepted social norms, which may lead to a situation where he might commit antisocial acts (3).

Age and psycho-physical characteristics of minors justify a social response to antisocial acts committed by them, different from that adopted in the case of adult offenders, starting from the conditions of the judicial process and all the way to the sanctions (4). Also, the minimum age of criminal responsibility under which minors cannot be held liable for offenses should be set according to their level of emotional, mental and intellectual maturity (4, 5).

Minority is a period of formation and completing the profile of one's personality and psychosocial maturation, which means that exterior rehabilitation interventions are justified and effective. They are intended to restructure and rebalance personality by reorganizing its components in order to establish a connection between the behavior model of the minor and that accepted by society and to prevent reiteration of antisocial acts (3).

Imprisonment of juvenile offenders should be an extreme measure, the place of detention of minors being separated from that of adults (4, 6, 7). The application of alternative measures to imprisonment, which allows juveniles to maintain positive connection with their family, school and community and increase their chances of re-socialization, is important if we were to consider a decrease of recidivism, as the increased risk of recidivism of juveniles detained is well known.

In this paper the authors analyze the Romanian legislation addressing juvenile delinquency in criminal temporal evolution. On the one hand the minority age limits are sought and modulation of legislative provisions according to these, and on the other hand, types of penalties for minors are sought.

MINORITY IN ROMANIAN CRIMINAL LAW

Age was always a criterion for customization and reduction of sentences in Romanian legislation since the Middle Ages, when the first legal regulations on punishment of minors emerged. But the age of majority, the age limits of criminal liability and the age quantifying penalties underwent variations over time.

The law from the 17th century stipulated that the age of majority was 25 years. This category included the juvenile age groups that allowed various methods of punishment to be applied, and one of the criteria for determining criminal liability was sex. "*Cucon / cocoon*" was a minor under the age of seven years. *Young person* was a term booked for boys aged between twelve and a half and fourteen and girls aged between nine and twelve and a half (8, 9).

Minority limits remain unchanged until the early 1800s, when the child aged up to 7 years was not criminally liable. Penalties could be applied to minors aged 7 to 14 years and those aged 14 to 20 years, increasing the severity of penalties imposed as the minor got older. In *Legiuirea Caragea*, in 1818, a new age emerged for which exemptions to punishments were allowed, which is that of the "the not old" young (20-25 years old) who were entitled to ask the ruler for a sentence reduction (10).

In the 19th century the age of criminal responsibility was increased to 8 years old and the juvenile period was divided into age groups of 8-15 years old and 15-21 years old, juveniles of the latter category facing judicial situations unconditionally (10).

The first Romanian Penal Code (1865), provided that the minor is not criminally liable until the age of 8, and the juvenile period was divided into two groups: 8-15 years old and 15-20 years old (11).

In the Criminal Code of 1936 the coming of age was set at 19 years old, and was divided into two categories- child and adolescent- periods separated by the age of 14 (12).

In the Criminal Code of 1948, the terms child and adolescent were replaced by the term *minor*. The minor was the person who had not turned 18, and the age of criminal responsibility was lowered 14 to 12. A minor aged 12 to 15 was not held responsible for an act committed injudiciously (13).

The current limits for juvenile criminal liability were established in the Criminal Code of 1969. This stipulated that minors under the age of 14 were not criminally liable, those aged 14 to 16 were criminally liable if they did have discernment and those over 16 were criminally responsible (14).

THE SANCTIONING OF THE UNDERAGE OFFENDER- FROM PUNISHMENT TO EDUCATIONAL MEASURES

The Romanian law that provides criminal penalties for offenses committed by juveniles has evolved over time from punishing to coming up with educational measures and reintegration of offenders into the community. The overwhelming importance of the factors regarding family, education and society in the genesis of juvenile delinquency has gained increasing

relevance in modern criminal law.

In the 17th century, in case of crimes such as murder, robbery, sexual offenses or counterfeiting, committed by juveniles, the punishments were reduced compared to those applied in the case of adults even if the defendant was tried in adulthood. Lower punishment of juveniles was not, however, applicable in case of acts such as heresy or murder of a parent. 17th century legislation provided that young people aged 18 could be sentenced to prison. The 14 year-old ones who made a mistake for which adults were sentenced to death were publicly beaten and then sent to prison, and young people aged 14 to 25 who consciously committed criminal acts were beaten and sent to salt mines to do hard labor (8, 9).

In the early 1800s they separated the place for serving the sentence in the case of juvenile offenders and that of adult offenders, making it possible for juveniles aged 7-14 to be punished by imprisonment in the monastery. Imprisonment was possible for a maximum period of 10 years for minors aged 14 to 20 years for committing acts that attracted the death penalty for adults (10).

In the 19th century, in harmony with international law, beating was waived, juvenile prisons and those for adults were separated and a special jurisdiction for juvenile delinquents was created. Then there appear the first legal provisions allowing the sanctioning of minors aged 8-15 years who committed acts unwittingly, by measures of education within families. For acts committed with premeditation, however, juveniles of the same age were punished with solitary monastery confinement for 3 months up to 3 years (10).

In the second half of the 19th century, educational measures and penalties were maintained. Minors aged 8-15 who lacked discernment were not punished, but were entrusted to the parents or were taken to the monastery for re-education until they turned 20. The discerning minors or those between 15 and 20 years old could be sentenced to 3-15 years in prison for a crime that drew sentences of hard labor for life for an adult. For other offenses, the minor could be sentenced to at least one third or to more than half of an adult's sentence. Minors were judged by special courts and the sentence was carried out in special places (11).

At the beginning of the 20th century the first institutions for the re-education of juvenile offenders appear, such as the disciplinary colony from Mălureni, Argeş, designed for beggars and homeless minors or the Romanian Reformatory Education Institute in Gherla, for non-criminal and criminal minors after serving their prison sentence. In this institute the sentences of beating with a rod and solitary confinement were kept (10).

The judicial reform after the World War I led to a system of modern approach of juvenile offenders. The *Law for defeating vagrancy and begging and for the protection of children* (1921) and the *Law on prisons and preventive institutes* (1929) allowed the establishment of schools for re-education and protection, of labor colonies and correctional institutions, in order to ensure the qualification in a trade and the social reintegration of juvenile offenders (15, 16).

The Criminal Code of 1936 (12) mentioned that discerning juvenile offenders could be entrusted with their family, a close relative or *"an honorable person, a society of patronage or a public or private institution authorized by the State."* The minor

may also be placed under supervised release or admitted to an educational institute. Minors with psychiatric disorders could be admitted to a medical facility (hospice or nursing home) with the surveillance of a specialist, until "complete recovery".

For the discerning teenager, the court could apply educational measures (corrective education, supervised release) or punishment (reprimand, correctional prison, simple imprisonment).

In this period juvenile courts were established and correctional punishment or detention were executed in institutes for corrective education, where children were separated from teenagers and boys were separated from girls. Minors who could not adapt to joint treatment were subjected to a type of treatment which allowed them to be assigned individually or in a group of hospitalized inadaptable. Depending on the seriousness of the offense, imprisonment was also individualized to solitary confinement- night time and day time.

In the Criminal Code of 1948 the term *corrective education* was replaced with that of *moral rehabilitation* (13).

Since 1951 (18) educational colonies for minors with social adjustment problems were created by the Ministry of Internal Affairs, as well as regional and interregional reception points for children aged 3-18 (19).

The Criminal Code of 1969 (14) maintained educational measures and punishment, sanctions that could be imposed on juvenile delinquents, depending on the seriousness of the offense committed and on their physical, intellectual and moral development. The educational measures were: reprimand, supervised freedom, hospitalization in a rehabilitation centre or in a medical-educational institute. The minor could also be admitted to these institutions after the age of 18, up to 2 years.

Punishment, applied when educational measures failed, was imprisonment or a fine. Imprisonment limits were reduced by one third, without this exceeding a period of 5 years. For offenses punishable by death in adults, juvenile imprisonment would be applied from 5 to 20 years without the possibility of complementary sanctions as well, with the possibility of parole.

Compulsory social inquiry appears (called the *evaluation report* from 2006) meant to provide the judicial body with data concerning the minor and the prospects for social reintegration, based on the information about behavior and his personal pathological history and his family and social environment (20, 22).

The 2004 Criminal Code sanctions maintained punishment and educational measures, supplementing the latter with freedom under strict supervision. Also, the duration changed: 5-15 years for a crime punishable by life imprisonment in adults, 3-12 years for severe crimes, or halving the sentence but not more than 3 years. The minor could perform community service and could waive the penalty for offenses punished with imprisonment for not more than 2 years, in the case of minors without criminal records, who covered the damage they had caused and proved they could change without the necessity of a sentence (21).

The new Criminal Code, effective since 1 February 2014 (23), radically changes the consequences of criminal liability with respect to minors, leaving out penalties and keeping only educational measures. Educational measures may mean non-custodial (civic training course, supervision, weekend confinement and daily assistance) or deprivation of liberty (placement in an educational centre or in a detention centre),

depending on the seriousness of the offense and on the minor's peculiarities, their implementation being coordinated by the probation service.

The civic training course consists of the minor's commitment to participate in a four-month program in order to understand the legal and social consequences he is exposed to in case of criminal offenses and to be more aware of his behavior in the future. Supervision consists of controlling and guiding the minor in his daily program for a period of 2-6 months. For the weekend confinement, the minor is not allowed to leave the house on Saturday and Sunday, for a period 4-12 weeks. Daily assistance may be ordered for a period of 3- 6 months and it requires the minor to follow a schedule set by the probation service. In addition, obligations may be imposed, such as enrolling in training courses, submission to control measures, medical treatment and care. Disrespecting all of the above may prolong or worsen the educational measure or may lead to admission to an educational centre.

Admission to an educational centre requires the minor's hospitalization for a period of 1- 3 years in a specialized institution where he will attend a training program for school, vocational training and social reintegration. Relapse leads to prolonging the hospitalization period or to a more severe measure, and good behavior can motivate a non-custodial measure or educational release from the centre if the person is 18.

In the detention centre, a specialized institution with security and surveillance regime, the minors attend an intensive program of re-education. Admission is disposed over a period of 2-5 years or 5 -14 years when the offense committed is punishable by imprisonment for more than 20 years or life imprisonment in adults. Relapse attracts an extended hospitalization and good behavior can lead to a non-custodial measure or release from detention centre if the person is 18 years old. If anti-social behavior is maintained at the age of 18, the court may order the continuation of the educational measure in a prison.

CONCLUSIONS

Our study shows that the approach to juvenile delinquency in the current Romanian Criminal Code is the result of a long process of reflection of the legislators on adopting a different system of punishments for juvenile offenders and on creating special regulations concerning the prosecution, trial and enforcement of the decisions regarding them.

By eliminating the punishment from the penalties in the case of juvenile offenders and by focusing on educational measures, the current criminal code adheres to the international approach of juvenile delinquency, emphasizing the education and re-integration into the community at the expense of custodial sentences.

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EPIDEMIOLOGY AND PATHOLOGY OF PAROTID TUMORS – FIVE-YEAR RETROSPECTIVE STUDY

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EPIDEMIOLOGY AND PATHOLOGY OF PAROTID TUMORS – FIVE-YEAR RETROSPECTIVE STUDY (Abstract) **Aim:** To retrospectively analyze the epidemiological, clinical and pathological data of parotid tumors. **Material and methods**: Reassessment of the histologic diagnosis of parotid tumor in the patients admitted to the Oral and Maxillofacial Surgery Unit of the Iasi "Sf. Spiridon" University Emergency Hospital during 2009-2013. Data on gender, age, place of residence, location, size, surgical procedure type and histopatological type were recorded. **Results**: The risk of developing pleomorphic adenoma is 7.40 times higher in women and 4.08 times higher before the age of 50 years. The risk of Warthin tumor is 16.47 times higher in male patients and 3.58 times higher in urban patients. People older than 50 years have a 7.14 times higher risk of developing malignancy and rural people have a 2.41 times higher risk of developing cancer. Diabetes, obesity and systemic hypertension were not important risk factors in our study. **Conclusions**: According to this study age and the place of residence could be important predictors for parotid gland tumors. Since in Romania there are few epidemiological data regarding the parotid gland pathology, further cohort studies are needed for a better understanding of their clinical and pathological behavior. **Keywords**: PAROTID GLAND TUMOR, EPIDEMIOLOGICAL STUDY, PATHOLOGY.

Parotid tumors are a heterogeneous group of tumors accounting for 85% of all salivary gland tumors, 2-6% of the head-neck tumors and about 1% of all tumors. Several studies have analyzed the epidemiology of salivary gland tumors but only a few have investigated the parotid tumor pathology. Most of them were conducted on less than 300 cases. In a study by Ali et al. (1) on the characteristics of parotid tumors in Pakistan population besides demographic and histopathological data the authors also described the clinical complaints, fine needle aspiration cytology results and complications. In another study, Moeller et al. (2) considered also the imaging findings. A study from China conducted by Chan et al. (3) described the demographic data and the management and complications of parotid tumors. In another study the epidemiological data were completed by a survival analysis (4). In the Romanian literature there are a

few studies about salivary gland pathology, some of them being case reports (5), some describing only pleomorphic adenoma of parotid gland (6), but there are no epidemiological studies.

The aim of this study was a descriptive analysis of the epidemiological and morphological aspects of benign and malignant tumors treated at the Oral and Maxillofacial Surgery Clinic and diagnosed in Pathology Department of "Sf. Spiridon" Emergency Hospital Iasi, Romania during the period 2009-2013.

MATERIAL AND METHODS

In present study were analyzed 162 tumors, of which 116 (67.46%) benign tumors and 46 (32.53%) malignant tumors.

The histologic diagnosis of the patients with parotid tumors admitted during 2009-2013 to the Department of Oral and Maxillofacial Surgery, "Sf. Spiridon" University Emergency Hospital, Iasi was reevaluated. After fixation in buffered formalin 10%, neutral pH, biopsy specimens were processed using paraffin embedding and hematoxylin-eosin, van Gieson, Alcian blue and PAS staining methods. The age of the study patients ranged between 13 and 90 years. To describe the morphological pattern of parotid tumors we used the 2005 World Health Organization classification (7).

Our analysis was based on demographic data (gender, place of residence, age), clinical data (location of the parotid gland, size of the tumor, type of surgical procedure) and histopathological type of tumor (benign/malignant). All patients with missing data on one or more variables used in the study were excluded.

For univariate analysis we used Mann-Whitney U to compare average age and chi square test to compare gender, place of residence, tumor location, size and type of surgical procedure. For multivariate analysis we used Multiple Logistic Regression considering as variables the gender, age (≤ 50 years/ > 50 years), place of residence (urban/rural), parotid gland location (left/right), tumor size (≤ 4 cm/ > 4 cm), and type of surgical intervention (superficial parotidectomy/total parotidectomy/ extended parotidectomy). Data were considered significant at $p < 0.05$.

RESULTS

The gender distribution showed a slight male predominance (55%) (fig.1A). No statistically significant gender differences were found between benign and malignant tumors (p=0.339). Most patients with benign tumors were living in urban areas (58.62%) and most patients with malignant tumors in rural areas (63.04%) (fig. 1B).

The difference between the two groups was statistically significant (p=0.012). There was a slight predominance of right parotid location of benign tumors (52.58%) and a slight predominance on left parotid location of malignant tumors (53.33%). The difference was not statistically significant (p=0.163). The mean size of malignant tumors was greater for (4.36 ± 3.14 cm) than that of benign tumors (3.49 ± 1.8 cm). The difference was statistically significant (p=0.025) (tab. I).

The age of patients with benign tumors ranged between 13 and 86 years, mean age 50.3 ± 15.81 years, and of the patients with malignant tumors between 20 and 90 years, mean age 67.17 ± 14.62 years. There were 2 cases under the age of 18 years, both with pleomorphic adenoma (tab. I). Age group distribution showed that most of benign tumors occurred in patients aged 41-70 years while malignant tumors were more

frequent in patients aged between 61 and 80 years (fig.2). Age group difference between benign and malignant tumors was statistically significant (p=0.025) (tab. I).

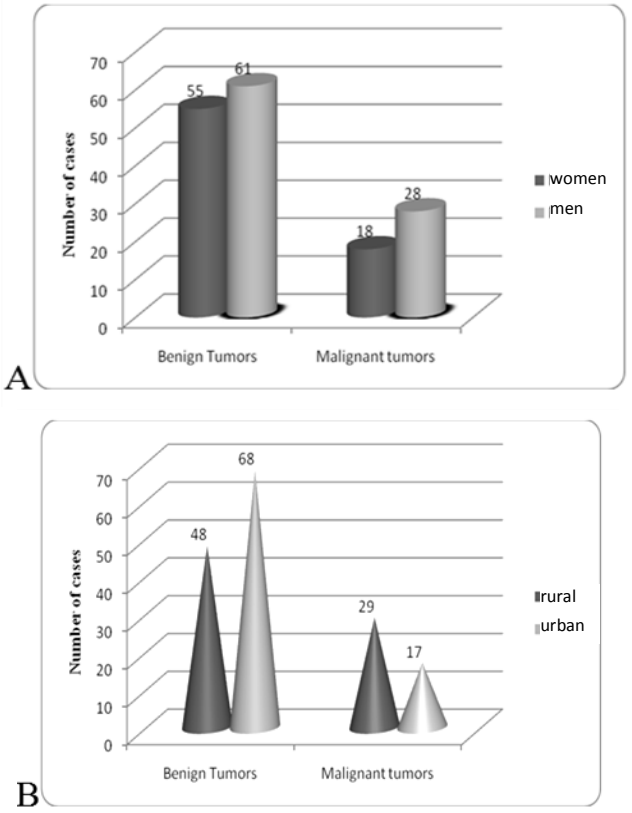

Fig. 1 Distribution of patients according to: A. Gender. B. Place of residence

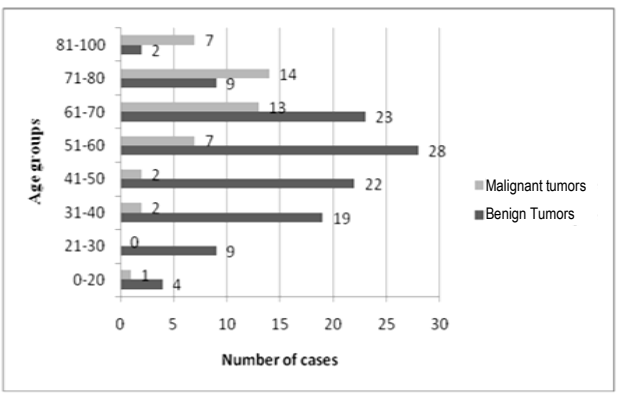

Fig. 2. Age group distribution of benign and malignant tumors

TABLE I
Comparative study of benign and malignant tumors

	Results	Benign tumors	Malignant tumors	p
Age	Mean ± SD, y	50.3±15.81	67.17±14.62	S (**p=0.039**) (Mann-Whitney U test)
	Min-Max, y	13-86	20-91	
Location	Left parotid No (%)	55 (47.41%)	24 (53.33%)	NS(p=0.163) (χ^2test)
	Right parotid No (%)	61 (52.58%)	21 (46.66%)	
Size	Mean ± SD, cm	3.49 ± 1.8	4.36 ± 3.14	S (**p=0.025**) (χ^2test)
	under 4 cm	82 (70.69%)	24 (52.17%)	
	over 4 cm	34 (29.31%)	22 (47.83%)	
Surgery type	Superficial parotidectomy No (%)	68 (72.34%)	13 (38.23%)	NS (p=0.355) (χ^2test)
	Total parotidectomy No (%)	4 (4.25%)	13 (38.23%)	
	Extended parotidectomy No (%)	22 (23.43%)	8 (23.53%)	

The multivariate analysis indicated a statistically significant difference between benign and malignant tumors in terms of age and place of residence and a significant difference between rural and urban area in terms of histopathological diagnosis.

Of the benign tumors the most common was pleomorphic adenoma (70%), followed by Warthin tumor (21%), and basal cell adenoma (7%) (fig. 3A).

In our study the most common primary malignant tumors were represented by acinic cell carcinoma (21%), squamous cell carcinoma (17%), adenoid cystic carcinoma (12%), adenocarcinoma NOS (12%), and carcinoma ex pleomorphic adenoma (12%) (fig. 3B).

In the present study the analysis of risk factors showed that gender, age and place of residence may influence the histologic type of parotid tumors. The risk of developing pleomorphic adenoma was 7.40 times higher in females than in males (OR=7.401, CI=2.76-19.82, p<0.0001) and 4.08 times higher before the age of 50 years (OR=4.085, CI=1.64-10.13, p=0.0016). The risk of developing Warthin tumor was 16.47 times higher in males than in females (OR=16.47, CI=3.65-74.17, p<0.0001) and 3.58 times higher in urban patients than in rural patients (OR=3.583, CI=1.23-10.37, p=0.01). The risk of developing cancer was 7.14 times higher in patients older than 50 years than in those under 50 years (OR=7.141, CI=2.63-19.36, p=0<.0001) and 2.41 times higher in rural patients than urban patients (OR=2.416, CI=1.19-4.88, p=0.012). Diabetes, obesity and systemic hypertension proved not to be important risk factors in our study. Due to incomplete data regarding the smoking habit this parameter could not be included in the study.

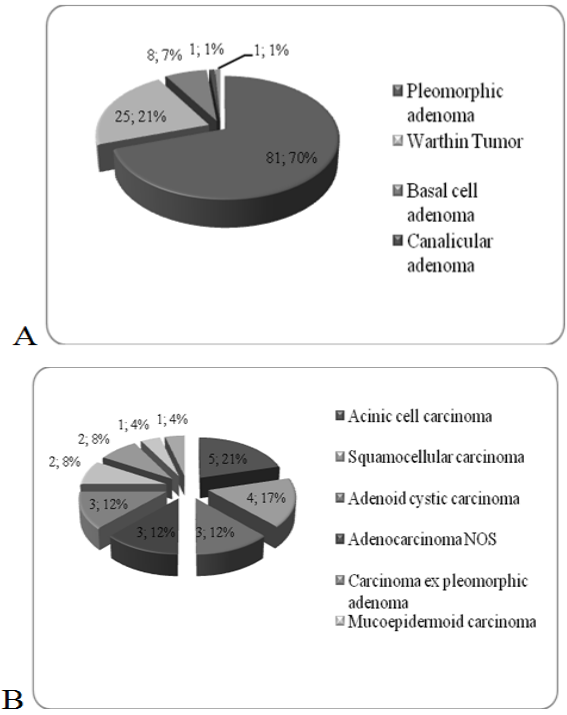

Fig. 3. Distribution of tumors: A. benign; B. malignant

DISCUSSION

There are many epidemiological studies regarding the salivary gland tumors, some of them the importance of parotid tumor pathology. Most of the studies are conducted on a limited number of cases and there are only few cohort series. Our paper presents the demographic data of the patients with parotid tumors diagnosed during the period 2009-2013 at the Oral and Maxillofacial Surgery Clinic and Pathology Department of the Iasi "Sf. Spiridon" University Emergency Hospital.

We found a male predominance which is consistent with other studies (1, 3, 4) but not with the study by Altinay et al. (8). Although, age distribution was similar with other studies (4, 8) in our patients mean age was a little higher. Unlike other reports (4) the majority of benign tumors were on the right side (52.58%) while most malignant tumors were on the left side (53.33%). The mean size of tumors in our study (3.49 cm for benign tumors and 4.36 cm for malignant tumors) was slightly greater than in other reports (3).

Using univariate and a multivariate analysis we found a predominance of benign tumor in urban areas (58.62%) and of malignant tumor in rural areas (63.04%), the difference being statistically significant (p=0.012). The lower utilization of medical services by the rural people could depend on their medical education, their trust in alternative medicine and a lower availability of medical services. This could be the cause for the delayed treatment of benign tumors with an increased risk of malignant transformation in these cases. Our study demonstrated that the risk factors were

represented by female gender and age less than 50 years for pleomorphic adenoma, male gender and urban residence for Warthin tumor, age over 50 years and rural residence for malignant tumors.

In terms of histopathological diagnosis the incidence of benign and malignant tumors is different in the international reports due to the great variability of study population. According to other studies (9, 10) 71% of the parotid gland tumors were benign and 29% malignant ones. In the present pleomorphic adenoma was the most common benign tumor, followed by Warthin tumor study which is agreement with other studies (1, 3). In case of malignant tumors the epidemiological data differed. Despite the low incidence of malignant tumors, five histopathological subtypes are considered the most frequent (11, 12). Thus, in some studies the most common types were mucoepidermoid carcinoma and adenoid cystic carcinoma (9, 13-16). Fonseca et al. considered adenocarcinoma Not Otherwise Specified (NOS) as the most common malignancy (10). A study in Taiwan indicated acinar cell carcinoma and mucoepidermoid carcinoma as the most frequent malignancies (3). The fifth more frequent carcinoma is carcinoma ex pleomorphic adenoma (12). Unlike these studies in our study the most common malignant tumors were acinic cell carcinoma, squamocellular carcinoma and adenoid cystic carcinoma. In our study there was a statistically significant difference ($p= 0.017$) between rural and urban residence in terms of tumor histological type.

CONCLUSIONS

Our study demonstrates that the age under 50 years is a risk factor for benign tumors, while the age over 50 years for malignant tumors. The patients residing in rural areas have a higher risk to develop malignant tumors due to a low utilization of health services. Since in Romania there are few epidemiological data on parotid gland pathology, further cohort studies are needed for a better understanding of their clinical and pathological behavior.

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NEWS

RELATIONSHIP BETWEEN OBESITY AND ORAL DISEASES

The aim of a study realized by a group of researchers from University of Benin, Nigeria was to determine the relationship between obesity and periodontal status and dental caries experience of a group of nigerian dental patients. Participants were selected from patients attending dental outpatient clinics of the University of Benin Teaching Hospital, Benin City, Nigeria. Their weight and height were measured and body mass index (BMI) estimated in kg/m^2, gingival health assessed using bleeding on probing index, oral hygiene estimated using the simplified Oral Hygiene Index (OHI-S), periodontal health estimated using the Basic Periodontal Examination (BPE) and caries experience was estimated with the Decayed, Missing, Filled teeth (DMFT) index. A few participants (3.8%) were underweight, 52.6% fell within the normal BMI range, 28.2% pre-obese, 12.2% obese class I and 3.2% obese class II. The mean OHI-S score was 2.16 ± 1.13 among the overweight participants and 2.05 ± 1.13 among those who are not ($P = 0.543$). The mean DMFT score was 3.03 ± 4.25 among the overweight participants and 2.32 ± 3.01 among those who are not ($P = 0.223$). Sixty-five percent of participants with BPE score of 0, considered to signify periodontal health, had normal BMI while all the participants with the worst BPE score recorded belong to the obese 1 group ($P = 0.070$). The binary logistic regression revealed that the likely predictor of gingival bleeding in the study is BMI between 35.0 and 39.9 (obese class 2) ($P = 0.046$, odds ratio = 0.07, 95% confidence interval = 0.01-0.96). It can be concluded that there was no statistically significant relationship between obesity and periodontal status and dental caries experience in the studied group of dental patients. Increased body mass index may however be a predictor of gingival bleeding (Sede MA, Ehizele AO. Relationship between obesity and oral diseases. *Niger J Clin Pract*, 2014 ; 17 (6) : 683-690)

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ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERN OF *S.AUREUS* STRAINS ISOLATED FROM THE ORTHOPEDICS–TRAUMATOLOGY SECTION OF "SF. SPIRIDON" CLINICAL EMERGENCY HOSPITAL, IAŞI

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ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERN OF *S.AUREUS* STRAINS ISOLATED FROM THE ORTHOPEDICS–TRAUMATOLOGY SECTION OF "SF. SPIRIDON" CLINICAL EMERGENCY HOSPITAL, IAŞI (Abstract): **Aim:** The retrospective analysis of antibiotic sensibility of *S.aureus* strains isolated from infected patients from the Orthopedics–Traumatology Clinic of "Sf. Spiridon" Clinical Emergency Hospital, Iaşi during January 2003–December 2013, in view of determining the evolution trend of the resistance phenomenon and of pinpointing the most useful treatment for these strains. **Material and method**: The antibiotic sensitivity test was carried out using two methods: diffusimetric–Kirby-Bauer and the MIC determination by E-test (for the strains isolated in 2013); the interpretation of the sensitivity was made in a standardized manner, in compliance with the CLSI (*Clinical and Laboratory Standards Institute*) standard for antibiotics testing in force. **Results:** The sensitivity testing for β-lactams proved that during the 11 years of the study, the average value of the frequency of resistant strains was of 41.59%±8.68. The highest frequency of MRSA (*Methicillin Restant S. aureus*) strains was noticed in 2012 (58.6%), followed by 2004 (50.7%). Even if in 2013 it dropped to 38.9%, the trend calculated for 2003-2013 is slightly rising (y=0.0073x+0.372). Out of the total of 495 *S.aureus* strains that were isolated, 164 (33.13%) were completely sensitive to the tested antibiotics and 26 (5.25%) were resistant only to β-lactams. The other MRSA strains associated multiple resistance and MIC for vancomycin varied between 0.5-2 mg/ml. Two strains whose MIC was of 0.5 mg/ml were sensitive to most classes of tested antibiotics, including β-lactams, except for macrolides (erythromycin), and the strain whose MIC was of 2 mg/ml, was resistant to all classes of tested antibiotics, except for glycopeptides and oxazolidiones. The other tested strains had a MIC for vancomycin equal to 1mg/ml. **Conclusions**: Due to the fact that there are infections with SAMR strains in a rather worrying percentage (53.9%) that are resistant to the other classes of antibiotics, the only therapeutic solution being the vancomycin treatment, its use should be limited solely to those cases when it is really necessary. Fortunately, no vancomycin resistant MRSA strains have been identified in our country, but this phenomenon should be kept under close surveillance. **Keywords**: MRSA, VANCOMYCIN, MINIMUM INHIBITORY CONCENTRATION, E-TEST

Hip or knee prostheses, fractures fixation, ligament or sinew reconstruction and other procedures requiring the use of an implant are very frequently used in view of repositioning the fractured bone segments and of recovering the articular function. Unfortunately, all these procedures are accompanied by a variable risk of infection, depending on the type of surgical intervention, ranging from 0.8% for arthroplasties and 53% for open fractures (type III Gustillo) (1). Osteoarticular infections are difficult to treat, being associated with a recurrence risk of 10-20% cases, especially in case of multi-resistant pathogens, such as methicilin resistant *Staphylococcus aureus* (MRSA). The possibility to come across a microorganism with antibiotic multi-resistance is a major concern for the clinician, in full awareness that an infection triggered by such a strain leads to the increase of morbidity and mortality (2). The retrospective analysis of the antibiotic sensitivity of *S.aureus* strains isolated from the infected patients in the Orthopedics–Traumatology Clinic of "St.Spiridon" Clinical Emergency Hospital, Iaşi from January 2003 to December 2013, in view of determining the evolution trend of the resistance phenomenon and of pinpointing the most useful treatment for these strains.

MATERIAL AND METHODS

The retrospective study was made for the period 01.01.2003–31.12.2012, and the result of the diffusimetric antibiograms was taken from the hospital's informatics system; the prospective component of the study implied the collection of pathological products from patients admitted during 01.01.2013–31.12.2013, for whom there was a suspicion of clinical infection, in compliance with the general norms of sampling products destined for the bacteriological exam. We excluded from the database the strains from the same bacterial species and with an identical phenotype of resistance to anti-infections chemo-therapeutic products as another isolated strain from the same patient during the same infectious episode. The strains were isolated and identified in the Microbiology Labs of "Sf. Spiridon" Hospital, Iaşi and of the Microbiology Discipline within "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi. Antibiotic sensitivity testing was carried out using two methods: diffusimetric–Kirby-Bauer and MIC determination by E-test (for strains isolated in 2013), and the interpretation of the sensitivity was conducted in a standardized manner, in compliance with the CLSI standard (*Clinical and Laboratory Standards Institute*) for antibiotic testing in force on that date (3).

RESULTS

From January 2003 to December 2013 a number of 555 strains of staphylococcus were identified, 495 belonging to the *S.aureus* species, the rest of 60 strains being coagulase-negative staphylococci (CoNS). The *S.aureus* strains isolated were tested for the following antibiotics: AMC (amoxicillin + clavulanic acid), OXA (oxacillin), RIF (rifampicin), CAM (chloramphenicol), TEI (teicoplanin), VAN (vancomycin), LNZ (linezolid), GEN (gentamicin), PFX (pefloxacin), LVX (levofloxacin), CIP (ciprofloxacin), CLI (clindamycin), MFX (moxifloxacin).

Sensitivity testing for β-lactams proved that during the 11 years of the study, the average value of the frequency of resistant strains was 41.59%±8.68 (tab.1).

The highest frequency of MRSA strains

was observed in 2012 (58.6%), followed by 2004 (50.7%). Even if in 2013 it dropped to 38.9%, the trend calculated for 2003-2013 is slightly rising (y=0.7373x+37.167) (fig. 1). Following rifampicin sensitivity testing of isolated strains of *S.aureus* the average of the frequency of resistant strains for the whole duration of the study was of 32.09±10, and the yearly distribution showed a peak in 2004 (50.7%), the trend calculated for 11 years being descending (y=-2.1609x+44.093) (fig. 2).

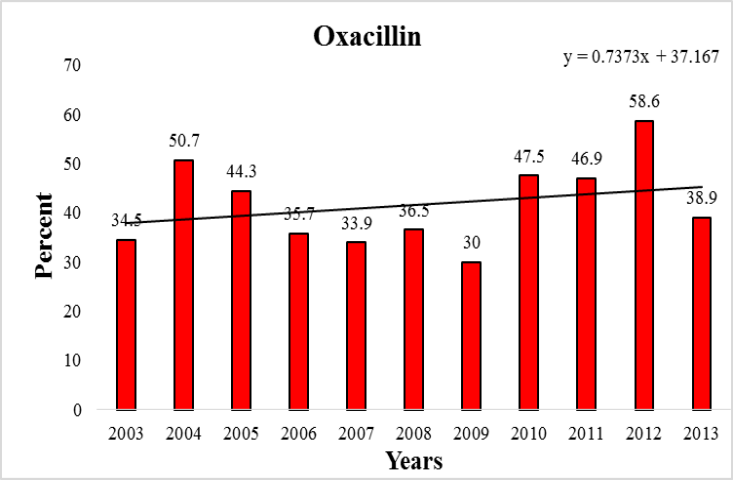

Fig. 1. Distribution of the frequency of MRSA strains on years of study

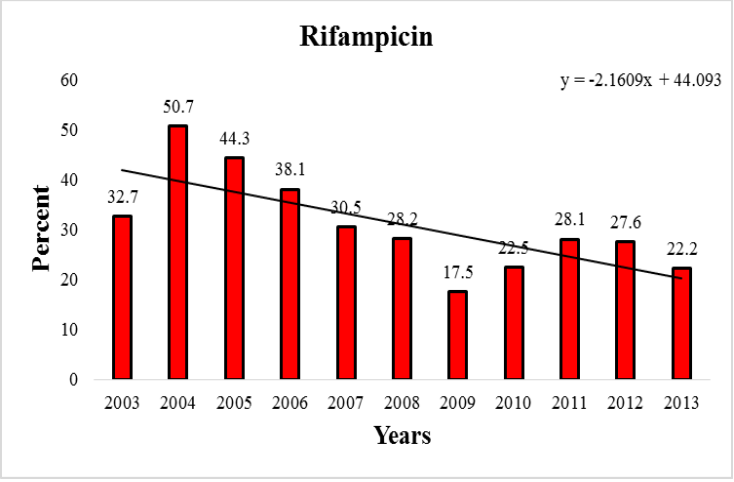

Fig. 2. Distribution of the frequency of rifampicin resistant *S.aureus* strains on years of study

Starting with 2004, the *S.aureus* strains were also tested for vancomycin and linezolid. Vancomycin sensitivity was 100% and for linezolid 100% during 2009-2013 and during 2004-2008 the percentage of resistant strains was extremely low (2004-

3%, 2005-4.9%, 2006-7.1%, 2007-1.7%, 2008-5%) (tab. I).

S.aureus strains were tested, depending on the year, for both 2nd generation quinolones, such as pefloxacin (2003-2006) and ciprofloxacin (2006-2013), and 3rd generation ones, such as levofloxacin (2004-2011) and moxifloxacin (2009-2011). The average of resistant strain frequency can be seen in table I. Both for ciprofloxacin, and for levofloxacin, the tendency of identifying resistant strains, calculated for the duration of the study, was descending (y=-4.45x±51.713 for levofloxacin and y=-1.83x±34.491 for ciprofloxacin).

TABLE I

Distribution on years of the frequency of *S.aureus* strains resistant to various antibiotics

Year	No. of isolated strains	AMC	OXA	TEI	VAN	LNZ	GEN	CIP	PFX	LVX	MFX	CLI	RIF	CAM
2003	55	52.7%	34.5%	3.6%	NT	NT	30.9%	NT	34.5%	NT	NT	NT	32.7%	18.2%
2004	67	56.7%	50.7%	0.0%	0.0%	3.0%	52.2%	NT	82.1%	55.2%	NT	NT	50.7%	50.7%
2005	61	62.3%	44.3%	0.0%	0.0%	4.9%	45.9%	NT	80.3%	45.9%	NT	NT	44.3%	44.3%
2006	42	69.0%	35.7%	0.0%	0.0%	7.1%	40.5%	42.9%	52.4%	35.7%	NT	NT	38.1%	NT
2007	59	35.6%	33.9%	0.0%	0.0%	1.7%	27.1%	30.5%	NT	25.4%	NT	10.2%	30.5%	15.3%
2008	52	36.5%	36.5%	0.0%	0.0%	1.9%	44.2%	21.2%	NT	23.1%	NT	5.8%	28.8%	NT
2009	40	30.0%	30.0%	0.0%	0.0%	5.0%	25.0%	22.5%	NT	22.5%	22.5%	10.0%	17.5%	NT
2010	40	47.5%	47.5%	2.5%	0.0%	0.0%	20.0%	20.0%	NT	17.5%	17.5%	12.5%	22.5%	NT
2011	32	50.0%	46.9%	0.0%	0.0%	0.0%	34.4%	28.1%	NT	28.1%	25.0%	18.8%	28.1%	NT
2012	29	NT	58.6%	0.0%	0.0%	0.0%	41.4%	17.2%	NT	NT	NT	44.8%	27.6%	NT
2013	18	NT	38.9%	0.0%	0.0%	0.0%	22.2%	27.8%	NT	NT	NT	38.9%	22.2%	NT
Average ± DS		48.92± 12.98	41.59± 8.68				34.92± 10.62	26.30± 8.09	62.82± 22.73	31.67± 12.99	21.66± 3.81	20.14± 15.42	32.0± 10.09	

The percentage of clindamycin resistant *S.aureus* strains was variable. Sensitivity testing for this antibiotic started in 2007 and continued until 2013. If, in the first years, the percentage of clindamycin resistant *S.aureus* strains was relatively low (5.8%-10.2%), one can see in figure 3 that during the last two years their frequency increased approximately four times (44.8%-2012 and 38.9%-2013), the rising trend of resistance to this antibiotic being obvious (fig. 3).

Multiple antibiotic resistance has registered an increased incidence starting with 1950. The antibiotic classes that were tested *S.aureus* strains were: β-lactams, aminoglycosides, fluoroquinolones, lincosamides, rifampicin, glycopeptides and oxazolidinones. Taking into consideration the fact that all tested strains were sensitive to glycopeptides, and oxazolidinone resistance was extremely low, cross resistance was analyzed only in the case of the first five classes of antibiotics. Out of the 495 isolated

S.aureus strains, 164 (33.13%) were completely sensitive to all tested antibiotics and 26 (5.25%) were only resistant to β-lactams. The other MRSA strains associated multiple resistances: 16 (3.23%) of them showed resistance to only one antibiotic class, mostly aminoglycosides (e.g. gentamicin) – 10 strains (2.02%); 103 strains (20.8%) associated resistance to two other antibiotic classes, the most frequently used being aminoglycosides and rifampicin – 92 strains (18.58%).

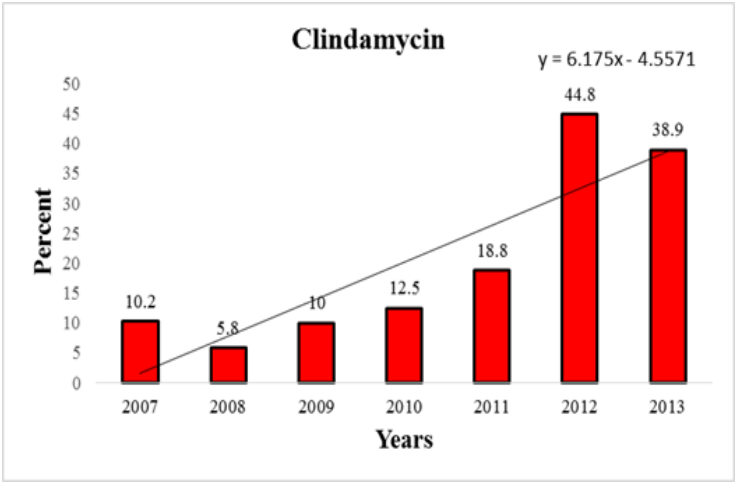

Fig. 3. Distribution of the frequency of clindamicyn resistant *S.aureus* strains on years of study

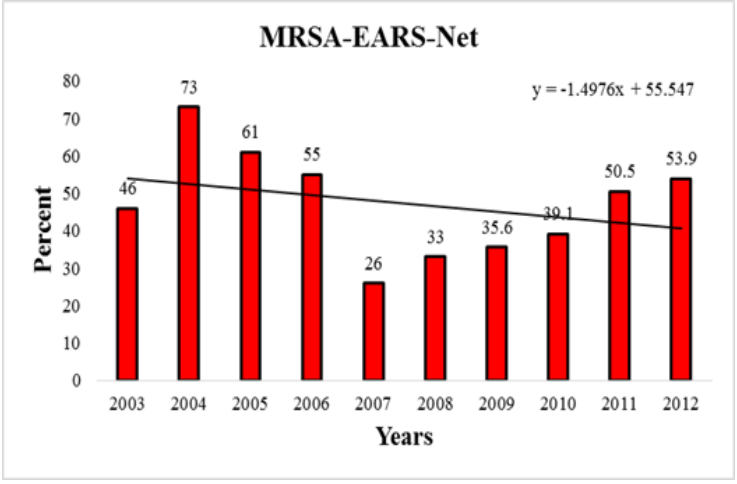

Fig. 4. Distribution of frequency of MRSA strains according to the data from EARS-Net

Associated resistance to four antibiotic classes could be determined only starting with 2007, when the *S.aureus* strains began to be tested for clindamycin. 35 strains (7.07%) fell into this category, most of them associating resistance to lincosamides

as well. We registered a number of 23 strains that were sensitive only to glycopeptides (4.64%).

For 18 strains of *S.aureus* isolated in 2013 we used the E-test to determine the MIC for vancomycin, which varied between 0.5 -2 mg/ml. Two strains whose MIC was of 0.5 mg/ml were sensitive to most tested antibiotic classes, including β-lactams, except for macrolides (erythromycin) and the strain whose MIC was of 2 mg/ml, was resistant to all tested antibiotic classes, except for glycopeptides and oxazolidinones. The other tested strains had a MIC for vancomycin equal to 1mg/ml.

DISCUSSION

The antibiotic sensitivity test showed high resistance levels to antimicrobial agents, with the presence of clinically and epidemiologically important phenotypes, among which the high incidence of MRSA strains (41.59%) stands out as well as the increased incidence of MDR phenotypes (31,11%). Methicillin resistant *Staphylococcus aureus* is the most important cause of infections associated to medical care round the world. In 2009, only six countries in Europe reported decreasing trends for MRSA. In 2012, although the problem seems to have been stabilized or even to have decreased in certain European countries, MRSA remains a public priority as the proportion is still over 25% in more than 1/3 of the countries (4). The latest data from EARS-Net (European Antimicrobial Resistance Surveillance Network) (2012) indicate for Romania and Portugal (the only countries in Europe with this level of resistance) a MRSA percentage of over 50% (Romania 53.9%, Portugal 53.8%). According to our study, in 2012, the percentage of MRSA strains was of 58.6%, but in 2013 it fell under 40% (38.9%).

Upon analysis of the EARS-Net reports for 2003-2012, we assessed that: the highest frequency of MRSA was registered in 2004 (73%), followed by a marked decreased until 2007, namely 26%, in turn followed by a continuous increase until 2012, when it exceeded 50% (namely 53.9%). However, it seems that the trend calculated for 2003-2012 is slightly decreasing (y=-1,4976x+55.547) (fig 4).

By comparing the data supplied by EARS-Net and the data obtained by us, there is no statistically significant difference (t=-1,495, GL=9, p=0,169). With regard to the results concerning RIF resistance, except for 2009, when the frequencies were roughly equal (17.5% in our study and 15.6% - EARS-Net), in the other two years an increase of the incidence of RIF resistant strains was registered in our area, as compared to the data supplied by EARS-Net (2010 – 22.5% vs. 13.3%, 2011 – 28.1% vs. 7.9%).

For 2012, in Europe, the percentage of MRSA strains varied from 0.7% in Sweden to 53.9% (Romania). Except for Sweden, there are five other countries in Europe which show a percentage of SAMR strains ranging between 1.3 – 2.1%, most of them having a MRSA frequency between 10 – 25% (14 countries) (4). In addition, the same 2012 report supplies data according to which the RIF resistance for MRSA strains was of 5-7% and for MSSA (*Methicillin Sensitive S. aureus*) strains of only 0.6%. In our study, RIF resistance associated to MRSA strains was of 73.52% for the whole duration of 11 years of the study and of 47.06% for 2012, while for MSSA strains for the same period it was of 9.34%, except for 2012, when all strains were sensitive.

According to the same EARSS report (from 2013) regarding the antimicrobial resistance in Europe for 2012, the MRSA strains associated resistance to fluoroquinolones of 81% as compared to MSSA strains, with only 6%. In our study, in 2012, isolated MRSA strains associated a resistance to fluoroquinolones of 35.3% and in 2013 of 71.42%, while MSSA strains were completely sensitive to fluoroquinolones, both in 2012 and in 2013.

Although there are certain countries which militate for the use of other antibiotics than VAN, there isn't sufficient evidence in the published randomized studies to indicate their superiority (5, 6). The data showing the efficiency of any antibiotic other that VAN in the treatment of serious infections caused by the strains whose MIC for vancomycin is situated in the "highly sensitive" area are even more limited. There is a study carried out by Soriano and col. which concluded that a MIC > 1mg/l to vancomycin through E-test is considered to be among the risk factors associated with a high mortality, but only in the case of patients who were treated empirically with vancomycin (7).

Similarly, Hidagat and col. reported that 12 out of 15 patients (80%) with MRSA determined infections, whose MIC for vancomycin was high and who failed to respond properly to the treatment with this drug, had good results when another antibiotic was used (8).

The guides for monitoring vancomycin therapy for 2009 of the Pharmacists' Society in USA recommend alternative solutions for the treatment o MRSA infections, if MIC $\geq$ 2mg/l (9). On the other hand, more recent guides (2011) recommend for strains whose MIC $\geq$ 2mg/l, that the decisional factor in continuing vancomycin therapy should be the patient's response, independently from MIC (10).

In conclusion, due to the fact that there are MRSA infections in a worrying percentage (53.9%) that are resistant to the other antibiotic classes, the only therapeutic solution being the vancomycin treatment, its use should be limited only to those cases when it is strictly necessary. Fortunately, in our countries vancomycin resistant MRSA strains haven't been yet identified, but the constant surveillance of this phenomenon is a must.

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NEWS

ANTIMICROBIAL ACTIVITY OF NOVEL 5-AMINOIMIDAZOLE-4-CARBOXAMIDRAZONES

Ribeiro et al. developed a method to prepare a series of fifteen 5-aminoimidazole 4-carboxamidrazones, starting from 5-amino-4-cyanoformimidoyl imidazoles. When testing the antimicrobial activity of these novel amidrazones against *Candida* sp. (*C. albicans*, *C. krusei*, *C. parapsilosis*) and Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, all inhibited the growth of *Candida* sp and a subset of compounds displayed fair-moderate activity against *S. aureus* and *E. coli*. The most potent antifungal compounds were also tested against *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum*, showing good activity aganist *C. krusei* and *C. neoformans*, but less on filamentous fungi when compared to their activity on yeasts. (Ribeiro AI, Gabriel C, Cerqueira F et al. Synthesis and antimicrobial activity of novel 5-aminoimidazole-4-carboxamidrazones. *Bioorg Med Chem Lett*. 2014. pii: S0960-894X(14)00856-7. doi: 10.1016/j.bmcl.2014. 08.025.)

Teodora Vremera

CLINICAL-EPIDEMIOLOGICAL STUDY ON ADVANCED NON-SMALL CELL LUNG CANCER

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CLINICAL-EPIDEMIOLOGICAL STUDY ON ADVANCED NON-SMALL CELL LUNG CANCER **(**Abstract). **Aim:** To assess the clinical and epidemiological features of all three main histological types of non-small cell lung cancer (NSCLC). **Materials and methods:** The study included 34 patients with NSCLC admitted to specialized units of the Iasi Regional Cancer Institute (IRCI) in the interval May 2012 - May 2014. Data were collected electronically based on a clinical-epidemiological survey form. The obtained data were then processed and interpreted by statistical-mathematical methods using the correlation index (significant at $p \leq 0.05$ and highly significant at $p < 0.005$), chi-square and Y tests. Data were analyzed using *SPSS 13.0* (SPSS Inc. Chicago, Illinois, USA). **Results:** Most NSCLC patients were aged 56-71 years, the Pearson correlation index between age and diagnosis being $p = 0.01$. Sex difference was highly significant ($p=< 0.001$) and so was the relationship between heavy smoking and age groups. Most cases were histogenetically classified as adenocarcinomas (22%). Four patients (21%) were in stage IIIA NSCLC, 4 (21%) IIIB, 9 (47.3%) IV, and 15 (79%) unspecified stage. All patients received primary chemotherapy. Single or associated complications included anemia and other hematologic disorders (26-74.6 %), respiratory failure, hemoptysis, cachexia, atelectasis, etc. Surgery was performed in 5 (14.7%) stage IIIA NSCLC patients. **Conclusions:** NSCLC showed a highly significant predominance in the age groups 56-71 years, and the most common histologic type was adenocarcinoma. All patients received primary chemotherapy, and patients with stage IIIA underwent surgery. Anemia and other hematologic disorders were the most common complications**.** **Keywords**: CLINICAL-EPIDEMIOLOGICAL, CANCER, NON-SMALL CELL LUNG CANCER.

By its high incidence and mortality lung cancer is one of the most common human malignancies. Thus, in the European Union the incidence of lung cancer exceeds annually 50/100,000 people, 82.5 in men and 23.9 in women.

In Romania, lung cancer has a mortality rate of about 60/100,000 people in men and 14/100,000 in women (1, 2). Of all lung cancers, 80-85% are non-small cell lung cancer (NSCLC), with the three major histological subtypes (squamocellular – 50%, adenocarcinoma – 15%, anaplastic – 15%), but with similar biological, clinical

and therapeutic features.

Clinical manifestations range widely from a small number of asymptomatic cases to cases presenting local tumor growth, regional spread, metastatic dissemination, and presence of paraneoplastic syndromes (3, 4, 5).

This study aimed to assess the clinical and epidemiological features of a series of patients with NSCLC admitted to specialized units of the Iasi Regional Cancer Institute (IRCI) (6, 7).

MATERIAL AND METHODS

This clinical-epidemiological study included a series of 34 patients with NSCLC admitted to IRCI in the interval May 2012 - May 2014. The patients were aged 52-81 years, 26 (76.4%) of them belonging to age group 56-75 years; 32 (94.1%) were men and 2 (5.9%) were women; over 80% resided in urban areas.

Data were collected electronically based on a clinical-epidemiological survey form, and were then processed and interpreted using statistical-mathematical methods: Pearson correlation index, significance ($p = 0.05$) and highly significant ($p < 0.005$), and chi-square, Y tests, CI = 95%_tests; data were analyzed using *SPSS 13.0* software.

RESULTS AND DISCUSSION

Age-group distribution showed that the majority of NSCLC patients were aged 56-71 years (26-76.4%), the study patients covering all 7 age groups (fig. 1).

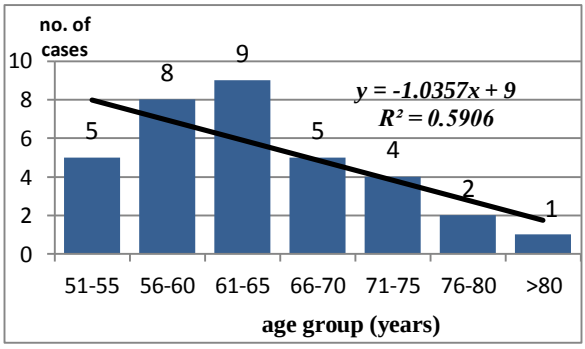

Fig. 1. Age-group distribution of the study patients

Pearson correlation index between age and diagnosis was $p=0.01$, which is consistent with our epidemiological data showing that 26 (76.4%) of our cases belonged to age group 56-75 years, and in agreement with data in other studies (32 - 94.1% *vs.* 2 - 5.9%) ($p < 0.001$).

Twenty-nine patients (85.3%) of the 34 study patients had a history of heavy smoking for at least 2-3 decades; all these patients were men, distributed quite evenly over all age groups, and with a very strong statistically significant correlation ($p < 0.0001$). Our data are consistent with those reported by many epidemiological studies (8, 9).

Adenocarcinoma was the most common type according to histogenetic classification (22 - 64.7%), followed by squamous cell carcinoma (8 - 23.5%) and large cell carcinoma (4 - 11.8%) (tab. I).

Histologic grading (WHO) was carried out in 7 (20.6%) cases (G2: 2-28% and G3: 5 - 71.4%).

TABLE I
Percentage validation of the cases based on histogenetic classification

Valid	Frequency	Percent	Valid percent	Cumulative percent
Adenocarcinoma	20	58.8	58.8	58.8
Squamous carcinoma	7	20.6	20.6	79.4
Others	7	20.6	20.6	100.0
Total	*34*	*100.0*	*100.0*	-

Staging at the time of diagnosis was carried out in 19 (55.9%) of the 34 patients enrolled in the study: 4 (21%) patients were in stage IIIA, 6 (31.6%) in stage IIIB, 9 (47.3%) in stage IV and 4 (21%) unspecified stage (tab. II).

TABLE II
Percentage validation of the cases based on disease staging

Valid	Frequency	Percent	Valid percent	Cumulative percent
IIIA	4	11.8	11.8	11.8
IIIB	6	17.6	17.6	29.4
IV	9	26.5	26.5	55.9
Unspecified	15	44.1	44.1	100.0
Total	*34*	*100.0*	*100.0*	-

Histological classification and staging together with the objective and subjective clinical findings guided the therapeutic approach.

All 34 patients included in the study and follow received primary chemotherapy with cytostatic agents active against NSCLC according to the guidelines of the sixth edition of AJCC Cancer Staging Manual, New York, Springer, 2000 (11, 12, 13, 14).

Various complications were recorded in 26 (76.4%) of the 34 study patients (15, 16, 17, 18) (tab. III, IV).

TABLE III
Complications recorded in the 34 patients with NSCLC (single or associated)

Category	No. of cases	%
Anemia	14	41.7
Other hematologic disorders	12	35.3
Respiratory failure	8	23.5
Hemoptysis	4	11.8
Cachexia	4	11.8
Other complications (lung abscess, atelectasis, lymphangitis, perithoracic phlegmons)	1 case each	-

TABLE IV
Percentage frequency of complications

Valid	Frequency	Percent	Valid percent	Cumulative percent
Yes	26	76.5	76.5	76.5
No	8	23.5	23.5	100.0
Total	*34*	*100.0*	*100.0*	-

Surgery was performed in 5 (14.7%) patients with stage IIIA resectable NSCLC as it is recognized to have superior therapeutic benefits (19, 20).

CONCLUSIONS

Lung cancer represents the most important neoplasia, in Romania.

The increased incidence of adenocarcinoma histologic subtype is highlighted in our study, too. Advanced stages are predominantly associated with co morbidities due to the evolution of neoplasia, but certain complications.

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NEWS

ELECTRONIC MEDICAL RECORDS - AN IMPERFECT SYSTEM WITH GOOD RESULTS FOR IDENTIFYING PATIENTS WITH A COMMON PHENOTYPE

The objective of this review was to summarize literature describing approaches aimed at automatically identifying patients with a common phenotype. The authors performed a review of studies describing systems or reporting techniques developed for identifying cohorts of patients with specific phenotypes. Every full text article published in (1) *Journal of American Medical Informatics Association*, (2) *Journal of Biomedical Informatics*, (3) *Proceedings of the Annual American Medical Informatics Association Symposium*, and (4) *Proceedings of Clinical Research Informatics Conference* within the past 3 years was assessed for inclusion in the review. Only articles using automated techniques were included. The identification of patients who satisfy predefined criteria from a large population in an institution has numerous use cases, including clinical trial recruitment, outcome prediction, survival analysis, and other kinds of retrospective studies. However, the process of distinguishing these patients on the basis of their patient records can be extremely time-consuming and challenging depending on the complexity of the criteria. This is because the data matching these criteria are buried within multiple documents and across multiple data points in the electronic health record (EHR) of a patient. Some data, such as laboratory results, medications, and diagnoses, have a structured format. The ability to extract meaningful pieces of information from the EHR and consolidate them into a coherent structure would provide great value for automatically identifying patient cohorts that satisfy complex criteria. Studies in this review addressed different diagnoses as the phenotype of interest. A large number of papers focused on diabetes, cancer, heart failure, rheumatoid arthritis, or cataract. A few studies described generic methods that could be applied to multiple diagnoses. Several papers addressed identification of adverse drug events. Some studies also analyzed genomic data of a predetermined phenotype to gain insight into other phenotypic properties of the same cohort. Authors have developed a top of the first 10 phenotypes of interest. Although cancer has been reported as a generic phenotype, there were 12 types of cancer (with breast cancer being most prevalent) studied in seven articles. Congestive heart failure and heart failure were counted as the same phenotype. Similarly, hypertension and resistant hypertension were considered to be one phenotype. Some articles also considered other observable characteristics such as smoking status and obesity among patients. Pneumonia and a variety of other infectious diseases were studied. This review is not without limitations, especially for the vagueness of the term phenotyping, meaning that there is no exhaustive search strategy for retrieving appropriate articles across the entire available body of biomedical literature. However, because of the venues considered and the recent popularity of this topic, this review is representative of past and present efforts in this domain. (Shivade C, Raghavan P, Fosler-Lussier E, Embi PJ, Elhadad N, Johnson SB, Lai AM. A review of approaches to identifying patient phenotype cohorts using electronic health records *J Am Med Inform Assoc* 2014;**21**:221-230 doi:10.1136/amiajnl-2013-001935).

Mihaela Mihai

EXAMINATION OF BOLTON INDEX COMPARING THE TRADITIONAL METHOD WITH A 2-DIMENSIONAL DIGITAL MEASUREMENT METHOD

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EXAMINATION OF BOLTON INDEX COMPARING THE TRADITIONAL METHOD WITH A 2-DIMENSIONAL DIGITAL MEASUREMENT METHOD (Abstract): The Bolton Index is one of the most important analyses in orthodontic treatment planning. It shows if there is a correct proportion in size between the maxillary and mandibular teeth. In this study we compared the digital and traditional method. **Methods**: 35 dental casts were measured using a Vernier caliper. The mean value was compared with the measurements from the scanned images of the dental casts. **Results**: The results showed that the digital method provided similar results with those of the traditional one with a mean difference of 0.23 mm. The Pearson correlation coefficients were very high and similar to each other: R=0.989 and R=0.988 for the Anterior Bolton Index (ABI) and Overall Bolton Index (OBI), respectively. No statistically significant differences were present from either the ANOVA or the paired t-tests. **Conclusion**: The used digital method is as accurate as the traditional one, it is faster and easier to carry out and it offers all the advantages of image storage and data for subsequent use. **Keywords**: BOLTON INDEX, TRADITIONAL METHOD, DIGITAL METHOD.

In 1958, Bolton (1) studied 55 patients with excellent occlusions and produced ratios for the mesiodistal sizes of maxillary and mandibular teeth. He introduced two indices: the Anterior Bolton Index (ABI) which is the percentage obtained by adding the mesiodistal size of the six mandibular anterior teeth (from canine to canine) divided by the mesiodistal size of the six maxillary anterior teeth (from canine to canine); and the Overall Bolton Index (OBI) which is the percentage obtained by dividing the total mesiodistal size of the 12 mandibular teeth (from first molar to first molar) by the mesiodistal size of the maxillary teeth (from first molar to first molar). The indices for a correct occlusion, extrapolated from Bolton`s studies. (2)

ABI= 77.2 per cent (74.5- 80.4 per cent)
OBI= 91.3 per cent (87.5- 94.8 per cent)

In 1962, Bolton (3) reviewed his original study. He presented several clinical cases to determine if his analysis was viable diagnostic aid and determined that by employing the analysis, there is rarely a need for a diagnostic setup. (4)

Computer aided analysis of tooth size discrepancy was investigated by Tomassetti et al., (2001) who compared manual measurements with Vernier callipers to the QuickCeph Image programme, the Hamilton Tooth Arch System software (HATS) and the OrthoCad software. Measurements with the HATS system had the highest degree of correlation to Vernier callipers, with 86.4% of the measurements for the overall ratio being within 1.5mm of the vernier calliper measurements, followed by OrthoCad and QuickCeph. QuickCeph was found to be the fastest method of measurement at 1.85minutes, followed by the HATS system at 3.4minutes, OrthoCad at 5.37minutes and finally Vernier callipers at 8.06 minutes. (4)

MATERIAL AND METHODS

Thirty five dental casts of patients attending the Orthodontic Department of the University of Medicine and Pharmacy Targu-Mures, were selected.

In order to compare the two measuring procedures under the best condition, the selection criteria of the casts were:

1. A permanent dentition from first molar to first molar
2. Good quality casts
3. No tooth agenesis or extractions
4. No large restorations that could change the mesiodistal diameters of the teeth
5. No teeth with anomalous shapes.

The mesiodistal sizes of the upper and lower teeth of each cast were measured by both methods as follows: for the traditional method callipers were used to measure the mesiodistal size of the cast. The ABI and OBI of these mesiodistal sizes were calculated by totalling the sizes of the teeth and determining the corresponding index. Three measurements were carried out in one month period with at least two weeks between each measurement and the order in which the casts were presented was changed.

For the digital method the casts were scanned. Digitization was carried out by placing the stone dental casts on a scanner (Konica Minolta, Bizhub C554), surrounded by a sheet of millimeter graph paper. This enabled the calibration of the two axes and thus calculation of the ABI and OBI. The scanned image was then scaled so that two different vertical and horizontal marks on the squared paper were selected at a specific distance of 30 mm apart, to establish both the horizontal and vertical transformation dimension factors. These transformation dimension factors (the horizontal factor `x` and the vertical `y`), which must have the same value, serve as verification. If the transformation dimension factors obtained are the same for the `x` and `y` axes, the calibration has been correctly carried out and the image has not been distorted; the equivalence relationship of the original cast model has been maintained but has simply been made larger.

STATISTICAL METHOD

A statistical package (IBM SPSS Statistics 20) was used to analyze the data obtained with the traditional and digital method which were presented as an Excel page. Absolute differences and Pearson correlation coefficients were used to compare the 3 sets of measurements made with Vernier calipers between each other and with the digital method. Repeated-measures analysis of variance (ANOVA) testing and

paired t-testing was performed on the same sets of data. A significance (P) value of .05 was used for each test.

RESULTS

Vernier calipers

Comparing the overall discrepancy for the first set of Vernier caliper measurements to the second set of Vernier caliper measurements showed that 85.7 % of the calculations were within 0.7 mm of each other. The mean difference was 0.38 mm, and the range was 0.00 mm to 1.28 mm. The Pearson correlation coefficient (R value) was 0.975. For the anterior discrepancy, the differences ranged from 0.00 mm to 1.18 mm with a mean of 0.47 mm twenty eight of 35 measurements (80 %) were within 0.7 mm, and the R value was 0.961.

Comparing the first and third set of models the results were similar. For the overall discrepancy, 94.2% of the measurements fell within 0.7 mm of each other with a range odd 0.02 mm to 0.82 mm and a mean of 0.28 mm. The R value was 0.985. For the anterior discrepancy, 91,4% of the measurements were within 0.7 mm, with a range of 0.00 mm to 0.84 mm and a mean of 0.29 mm. The Pearson correlation coefficient was 0.979.

The second and third sets of measurements were compared. For the overall discrepancy 94,2% of the differences were within 0.7 mm, and the differences ranged from 0.00 mm to 0.96 mm. The mean difference was 0.31 mm and the R value was 0.985. For the anterior discrepancy, 94,2 % of the results were within 0.7 mm. The mean difference was 0.33 mm, and the range was 0.02 mm to 1.18 mm. The Pearson coefficient was 0.981.

Digital method *vs*. Vernier calipers

The digital overall analysis differed from the ones made with caliper by a mean of 0.23 mm. The variations ranged from 0.00 mm to 0.95 mm, with 97.1 % within 0.7 mm.

For the anterior analysis the mean difference was 0.27 mm, with a range from 0.02 mm to 0.74 mm. Thirty four of 35 (97.1%) were within 0.7 mm.

The R value for the overall discrepancy was 0.989 and for the anterior discrepancy was 0.988.

No statistically significant differences were present from either the ANOVA or the paired t-tests. The P values for the paired t-tests were .091 for the overall discrepancy and .080 for the anterior discrepancy. For the ANOVA, the P value was .061 for the anterior discrepancy and for the overall there were no statistical differences between groups.

DISCUSSION

The importance of tooth size discrepancies in orthodontic diagnosis has been widely reported in the literature and accepted by the orthodontic community because the relationship between the upper and lower anterior dentitions is related to orthodontic finishing excellence.

Traditionally a Vernier caliper or needle-point divider is used to measure teeth and complete a tooth size analysis. Sheridan (2000) (5), reported that the Bolton Index was the most widely used diagnostic tool in clinical practice. However as determining this index with traditional measuring methods is laborious, it is not undertaken for more than half of the cases in clinical practice.

The OBI results with both methods showed concordance for all thirty-five patients (four patients with and thrity-one without discrepancies in the OBI). The ABI results were similarly very good, the find-

ings for all thirty-five patients concurring with both methods (six patients with and twenty-nine without discrepancies for the ABI). For the deviations in the measurements a variety of reasons could be responsible. First, the operator was less familiar with the digital system than with the calipers. The investigator found it difficult to pinpoint the exact mesial and distal points to be used for the measurements on the scanned images. Additionally, the closer the points were on adjacent teeth, the harder the task of distinguishing them. (4)

The aim of this study was to determine the accuracy and efficiency of performing Bolton`s tooth-size analysis using manual measurements with a Vernier caliper with computerized methods. The results obtained are in correlation with those in dental literature. Paredes (2006) (2) made a similar study and found that the correlation coefficients of the two methods were very high and similar to each other R=0.976 for ABI and R= 0.979 for OBI. The results also showed more discrepancies in the ABI than in the OBI using both methods. Tomasetti et al. (4) compared the traditional method with three digital systems: OrthoCad, HATS and QuickCeph. The HATS analysis had very similar results when compared with the averaged Vernier caliper measurements, The R value for overall discrepancy was 0.885 and for the anterior discrepancy 0.825. OrthoCad has slightly less correlated results, and the differences were of a greater magnitude. The R values for the overall and anterior discrepancies were 0.715 and 0.574 respectively. QuickCeph analyses were the least correlated of the 3 methods compared with the Vernier calipers. The R values for the overall and anterior discrepancies were 0.432 and 0.439 respectively. Distinguishing the proper landmarks was difficult with both the OrthoCad ad QuickCeph systems. In his review, Houston (6) stated that perhaps the greatest source of random errors is difficulty in identifying a particular landmark or imprecision in its definition.

CONCLUSION

The used digital method is as accurate as the traditional method for determining the Bolton indices. It is faster and easier to carry out and it offers all the advantages of a computer method, such as the storage of images and data for subsequent use

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STUDY ON ERYTHROCYTE AGGREGATION USING COMPUTERIZED IMAGE ANALYSIS METHODS

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STUDY ON ERYTHROCYTE AGGREGATION USING COMPUTERIZED IMAGE ANALYSIS METHODS (Abstract). **Aim**: To study the relationship between erythrocyte aggregation and physical parameters: temperature and pH. **Material and methods:** Aggregation was evaluated using computerized image analysis by Aggregate Shape Parameter (ASP), defined as the ratio of the aggregate projected area to its square perimeter, and erythrocyte aggregation index (EAI). EAI was measured and compared at different temperatures (4ºC, 20ºC, 37ºC) and different pH values (3, 5, 7 and 9). **Results and discussion:** The results show that the increase in temperature induces EAI increase and a decrease in Ph, a decrease of erythrocyte aggregation. It was found that, at pH=3 and 5, erythrocytes are destroyed and, at pH=9 RBC clusters are formed. **Conclusions:** RBC aggregate shape reflects intercellular interaction and its quantification may thus be another characteristic of RBC aggregation. Increased RBC aggregation was observed in various pathological states, such as diabetes and myocardial infarction, or following trauma. **Keywords:** ERYTHROCYTE AGREGATION, COMPUTERIZED IMAGE ANALYSIS, TEMPERATURE, pH.

Red blood cell (RBC) aggregation has an important role in blood flow, particularly in the microvascular system. The flow in small blood vessels is particularly sensitive to the aggregate size and shape.

Normal RBCs in low-flow states or stasis form rouleau aggregates, while abnormal RBC aggregation may form an irregular aggregate structure. The irregular shapes may reflect abnormal cell-cell interactions between the RBCs. These abnormalities may be induced by cell-associated factors, such as reduced levels of membrane sialic acid (1), or extracellular factors, such as high levels of plasma immunoglobulin on the cell surface (2), as well as by temperature, pH, osmolarity (3,4), and ionic strength of the extracellular medium.

The morphology of RBC aggregates is related also to RBC deformability. When two cells collide, the point-to-point attachment between RBCs develops into surface-to-surface contact by membrane rotation and a rolling movement of the attached cells to form the rouleau structure (1).

When the deformability of the attached cells is reduced, or the rolling process is too slow to be completed before collision with the next cell occurs, an irregular aggregate will be formed. Thus, RBC aggregate morphology may reflect the cell deformability (5). It may be concluded that RBC aggregate shape reflects intercellular interaction, and its quantification may thus be another characteristic of RBC aggregation.

In this study, the morphology of RBC aggregates was studied by using the computerized image analysis method.

MATERIAL AND METHODS

For this study we used computerized image acquisition and processing system (fig.1). The main part is an optical microscope to which a digital video camera is attached.

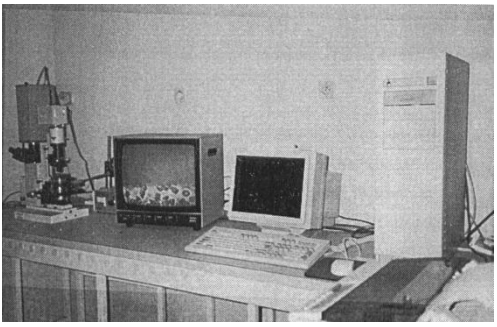


Fig. 1. Analysis system

EAI was determined using the Mchedlishvili's relation which establishes the total area of aggregated and not aggregated cells.

EAI is defined by equation no.1.

$$EAI = \frac{S_{agrg}}{S_{tot}} \cdot 100 \qquad (1)$$

The aggregate morphology is characterized by a dimensionless RBC Aggregate Shape Parameter (ASP) defined by equation no. 2:

$$ASP = 4\pi A/P^2, \qquad (2)$$

where A is the projected area of the aggregate and P is the perimeter of the projected aggregate. Since a circle has the maximal ratio of area to perimeter, this definition attributes to all aggregate structures ASP values between 0 and 1.

We studied the modifications that interfere in the process of erythrocyte aggregation due to variation in temperature and pH. In order to do the test we took into consideration 3 temperature values: 4 ºC, 20 ºC, 37 ºC. For each temperature we used tests from 6 patients. To determine EAI and ASP, 10 images from each test were processed.

In order to determinate the EAI we had to take into consideration the areas of the surfaces occupied by the aggregated and not aggregated cells. First, the total aria S_{tot} occupied by erythrocyte (aggregated and not aggregated) was calculated and then, through the elimination of the isolated cells (not aggregated), the area of the aggregated cells S_{agreg} was determined. Using the Simagis 3.0 (Smart Imaging Technologies) software, these arias were found beginning with the initial image.

RESULTS

Study of erythrocyte aggregation for different values of temperature.

This is a relevant example for 4 ºC temperature (fig. 2). The images were processed using Simagis (fig. 3).

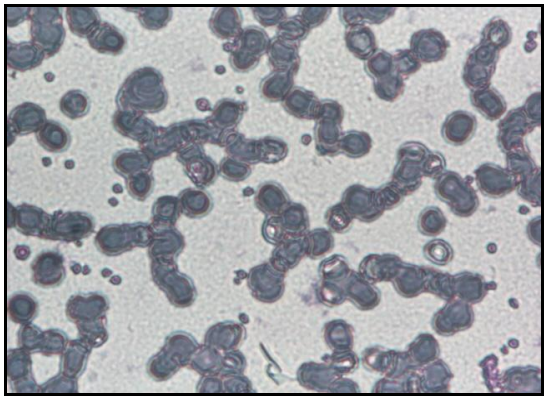
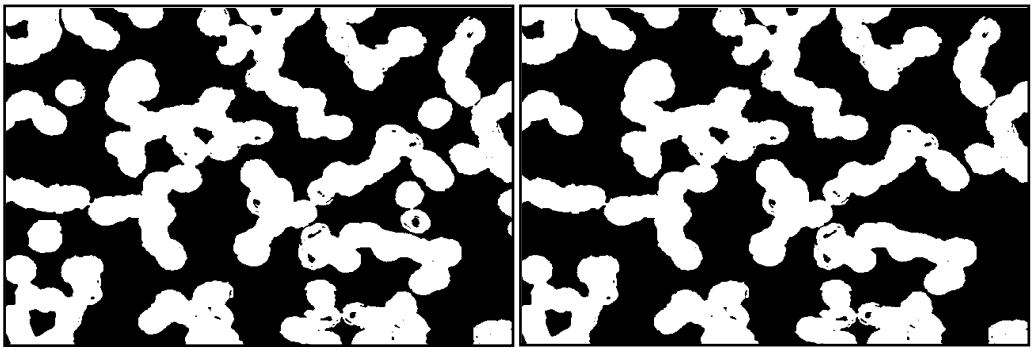


Fig. 2. Image of a blood test at 4 ºC



a. **b.**

Fig. 3. Processed images using Simagis for figure 2
a. for the determination of the total area;
b. for the determination of the surface occupied by the aggregated cells.

TABLE I.
Mean EAI values depending on temperature

Erythrocyte Aggregation Index		
4° C (n = 60)	20° C (n = 60)	37° C (n = 60)
94.5482	98.2622	99.5559

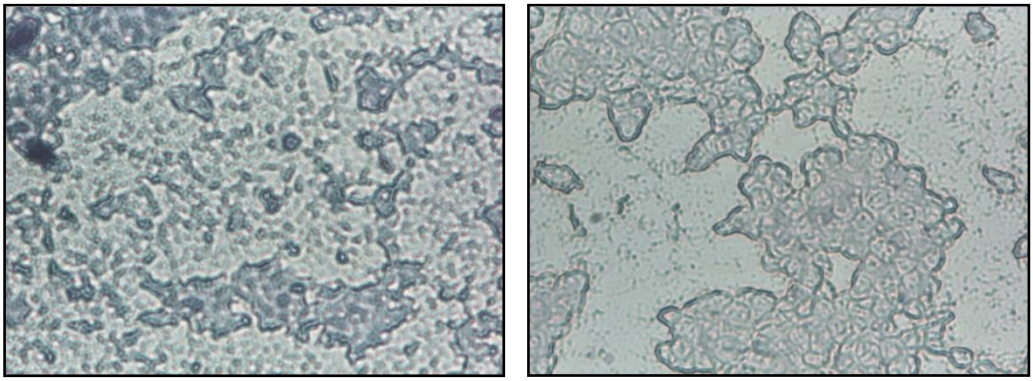
Study of the erythrocyte aggregation for different pH values.

Another study was conducted in order to determine the changes in erythrocyte aggregation depending on pH. In order to do the test we took into consideration 4 pH values: 3, 5, 7 and 9.

Like in the temperature study we processed 10 images each. At pH = 3 and 5 the cells were destroyed and EAI could not be detected (fig. 4). No rouleaux were formed.

For a normal pH = 7 RBC rouleaux were formed. EAI was determined using the same procedure as in the temperature study.

It is to be noticed that at pH = 9 tridimensional rouleaux could form (fig. 5). In this case clusters were also found.



a. **b.**

Fig. 4. Images of RBC aggregates at **a.** pH = 3 and **b.** pH = 5

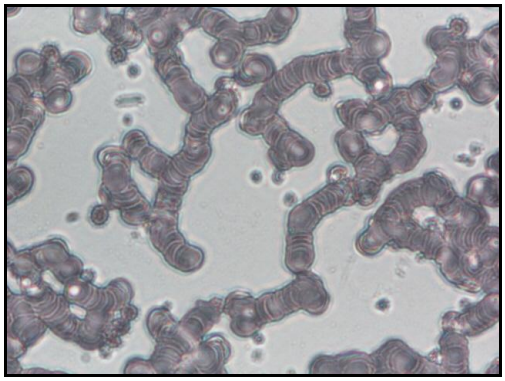


Fig. 5. Image of RBC aggregates at pH = 9

TABLE II.
Mean EAI values depending on Ph

Erythrocyte Aggregation Index	
pH = 7 (n = 60)	pH = 9 (n = 30)
98.5916	99.4342

DISCUSSION

The results show that the increase in temperature induces EAI increase (tab. I) and a decrease in pH a decrease of erythrocyte aggregation (tab. II). It was found that at pH=3 and 5 erythrocytes are destroyed, and at pH=9 RBC clusters were formed. It may be concluded that RBC aggregate shape reflects intercellular interaction and its quantification may thus be another characteristic of RBC aggregation.

As demonstrated, a globular aggregate has an ASP near 1; the longer the aggregate, the smaller the ASP (fig. 6).

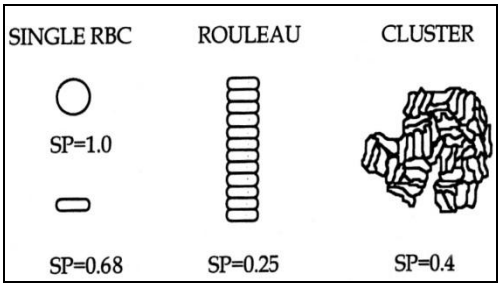


Fig. 6. Different aggregate shapes and corresponding ASP calculated according to equation no. 2 (1).

The theoretical model for rouleau aggregates (fig. 6) provides a useful reference for measuring deviations of RBC aggregate morphology from that of the rouleau structure, since normal blood is characterized by rouleau aggregates, and the formation of RBC clusters characterizes disease states (5).

Increased RBC aggregation was observed in various pathological states, such as diabetes and myocardial infarction, or following trauma.

Although erythrocyte aggregation phenomena were observed for a long time and several results were obtained, this phenomenon is not completely elucidated (6).

It is well known that erythrocyte aggregation is modified by cell deterioration in some physiological or pathological states (7).

Literature data show that the increase in temperature leads to the increase of erythrocyte aggregation. This was supported by Maeda (3) who demonstrated that the increase of temperature between 10°C and 40°C leads to the decrease of blood viscosity and of the stiffness of erythrocyte membrane responsible for the increase of erythrocyte aggregation.

The results on erythrocyte aggregation depending on pH are also satisfactory, being in agreement with Maeda's results (4) that evidence a RBC stiffness at low pH values and an increased aggregation at pH interval 6-9.

CONCLUSIONS

The computerized image analysis method to study the erythrocyte aggregation depending on temperature and pH could be successfully applied, having satisfactory results. We may conclude that the method has the following advantages:

- This method of image analysis is a direct method for measurement of erythrocyte aggregation;
- The Erythrocyte Aggregation Index (EAI) and the Aggregate Shape Parameter (ASP) values are quantitative;
- The method permits the comparison of the results obtained in different conditions (of temperature and pH);
- The method permits the comparison of the results obtained for different patients in a normal state of health or in diverse pathological states.

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CONSERVATIVE TREATMENT OF DENTAL TRAUMA: CASE PRESENTATION-DIRECT ADHESIVE RESTORATION TECHNIQUES OF THE 4 UPPER INCISORS WITH DIFFERENT TYPES OF FRACTURES ON THE CORONAL LEVEL

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CONSERVATIVE TREATMENT OF DENTAL TRAUMA: CASE PRESENTATION-DIRECT ADHESIVE RESTORATION TECHNIQUES OF THE 4 UPPER INCISORS WITH DIFFERENT TYPES OF FRACTURES ON THE CORONAL LEVEL (Abstract): Dental trauma creates serious functional, aesthetic and psychological problems for the patient. The known developments in recent years in the area of materials and techniques related to direct composite resin restorations provide for the dentists direct conservative solutions that allow both morphology and aesthetic restoration and preservation of pulp vitality in many of the clinical situations encountered. A careful clinical examination and an accurate diagnosis (in terms of classification of the trauma) will allow the dentist to choose the most conservative therapeutic solution. We recommend the use of the diagnostic and therapeutic guidelines mentioned in the article. **Keywords:** DENTAL TRAUMA, CORONAL FRACTURE, DIRECTS COMPOSITE RESIN, CORONAL RECONSTRUCTION, and AESTHETICS.

Dental trauma is a fairly frequent condition among school age children and youth. Clinical studies have shown the most common causes of injuries are accidents during playing or sport events and rarely aggression. Moreover, dental traumas create functional, aesthetic and psychological problems for the patients. In these cases the challenge is doubled for the dentist who must restore the affected teeth both on functionally and esthetically. This requires a good understanding of direct restorative techniques including knowledge of color, form, adhesive protocol and laminating techniques.

The progress in recent years in the field of materials and techniques related to direct composite resin restorations provides advantageous solutions for the practitioner versus the indirect restorative therapy used before.

The advantages and disadvantages of dental restorations are as follows:

Direct composite resin restorations: *advantages*: available to any dentist, provides a conservative therapeutic approach, great aesthetic results, very good periodontal tolerance, easy to repair, affordable price; *disadvantages*: the technique is sensitive to any small errors and requires the dentist to have some experience, does not provide good results in case of intense tooth discoloration .

Indirect restorations with ceramic veneers: *advantages:* sufficiently conservative approach, great aesthetic results, great periodontal tolerance; *disadvantages:* minimum two sessions, greater "sacrifice" of tooth substance than with direct methods, several sources for error in technical and clinical stages (preparation, imprinting, and collage), difficult to repair, higher price.

Direct-indirect restorations with composite resins: *advantages:* conservative approach, great aesthetic results, very good periodontal tolerance, better polymerization, easy to repair, affordable price; *disadvantages:* requires technical skills, extended working time, instruments and additional equipment required (specific to dental laboratory).

The types of dental trauma that we can meet are extremely varied: bruises, subluxations, extrusions, lateral luxation, intrusions, avulsion, fractures of the enamel, dentin and enamel fractures, and fractures with coronal pulp chamber opening, crown-root fractures with or without affecting the pulp, root fractures, alveolar fractures.

Recently, a new classification of these injuries was published along with a therapeutic guide for every clinical situation. More information is available on the website of the International Association of Dental Traumatology (IADT):

www.dentaltraumaguide.org

CASE REPORT

A 16 years old male who had a skateboard accident resulting in multiple trauma of the upper incisor group.

Clinical examination revealed the following types of injuries: 1.2 -limited oblique fracture of the enamel at the level of mesio-incisal angle; 1.1- horizontal fracture at the mesial half of the crown, restricted to the enamel; 2.1 horizontal crown fracture of the enamel and dentin; 2.2 crown and root fracture with pulp chamber opening. (fig. 1, 2).

Fig. 1. Front view of the upper traumatized incisors group.

Fig. 2. Pulp exposed 2.2

In accordance with the recommendations of IADT, the treatment plan included three steps: step 1 – emergency treatment; step 2 – rehabilitation treatment; step 3 - clinical and radiological control at 6 months and 1 year for assessment of the

vitality of teeth 1.2, 1.1, 2.1.

During step 1, the treatment included: protection of fracture zones of teeth 1.2, 1.1, 2.1 using composite flow; performing endodontic treatment on tooth 2.2. Due to the destruction of the crown with great loss of tooth substance, this tooth requires the use of a pivot root during the crown restoration stage. Therefore, pulp ablation was performed in this stage, although the literature and treatment guidelines recommend only pulpohtomy (fig. 3, 4); contention tray was made for the patient to wear 7-10 days until the relief of the acute symptoms (fig. 5).

Fig. 3. Checkup of the endo treatment 2.2

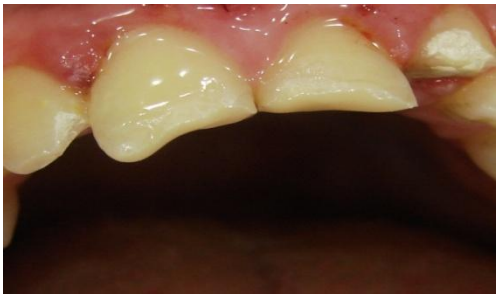

Fig. 4. The clinical aspect after endodontic treatment of tooth 2.2 and sealing the fracture zones in teeth 1.1 and 2.1 with composite flow.

Fortunately, the patient had just completed orthodontic treatment and the orthodontic department was able to provide us with the case study and cast models that have been used to make both the contention tray and the silicon guide during the second restorative stage (fig. 6, 7).

Fig. 5, 6. Mouth guards. Study models.

Fig. 7. Checking the correct positioning of the silicon guide.

Anti-inflammatory and analgesic medication was prescribed.

Second stage of treatment started 10 days later. All four incisors were restored. According to the IADT guidelines our treatment plan included: a silicon guide to

achieve the same shape and size of the incisors. (In order to make this silicon guide we used the casts from the orthodontic department); direct composite resin restorations to the teeth 1.2, 1.1, 2.1 presenting fractures of the enamel and enamel plus dentin areas. Mouth guards protected the teeth during the acute inflammatory period.

The restoration phase involved the specific stages of the adhesive technique:

1. dental surface preparation for collage (beveling the margins, Arkansas Type stones finishing, professional brushing);

2. adhesive stage- IV generation adhesive was used, in three steps: etching - primer adhesive;

3. direct composite resin stratification according to aesthetic principles using composite for each type of restored tissue: dentin, enamel, translucent (fig. 8, 9);

4. finishing and polishing

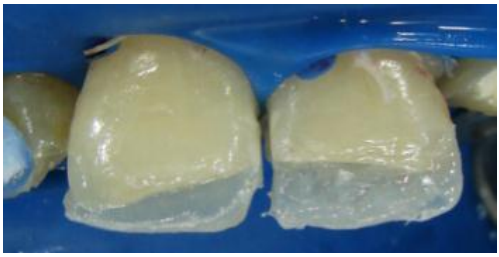

Fig. 8. Palatal wall view- translucent type composite.

Fig. 9. Intermediate phase during the restoration of tooth 1.1

A fiberglass root pivot was used for tooth 2.2 and the tooth crown was restored by direct stratification technique. The loss of substance in tooth 2.2 was at the gingival margin on the palatal side. This required an additional intervention using the electro cautery to highlight the extent of the fracture and achieve appropriate restoration at this level. 1.1, 2.1 were also restored using composite (fig. 10, 11, 12). Check-ups at one and two and a half years later showed satisfactory aesthetical and functional outcomes (fig. 13, 14).

Fig. 10., 11. Buccal and palatal view at the end of the restoration

Fig. 12 Final appearance after completing the restoration of all four upper incisors.

Fig. 13. Clinical aspect 14 months later.

Fig. 14. Follow-up 30 months later.

CONCLUSIONS

Regarding our case and the existing theoretical support, for most dental trauma, current conservative therapeutic solutions allow rapid recovery of the patient and satisfactory aesthetical and functional outcomes.

A careful clinical examination and accurate diagnosis (in terms of trauma classification) allow the clinician to choose the most conservative therapeutic solution. We recommend the use of guidelines for diagnosis and therapy listed above.

The practical application reported in this study confirms the previous studies related to restorative treatment of dental trauma through direct restorative technique. Furthermore, our results showed that implementation of this method for restorative treatments solve the aesthetic and functional problems of a trauma especially in case of the frontal teeth.

Improvement in the quality of materials used for direct restorations and their associated techniques create conditions for the clinician to act quickly and effectively avoiding the indirect restorative solutions (prosthetic), which are more expensive and require longer time.

The study can be a useful tool for researchers and practitioners keen to apply this technique in patients with dental trauma.

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THE MRI FINE EVALUATION OF CERVICAL LYMPHADENOPATHIES AND ASSOCIATED ORAL CAVITY PATHOLOGICAL CONDITIONS

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THE MRI FINE EVALUATION OF CERVICAL LYMPHADENOPATHIES AND ASSOCIATED ORAL CAVITY PATHOLOGICAL CONDITIONS (Abstract): The **aim** of this paper is to point out the value of MRI in diagnostic assessment of cervical lymphadenopathies and associated oral cavity pathological conditions. We used a statistic retrospective method to evaluate the results obtained after MRI examinations of 64 patients diagnosed with oral cavity pathology and cervical lymphadenopathies. In all cases the MRI exams detected the presence of cervical lymphadenopathies, their topoanatomic sites and oral cavity pathological entities that produced them. Due to fine characterization abilities by internal signal variation, MRI is the method of choice in daily practice for diagnosing, evaluation and staging of oral cavity pathology and cervical lymphadenopathies. Due to fine capacities of detecting internal signal variations in soft tissues or visceral parenchyma, MRI has high abilities in diagnostic assessment of oral cavity pathologies being a daily method of choice for diagnosing, evaluation and staging, with great value in any morbid entity. **Keywords:** CERVICAL LYMPHADENOPATHIES, ORAL CAVITY PATHOLOGY, MRI.

Aroused, as a general rule, by infectious lymphatic spread or by granulomatous or tumoral infiltration, cervical lymph-adenopathies are common marks, in daily practice, for diagnosing, evaluation and staging of oral cavity pathology (1).

Since cervical lymphadenopathies are very close related to oral cavity pathology, their presences affect the treatment and in case of malignancy reduce the 5-year survival following the rule of 50%. In a case of oral cavity squamous cell carcinoma the presence of a single cervical lymphadenopathy reduces the 5-year survival by 50%. If cervical lymph-adenopathies are bilateral, 5-year survival is reduced by another 50%. If there is clinically determined cervical lymph-adenopathy fixation or imaging data of extranodal extension expected survival goes down yet another 50% (2, 3).

In fact the clinician and the radiologist must work together as a team in staging oral cavity pathological entities since combined data can gain an 85% diagnostic accuracy (3).

Detecting internal signal variations MRI has high abilities in diagnostic assessment of oral cavity pathologies together with fine staging and evaluation of cervical lymphatic nodes spread (3).

MRI examination of cervical lymph-adenopathies can accurately detect the morphologic and volume changes from the local lymph nodes as a common result of any related anatomic pathologies (3).

The aim of this paper is to point out the value of MRI in cervical lymph-adenopathies examination and to underline the ability of this method, in daily practice for diagnosing, evaluation and staging of oral cavity pathology.

MATERIAL AND METHODS

MRI examinations were performed in cervical lymphadenopathies of 64 patients with oral cavity pathology clinically and biologically assessed and in cases of malignant diseases even with histo-pathological attestation.

The patients were MRI examined at Clinical Image Medical Imaging Center Timisoara using a 1.5 T Siemens unit and a dedicated investigation protocol. The MRI images were achieved as multiplanar SPIN-ECO in T1-wheighted acquisition with fine anatomical details and in T2 or Proton Density acquisition with great value in pathological changes detection. Fast imaging techniques as fast spin-eco and gradient - eco were sequences obtained very rapidly used in pre and postcontrast MRI evaluations. STIR - short tau inversion recovery - and DIFFUSION are very sensible techniques used for imaging subtle lesions detection (4).

The morphologic data: diameters, shape, contour, the presence or absence of central necrosis or cystic degeneration and extra capsular cervical lymph node extension are the MRI used diagnostic criteria of cervical lymphadenopathies (4).

The size criteria used depends on cervical lymph nodes location in one of the seven levels that they are anatomically classified. Level I nodes are the submental and submandibular nodes. Level II, III and IV are the nodes in the internal jugular chain, with level II nodes located from the base of the skull to the hyoid bone, level III nodes located between the hyoid bone and cricoid cartilage and level IV nodes located below the cricoid cartilage. Level V nodes are posterior to the sternocleidomastoid muscle. Level VI nodes are in the deep visceral chain and level VII nodes are in the superior mediastinum. Level II-VII nodes are considered pathologic in size if they are greater than 1 cm. Level I and the jugulo-digastric nodes are considered pathologic in size if they are greater than 1, 5 cm (5).

We used for malignant Hodgkin lymfoma RYE histologic classification and for malignant Non-Hodgkin lymphoma Working Group histological classification with Rappaport terminology equivalents. For precise evaluation of lymphatic nodal spread in malignant lymfoma we used Ann Arbor staging criteria (1).

In cases of oral cavity malignancy we used TNM classification in order to precise imaging tumoral, lymphatic nodes and metastasis staging.

RESULTS AND DISCUSSION

We diagnostic assessed cervical lymph-adenopathies according to size and architecture imaging criteria and we found in almost all cases nodal diameters between 1 and 3 cm corresponding to TNM stage NI.

In a case of malignant Hodgkin lymphoma (fig. 1) we detect and characterized

a group of cervical lymphadenopathies surrounding left carotid artery and adjacent vessels and muscles, with extra capsular spread in regional fatty tissue, as a mass of 6.7/2.4/4.4 cm in so termed carotid fixation.

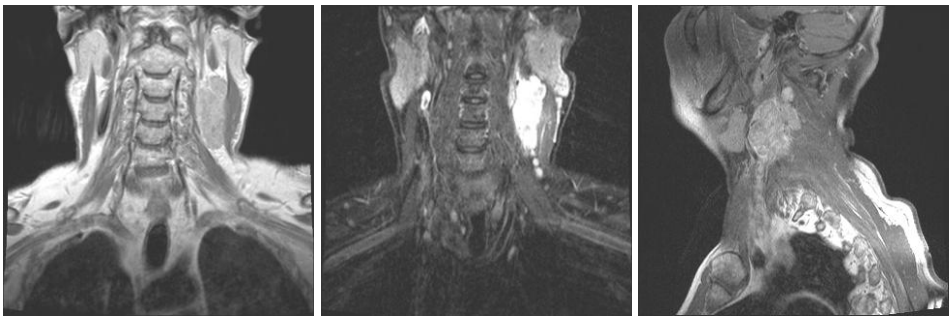

Fig 1. MRI exam of a patient with malignant Hodgkin lymphoma assessed a group of left cervical lymphadenopathies with carotid fixation

MRI cervical lymphadenopathy exam detected 22 cases of malignant cervical lymphadenopathies in patients with malignant lymphoma or oral cavity malignant tumors and 42 cases of reactive lymphadenopathies due to local inflammatory conditions induced by infectious lymphatic viral or microbial spread.

TABLE I

The oral cavity pathology associated with cervical lymphadenopathy

Oral cavity pathology	Number of cases
Malignant lymphadenopathies:	**22**
-carcinoma of the oropharynx	3
-carcinoma of the floor the mouth	3
-carcinoma of the salivary glands	2
-carcinoma of the tongue	2
-malignant Hodgkin lymphoma	
-mixt celularity	2
-nodular sclerosis	3
-limphocitic depletion	1
-malignant non - Hodgkin lymphoma	
-small grade of malignity	2
-intermediate grade of malignity	4
Reactive lymphadenopathies:	**42**
-viral parotidites	8
-tonsilites	14
-vestibular abcesses	8
-acute pharingites	10
-citomegalic inclusion disease	2

From 22 cases of malignant lymphadenopathies, 12 were latero-cervical forms of malignant lymphoma clinically, biologically and histopathological assessed at Hema-

tolology Clinic Timisoara and MRI evaluated at Clinical Image Medical Imaging Center (tab. I).

MRI-scan evaluated the extent of latero-cervical lymphadenopathies in 6 cases of malignant Hodgkin lymphoma: 2 in stage IIA and 4 in stage IIB and in 6 cases of malignant non - Hodgkin lymphoma all in stage IIA.

We evaluated by MRI 10 cases of oral cavity malignant tumors with lymphatic spread cervical lymphadenopathy clinically, biologically and histo-pathological assessed at Clinic of Cranio-Maxilo-Facial Surgery, Timisoara.

In the 3 cases of oropharingeal carcinoma we characterized aT2N1M0 palatine left tonsil localization and a T3N1M0 and a T4N1M base of tongue carcinoma (fig. 2, 3).

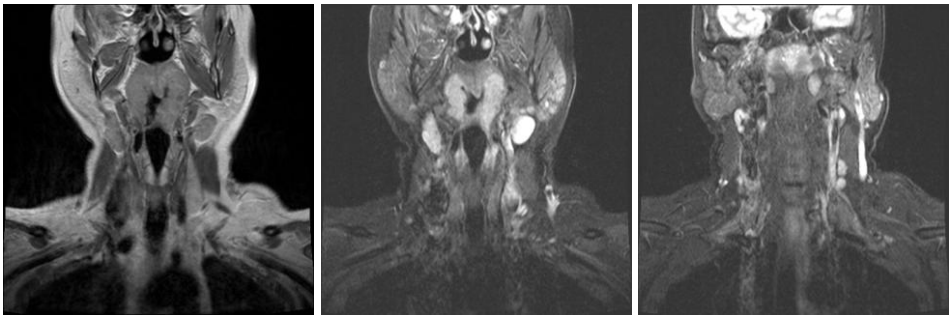

Fig. 2. A case of T2N1M0 palatine left tonsil carcinoma MRI clearly delineated and evaluated together with associated cervical lymphadenopathies

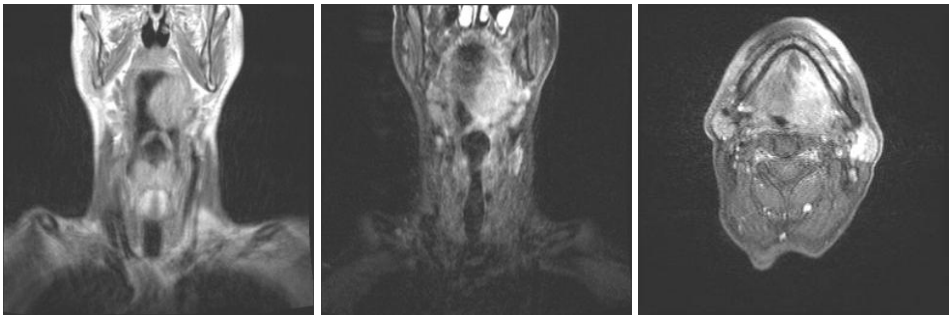

Fig. 3. MRI evaluation of a T3 left base of tongue carcinoma with N1 cervical lymphadenopathies

We detected 2 cases with T2N1M0 carcinoma of the floor the mouth and a T3N1M0 carcinoma with the same localization. MRI salivary glands evaluation revealed 2 cases of parotid gland carcinoma assessed as T2N1M0 and T3N1M0. In 2 cases we detected and evaluated by MRI-scan T2N1M0 carcinomas of the tongue.

We proceeded to MRI exam in 42 cases of nonmalignant oral cavity pathology at clinician indications to exclude tumoral pathology with fine characterization of inflammatory visceral changes and reactive lymphadenopathies detection. The vast majority of patients reclaimed clinical symptoms of viral or microbial infection together with latero-cervical mass or asymmetry due to cervical lymph-adenopathies.

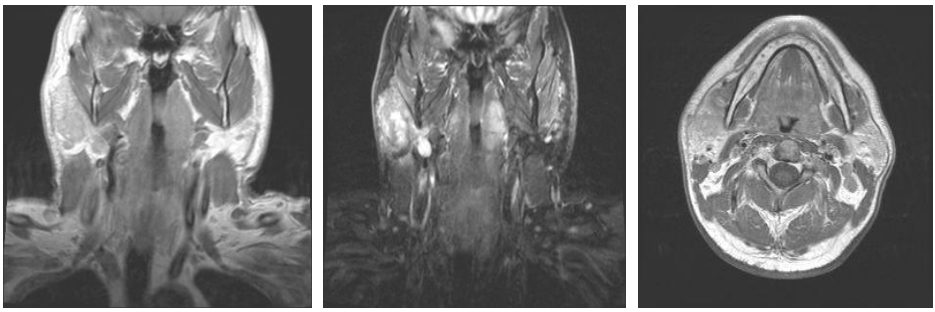

Fig. 4. MRI exam of a T2 right parotid gland carcinoma with N1 cervical lymphadenopathies

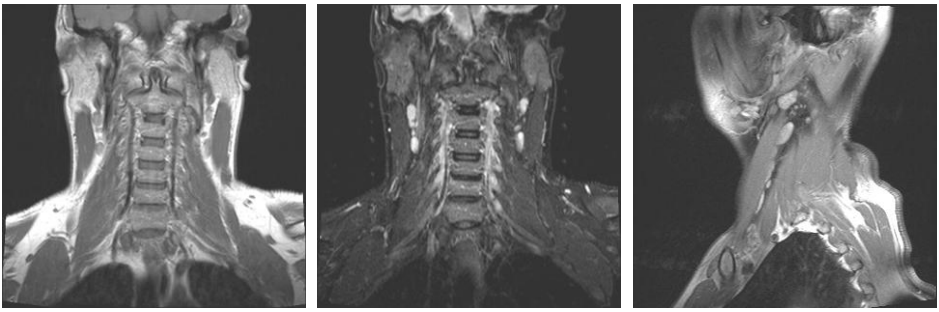

Fig. 5. MRI of a patient with latero-cervical asymmetry due to level III reactive internal jugular chain lymphadenopathies with greater transverse diameter on right side caused by microbial infection spread from a homolateral vestibular abcess

CONCLUSIONS

The size and architecture criteria for cervical lymphadenopathies imaging diagnostic assessment are the best used by MRI to detect, stage and topographic localize their pathological involvement.

MRI has gained a large known indication in malignant lymphoma for precise identification of lymphatic nodal spread, staging and accurately pre and post treatment evaluations.

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USE OF BLOOD MARKERS IN EARLY DIAGNOSIS OF OXIDATIVE STRESS IN AGE RELATED MACULAR DEGENERATION

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USE OF BLOOD MARKERS IN EARLY DIAGNOSIS OF OXIDATIVE STRESS IN AGE RELATED MACULAR DEGENERATION (Abstract): Age related macular degeneration (AMD) is the most frequent cause of irreversible blindness in the elderly population, affecting approximately 30-50 million people around the world. **Aim**: to establish the role of oxidative stress in retinal structural lesions. **Materials and methods**: It is a case-control study that included 19 patients diagnosed with AMD. Depending on the severity of the diagnosis, patients were divided into 3 groups: group 1 - mild AMD, group 2 – moderate, atrophic AMD, group 3 – severe, neovascular AMD. They were followed by assessment of visual acuity, optical coherence tomography (OCT) and oxidative stress markers like superoxide-dismutase (SOD), thiobarbituric acid reactive substances (TBARS) and inflammatory marker – C-reactive protein (CRP). **Results**: Risk factors involved in patients with AMD are arterial hypertension, smoking, hyperlipidemia and diet poor in antioxidants, as revealed in the questionnaire. Retinal thickness assessed by optical coherence tomography showed that values in patients with severity level 2 is within normal limits, while in patients with severity level 3, these values are significantly increased due to macular edema. The mean values of SOD, TBARS and CRP in the studied group were significantly higher compared to controls, being higher in group severity level 3. **Conclusion**s: This study shows the role of oxidative stress and inflammation in retinal structural lesions in AMD and the importance of blood markers in early detection of oxidative stress and thus of retinal lesions in this disease. **Keywords**: AGE RELATED MACULAR DEGENERATION, OXIDATIVE STRESS, RETINAL THICKNESS.

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the elderly population, affecting approximately 30-50 million people (1). AMD consists of degenerative and inflammatory lesions of the macular region, occurring in a previously normal eye and resulting in deterioration of central vision and macular function (2). The disease begins as a non-neovascular or dry stage, which can progress to geographic atrophy or macular neovascularisation. Recently, the research has focused on the risk factors that could be involved in the development of AMD, such as oxidative stress, inflammation and phototoxic stress (3, 4).

The aim of this study is to establish the role of oxidative stress in retinal structural

and functional changes in AMD. Also, it is meant to highlight the role of blood markers in early diagnosis of oxidative stress and consequently in early identification of retinal lesions.

MATERIAL AND METHODS

This is a case-control study that included a group of 19 patients diagnosed with AMD, selected from the „N. Oblu Hospital" in Iaşi, and two control groups, one with younger controls and another with controls with similar ages as the cases. Patients with systemic and ocular diseases that could increase oxidative stress, such as diabetes, inflammatory and infectious diseases, and vascular occlusions were excluded.

According to the law of patients' rights, they signed an informed consent and completed a questionnaire concerning risk factors, associated diseases, living conditions, subjective complaints and an Amsler grid. A score was calculated for each patient.

During a follow-up period for a median of 8 months, we recorded the visual acuity, anterior pole biomicroscopy, posterior pole appearance (Volk 78 D lens), Optical Coherence Tomography (OCT) and laboratory tests. As regards the laboratory tests, we followed the oxidative stress markers such as thiobarbituric acid reactive substances (TBARS), antioxidant enzymes - superoxide dismutase (SOD), and markers of inflammation - C-reactive protein (CRP).

Depending on the severity of the diagnosis, patients were divided into 3 groups according to the classification of "Age-Related Eye Disease Study" (AREDS) of the U.S. National Eye Institute: group 1 - mild AMD, group 2 – moderate, atrophic AMD, group 3 - severe neovascular AMD. According to the appearance of the posterior pole and the OCT results, we maintained the same classes. In the study group there were included only patients with severity level 2 and 3. The retinal thickness and retinal nerve fiber layer were measured with Spectral OCT OPTOPOL in 9 quadrants.

SOD activity was determined by xanthine oxidase method, TBARS were quantified by colorimetric method and CRP measurement was made by imun-turbidometry with calibrator.

The statistical analysis (performed with the Statistical Programme 7.0), was intended to highlight significant correlations between the severity of the diagnosis and results of the OCT, visual acuity and results of the blood tests. The statistical comparison was made with "Difference tests" from the sub-set of descriptive statistic programs, being significant those differences which stood at a safe threshold of 95% ($p < 0.05$).

RESULTS

We investigated a group of 19 subjects, 8 males (42.11%) and 11 women (57.89%) with a mean age of 72.47 ± 7.74 years (59 to 88). The first control group (older group) included 11 subjects (3 men and 8 women), with ages ranging between 60 and 85 years. We used the second control group (young group), which comprised of 40 subjects (11 men and 29 women), aged between 28 and 60 years, in order to compare the laboratory results.

We evaluated the risk factors in the study group and in the control group and we showed that there was a higher frequency of arterial hypertension (HTA), smoking, lack of antioxidants and UV exposure in the study group (fig. 1).

There were enrolled 16 patients with ne-

ovascular AMD, severity level 3, and 3 patients with dry form, severity level 2. We present the average retinal thickness, depending on the severity of diagnosis (tab. I).

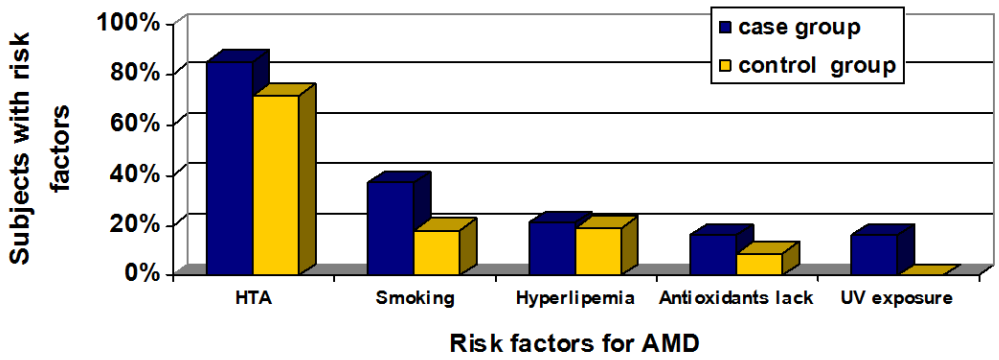

Fig. 1. Risk factors in the study and control groups

There were enrolled 16 patients with neovascular AMD, severity level 3, and 3 patients with dry form, severity level 2. We present the average retinal thickness, depending on the severity of diagnosis (tab. I).

TABLE I

Indicators of the average retinal thickness in the two severity groups

	Severity level 3 – retinal thickness			**Severity level 2 – retinal thickness**		
Quadrant	**Average** (μ)	**Minimum** (μ)	**Maximum** (μ)	**Average** (μ)	**Minimum** (μ)	**Maximum** (μ)
Quadrant 1	381.62	164.00	620.00	241.33	173.00	307.00
Quadrant 2	372.68	224.00	547.00	263.33	205.00	327.00
Quadrant 3	357.00	180.00	623.00	245.33	198.00	323.00
Quadrant 4	377.81	257.00	549.00	265.66	247.00	302.00
Quadrant 5	358.62	187.00	571.00	306.66	247.00	350.00
Quadrant 6	307.06	185.00	444.00	228.33	214.00	252.00
Quadrant 7	317.37	156.00	592.00	226.00	158.00	297.00
Quadrant 8	318.87	210.00	472.00	244.66	225.00	263.00
Quadrant 9	340.75	266.00	481.00	280.33	246.00	318.00
Average	347.27	228.88	495.00	257.46	231.12	295.50

In patients with the neovascular form, mean retinal thickness was 347.27 μ, signifying the presence of macular edema (over 340μ), with a maximum of 495μ.

In patients with dry form, mean retinal thickness was 257.47μ, falling within the normal range. Next we evaluated the retinal nerve fiber layer thickness, depending on the severity of diagnosis. The average thickness was much lower than normal both in severity level 2 group and severity level 3 group.

The average visual acuity is significantly higher in subjects with a diagnosis of severity level 2 (0.67) compared with 0.25 in those with level 3 severity (p = 0.005). The score was significantly higher in group severity 3 (39) compared to group severity 2 (28).

Depending on the aspect of the posterior pole and the OCT results, the average thickness of the retina is significantly higher in cases with severity level 3 compared to those with severity level 2 (fig. 2).

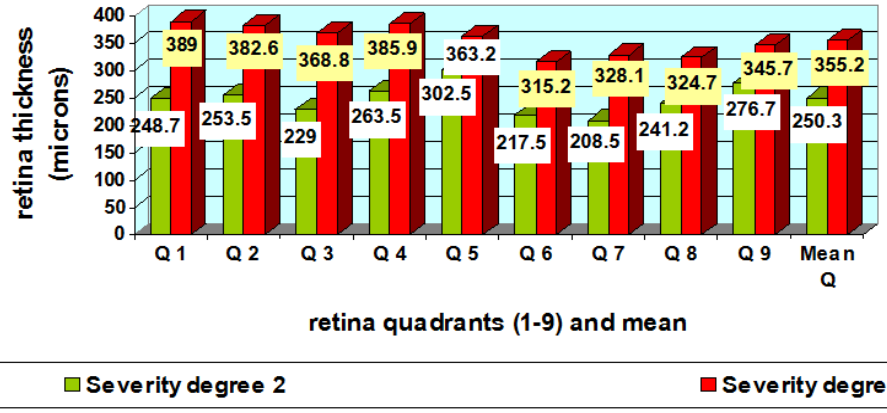

Fig. 2. The average thickness depending of the OCT results

We determined the levels of SOD, TBARS and CRP in the study group, older control group, and in the younger control group. To highlight the differences between groups it was used the t Student test, appreciating as significant those with p <0.05.

TABLE II

The mean values of laboratory tests

Groups	Number	SOD (U/ml)	TBARS (nmols/ml)	CRP (mg/dl)
Study group	19	331.8	4.6	3.2
Older control group	11	269.5	3.9	1.5
Younger control group	40	196.1	3.1	0.9

Comparing the mean values in the three groups, we found that the mean values of SOD, TBARS and CRP were significantly higher in the study group compared to con-

trols; the mean values of SOD and TBARS were significantly higher in the older control group compared to younger control group (tab. II). We also found that the values were higher in the group with severity 3 diagnosis, compared to severity 2 (fig. 3, 4).

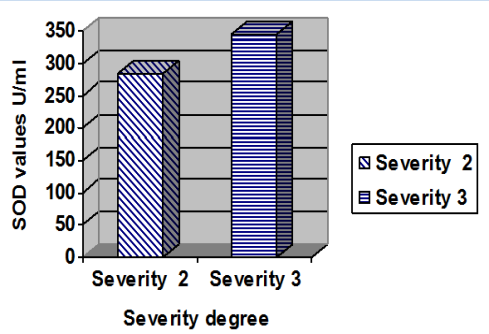

Fig. 3. The mean values of SOD in group severity 3 and 2

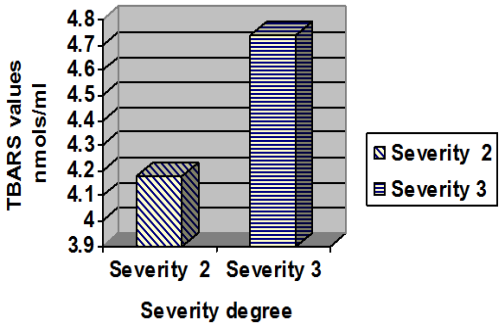

Fig. 4. The mean values of TBARS in group severity 3 and 2

DISCUSSION

We found that AMD correlated with certain risk factors, like arterial hypertension, smoking, hyperlipidemia and low antioxidants diet, which is consistent with the literature (1, 2).

Comparing the mean values of retinal thickness depending on the severity of diagnosis, we found an average value of 347.27 μ in the group of patients with severity level 3, significantly higher ($p = 0.02$) compared to those with severity level 2 (257.47 μ). The average thickness of the nerve fibers layer is 32.29 μ (ranging between 16.78 and 49.78 μ), being much lower than normal (90μ). It can be noticed that in severity level 2 group the average thickness of the retina is normal in all quadrants, while in those with severity level 3, it is above the upper limit in 5 of the 9 quadrants. These data are consistent with other studies published in the literature. Kolb (5) studied 75 eyes with neovascular AMD, treated with intravitrean drugs against vascular endothelial growth factor (anti-VEGF). Mean retinal thickness was higher than normal and its reduction was observed after treatment. Garas (6) has shown that AMD affects the macular thickness parameters in eyes without glaucoma.

We showed an increase in antioxidant enzymes (SOD) in the study group (331.8 U/ml) compared with the two control groups (young - 196.1 U/ml and older - 296.5 U/ml). SOD is one of the main antioxidant systems, that increased by a feedback mechanism, trying to keep the balance between antioxidants and free radicals. Dong (7) hypothesized that SOD protects retinal cells from oxidative stress. The results are consistent with the study by Imamura (8) who showed that the retinas of mice deficient in SOD1 had several key elements of human AMD. Kasahara (9) showed that homozygous mice deficient in SOD2 present morphological changes in the RPE and Bruch membrane and accumulation of oxidized proteins. Jia (10) found elevated levels of SOD and malondialdehide (MDA) in patients with AMD as a compensatory response. Shen (11) showed an increase in SOD activity compared to

the control group, along with a decrease in antioxidants. In the POLA study (12) the authors showed that another antioxidant enzyme, glutationperoxidase, increases in patients with AMD.

In this study we evaluated TBARS values, one of the earliest markers of oxidative stress. However, this marker has been less studied in relation to AMD. The mean value of TBARS in the study group was significantly higher (4.6 nmols/ml) than that in the control groups (young control group: 3.0 nmols/ml and older control group: 3.9 nmols/ml), which supports the idea that oxidative stress is involved in the etiopathogenesis of this disease. The mean SOD and TBARS values were significantly higher in elder control group compared with younger control group (SOD: 269.5 vs. 196.05 U/ml, TBARS: 3.9 *vs.* 3.0 nmols/ml). The mean values of laboratory tests were significantly higher in the group of subjects with severity level 3 compared to severity level 2.

To demonstrate the role of inflammation in AMD, we compared the mean values of CRP. Mean CRP was significantly higher in the study group (3.2 mg/dl) compared with controls (1.5 mg/dl). These data are consistent with other studies, showing an increased value of this marker (13, 14, 15, 16). The Rotterdam Study (15) showed that elevated CRP levels at baseline were associated with progression of AMD. Our study confirmed that elevated CRP levels were associated particularly with advanced form of AMD. Seddon (17) showed that CRP values were higher in subjects with severe maculopathies.

CONCLUSIONS

This research highlights the role of oxidative stress and inflammation in retinal structural lesions of age-related macular degeneration and the role of antioxidant enzymes in the early detection of oxidative stress. Risk factors involved in patients with age-related macular degeneration are arterial hypertension, smoking, hyperlipidemia and diet poor in antioxidants, as revealed in the questionnaire. Retinal thickness assessed by optical coherence tomography showed that values in patients with severity level 2 is within normal limits, while in patients with severity level 3, these values are significantly increased due to macular edema. Oxidative stress and inflammatory markers are elevated in the study group compared with control groups. This demonstrates that oxidative stress in relation to inflammatory stress is involved in the pathogenesis of age-related macular degeneration.

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PERIPROSTHETIC FEMORAL FRACTURES – EVALUATION OF RISK FACTORS

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PERIPROSTHETIC FEMORAL FRACTURES – EVALUATION OF RISK FACTORS (Abstract): Endoprosthetic hip arthroplasty is a surgical intervention that significantly improves the quality of the patient's life, with excellent long term results, but with an increased risk of major complications. One such complication is the periprosthetic femoral fracture, which may occur in both young and old patients due to several risk factors typical to the elderly (osteoporosis, fall risk), cementless press-fit implants, implant loosening, and revision arthroplasty. **Material and methods:** This study tries to evaluate the risk factors for periprosthetic femoral fractures treated at the Orthopedics and Trauma Department of the Iaşi Rehabilitation Hospital. Retrospective evaluations were conducted on 3454 patients with primary hip arthroplasty and 167 patients with revision hip arthroplasty, the surgical procedures being performed between January 1994 and March 2012. The study was conducted on 47 patients (17 males and 30 females, mean age 68 years) with periprosthetic fracture. **Results:** The incidence of periprosthetic femoral fractures in primary arthroplasty group was 0.89% (0.63% intraoperative and 0.26% postoperative), with a considerably higher incidence for cementless implants (81.39%), and 9.58% in revision arthroplasty group (8.38% intraoperative and 1.19% postoperative). Vancouver type B was the most frequent, both in operative and postoperative revision arthroplasty and in postoperative primary arthroplasty. 88% of the fractures were surgically treated, with a satisfactory postoperative course both in early and long-term follow-up. **Conclusions:** Periprosthetic femoral fractures result in increased morbidity and mortality rate, are difficult to treat, and require complex therapeutic strategies depending on risk factors, fracture type, bone stock, and implant stability. **Key words:** HIP ARTHROPLASTY, PERIPROSTHETIC FRACTURES, REVISION ARTHROPLASTY

The improvement in lifestyle entailed the necessity to improve the quality of patient's life up to an advanced age by means of pain relief and a normal functioning of the hip joint, two goals achieved by endoprosthetic hip arthroplasty. There is an increase in the number of postoperative complications directly proportional to the number of arthroplasties performed given the extension of its indications in both aged and young patients suffering from congenital dysplasia or aseptic necrosis of femoral head. One of the major complications is periprosthetic femoral fracture, with an increased incidence in both age groups, due to the significant risk of osteoporosis in the elderly, high-energy trauma in the young patients who are physically very active, and to a rise in the number of revision arthroplasties (1).

The risk factors for these fractures share

a common feature, namely a poor bone quality and reduced mechanical strength. An etiological classification of these risk factors as local and systemic is generally useful, although they often coexist.

Periprosthetic femoral fractures are more frequent in patients suffering from rheumatoid polyarthritis and severe osteoporosis, but may also occur against the background of other diseases such as osteomalacia, Paget's disease, osteogenesis imperfect, thalassemia, and some neuromuscular disorders such as poliomyelitis or Parkinsonism. Local risk factors for operative fractures include the use of cementless press-fit femoral components, proximal femur deformity, and revision arthroplasty.

Local risk factors for postoperative fractures are osteoporosis, periprosthetic osteolysis, femoral component loosening (2), femoral component malalignment, cortical perforation undetected during surgery, and infection. The most frequent cause of periprosthetic femoral fracture is a minor trauma caused by a same height fall. Adolphson et al. have reported a low-energy trauma in 88% of the fractures studied, (28 out of 35) (3). Cooke and Newman have reported a major trauma only in 8% (6 out of 75) of the cases (4). The incidence reported for periprosthetic femoral fractures is of 0.1% - 7.8%, depending on the reported series (1, 5, 6, and 7). Berry noted an incidence of postoperative periprosthetic femoral fractures of <1% after primary arthroplasty and of >7.8% after revision arthroplasty (5). Mayo Clinic reported an incidence of 0.6% in cemented total hip arthroplasty and 0.4% in the cementless ones (6). Lindahl et al. reported an incidence of 0.4% after primary arthroplasty and 2.1% after revision arthroplasty (1). In a study of 1442 cemented primary arthroplasties with a risk factor of 2.5%, Löwenhielm et al. reported an annual incidence between 0% and 1.2% (7). During primary arthroplasties, intraoperative periprosthetic fractures have been estimated to approximately 1% of the cemented stems (8, 9, 10) and between 3% and 20% of the cementless ones (11, 12, 13). During revision arthroplasties, periprosthetic fractures seem to follow similar patterns, but their occurrence is more frequent, the incidence of operative fractures being of 6.3% (14) for the cemented cases and 17.6% for the cementless ones (15).

MATERIAL AND METHODS

The study was conducted on 3454 patients with primary endoprosthetic hip arthroplasty and 167 patients with revision arthroplasty admitted to the Orthopedics and Trauma Department of the Iaşi Rehabilitation Hospital between January 1994 and March 2012. The retrospective evaluation was done on 47 patients with periprosthetic fractures, 36 intraoperative and 11 postoperative. This study investigated the incidence of periprosthetic fractures in relation with various local and general risk factors, type of arthroplasty, pathology requiring the primary surgical intervention, demographic data (age and sex), and time of postoperative fracture.

Periprosthetic fractures were assessed based on standard radiographs and categorized according to the Vancouver Classification System. Operative periprosthetic femoral fractures are divided into three types based on fracture location: A – proximal metaphyseal without diaphyseal impaction; B – diaphyseal; C – diaphyseal-metaphyseal distal fracture, further subdivided into three subtypes: 1). cortical perforation, 2). non-displaced fracture, and 3). displaced unstable fracture.

Postoperative periprosthetic fractures are classified into:

- A - trochanteric region with A(G) - Greater trochanter and A(L) – Lesser trochanter;
- B – around or distal to the stem, further divided into: B1 (stable prosthesis), B2 (unstable prosthesis), B3 (bone stock inadequate);
- C – well distal to the stem tip.

Other classification systems were used to assess the stability of the cemented and cementless femoral stems. Stability of cemented stem was evaluated according to the classification criteria defined by Harris et al. (16), while Engh and Massin (17) classification was used to assess stability of the cementless stem. According to these classifications, *loosening* is defined as stem displacement, visible on radiograph plates by means of either radiotransparent lines between the cement or the femoral stem and bone, or the presence of cement fracture, and/or the presence of a radiotransparent area which comprises 50 to 100% of the bone-cement gap size.

RESULTS

The prevalence of periprosthetic fractures was 1.29% of the total number of primary and revision arthroplasties, with an incidence of 0.89% (31 cases) in primary arthroplasty and 9.58% (16 cases) in revision arthroplasty. According to the time of occurrence, in primary arthroplasty there were 22 intraoperative (0.63%) and 9 postoperative periprosthetic fractures (0.26%). As to revision arthroplasty of the hip, 14 operative (8.38%) and 2 postoperative fractures (1.19%) were found. These results are in agreement with the statistical data reported in the literature (5).

The etiological assessment of hip arthroplasty showed degenerative pathology in 31 cases (65.95%), stage III-IV aseptic necrosis in 5 cases (10.63%), and femoral neck fracture in 11 cases (23.40%).

According to the type of implant used, periprosthetic fractures occurred in 37 total hip arthroplasties (THA) and in 10 hemiarthroplasties of which 4 bipolar and 6 cervocephalic (unipolar). The incidence of periprosthetic fractures was significantly higher in cementless (cless) arthroplasty – 81.39% (38 cases) compared with cemented (cem) arthroplasty– 18.7% (9 cases), as can be seen in the graphical representations below (fig. 1, 2).

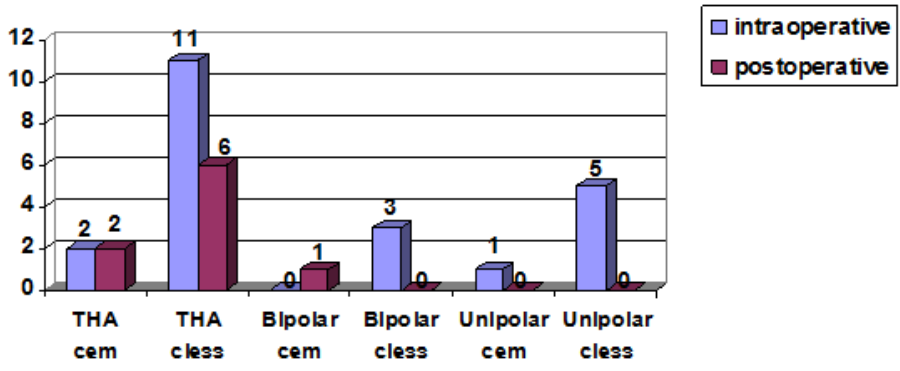

Fig. 1. Incidence of fractures in primary arthroplasty according to the implant type

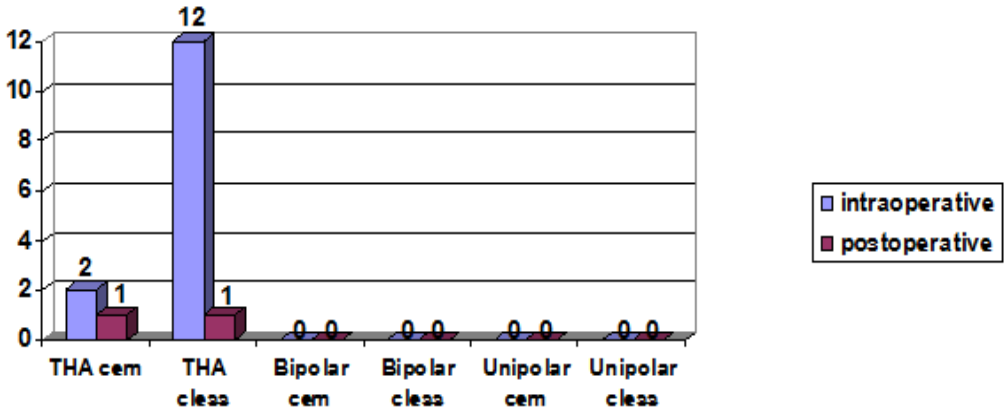

Fig. 2. Incidence of fractures in revision arthroplasty according to the implant type

The time of occurrence of postoperative periprosthetic fractures was: early in 5 cases (the first six weeks), 1-3 years in 3 cases, and after 6 years in 3 cases. In all these 13 cases fractures were caused by low-energy trauma, by same height falls.

In addition, the following associated risk factors have been monitored: osteoporosis in 19 cases (40.42%), rheumatoid polyarthritis in 5 cases, ankylosing spondylitis in 3 cases. Early or late postoperative periprosthetic fractures have the following local risk factors: cementless press-fit stems with cortical perforation undetected during surgery, osteolysis and loosening. Operative periprosthetic fractures occur as a result of press-fit femoral components, local osteoporosis, and brutal manipulation. In revision arthroplasty the most frequently met are osteolysis and loosening with a more or less severe associated cortical defect. Another cause is a specific infection (TB) (fig. 3).

Patients' mean age was 68 years (range 53 - 79 years) for postoperative fractures, 62 (range 40 – 82 eyars) for intraoperative fractures in primary arthroplasties, and 58 (40 - 67 years) in revision arthroplasties. Thirty patients (63.8%) were females.

Using the Vancouver Classification System the fractures were categorized as follows: 1). intraoperative in primary arthroplasties: 9 A2 fractures, 6 B1 fractures, 7 B2 fractures; 2). intraoperative in revision arthroplasties: 2 A2 fractures, 2 B2 fractures, 6 B3 fractures, and 1 C2 fracture; 3). postoperative in primary arthroplasties: 6 B1 fractures, 3 B2 fractures; and 4). postoperative in revision arthroplasties: 1 B3 fracture and 1 C fracture.

The therapeutical strategy depended on fracture type, time of fracture, implant and fracture stability, and bone stock. Consequently, 5 intraoperative B1 fractures were treated conservatively by forbidding leaning, 4 revision arthroplasties necessitated osteoplasty with structural allograft (25%), one revision arthroplasty required osteosynthesis with screwed plate, but most frequent was open reduction and fixation with metallic wires or Dahl Miles cables. The course was satisfactory in all cases, with consolidation and progressive regaining of articular mobility.

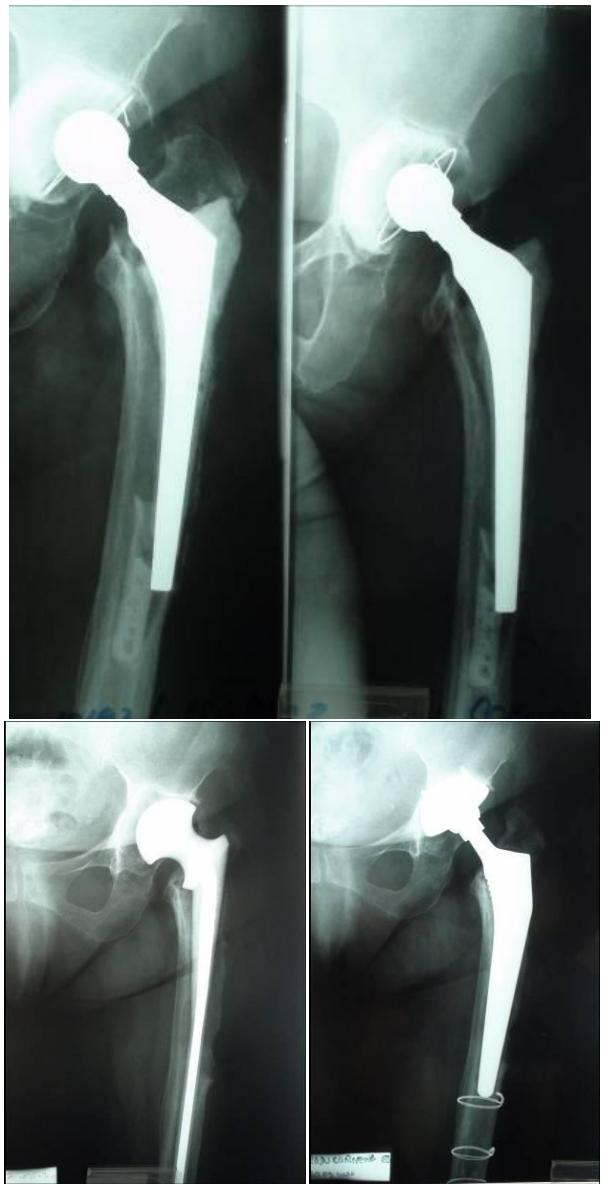

Fig. 3. Septic (specific) loosening; 1st step revision: acrylic spacer; 2nd step revision: uncemented total arthroplasty with intraoperative periprosthetic femoral fracture Vancouver B3.

DISCUSSION

The management of periprosthetic femoral fractures includes the identification of local and systemic risk factors, an accurate preoperative assessment, and an adequate therapeutical indication.

The systemic risk factors determine a decrease in bone quality and mechanical

strength and increase the likelihood of intra- and postoperative femoral fractures occurrence. Among these, the most frequent are postmenopausal and senile osteoporosis and rheumatic conditions (rheumatoid polyarthritis).

The local risk factors for intraoperative fractures include the use of cementless press-fit femoral components, proximal femur deformity, and revision surgery. The cementless femoral component may cause fracture as a result of overworking and additional forces distributed to the proximal femur during the press fit impaction. The deformities of the proximal femur, as can be seen in hip dysplasia, pseudoarthritis, and prior surgical interventions, may also increase the risk for intraoperative fracture. Revision arthroplasty has a higher incidence of intraoperative fracture than primary arthroplasty. During revision arthroplasty the femoral bone mass is often deficient due to multiple prior interventions or following infections or osteolysis. A narrowed cortical vein in relation to the medullar duct of the femur represents a risk factor in intraoperative fractures. Revision arthroplasty requires reconstruction with prostheses larger in diameter and length, often resulting in a higher intraoperative fracture rate due to excessive femoral hole and perforation.

The unrecognized cortical perforation during hip arthroplasty is an important risk factor in postoperative femoral fractures. Accidental perforation is most likely to occur during channel preparation and drilling. The impingement of femoral stem against the endosteal cortex may also increase the stress levels. This situation can occur as a result of the varus malalignment of femoral prosthesis and impingement in the lateral cortex.

Periprosthetic osteolysis is another local risk factor associated with postoperative fractures and it has become increasingly more frequent. The loosening of the femoral component is another significant local risk factor and it has been identified in 75% of the patients with postoperative periprosthetic femoral fractures in a study conducted by Bethea et al. (2). Duncan and Masri have reported loosening in 82 % of their cases (18).

CONCLUSIONS

Our study confirms the importance of risk factors assessment in periprosthetic femoral fractures in primary and revision hip arthroplasty, and points out the high risk of fracture when using cementless implants.

The main goal in the prevention of periprosthetic fractures is a rigorous preoperative planning in order to anticipate any potential trap or problem that may appear during surgery. Preparation is paramount in dealing with these problems. Meticulous history, rigorous clinical investigation (scars and articular rigidities which may require special techniques for surgical exposure and intraoperative manipulation of the limbs), as well as an accurate specific imaging examination with assessment of deformities, hip architecture and degree of osteoporosis are essential in the prevention of intraoperative fractures in primary arthroplasty.

In revision arthroplasties radiographic evaluation must focus on the degree of bone deficit, cortical vein discontinuity, and possible deformities.

Osteodensiometry scans and an adequate antiosteoporotic treatment are the

main means of preventing postoperative fractures.

The decision concerning the therapeutical strategy and surgical indications for periprosthetic femoral fractures is based on fracture level, patient's remaining bone stock, and stem stability. The objectives of periprosthetic fracture treatment include: restoring the anatomical alignment, recovery of the adequate bone stock, creation of a stable prosthesis, early mobilization of the patient, quick consolidation of the fracture, and the return to the original function prior to fracture.

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POSTTRAUMATIC STRESS DISORDER: NEUROENDOCRINE AND PHARMACOTHERAPEUTIC APPROACH

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POSTTRAUMATIC STRESS DISORDER: NEUROENDOCRINE AND PHARMACOTHERAPEUTIC APPROACH (Abstract): Posttraumatic stress disorder (PTSD) is represented by the development of characteristic symptoms, that appear following direct/indirect exposure to a traumatic event in which physical harm was threatened, witnessed or experienced. PTSD can also occur after the unexpected death of a family member or close friend, following a serious harm or threat of death or injury to a loved one, or in case of divorce or unemplyoement. It occurs in 1% - 4% of the population. As neuroendocrine pattern, PTSD is characterized by abnormal low cortisol levels and higher than normal epinephrine and norepinephrine levels. In chronique forms a total decrease of the hippocampal volume, was found, region of the brain involved in processing memories and in the memorization process. Symptoms are grouped in three main categories: re-experiencing the event, accompanied by anxiety, nightmares and flashbacks; persistent avoidance of any reminders of the event, feeling detached or estranged from others; persistent anxiety and/or physical reactivity. As treatment, besides various psychotherapy techniques, various classes of psychotropic drugs are used, such as morphine, antipsychotics, usual or atypical antidepressants, anticonvulsants, to reduce anxiety, avoidance, nightmares and hyperexcitability. **Key-words:** POSTTRAUMATIC STRESS DISORDER (PTSD), CORTISOL

DEFINITION AND HISTORY

Posttraumatic stress disorder is a severe anxiety disorder that may develop after exposure to any event those results in psychological trauma. The event involves the threat of death to oneself or to someone else or to one's own or someone else's physical, sexual, or psychological integrity, overwhelming the individual's ability to cope.

One of the first descriptions of PTSD was made by Herodotus, who described an Athenian soldier with no war injury, but who became blind after witnessing the death of a fellow soldier, during the Battle of Marathon.

In the early 1800's military medical practitioners diagnosed soldiers with "exhaustion" after the stress of battle. One-tenth of the mobilized Americans were hospitalized for mental problems, between 1942 and 1945, and after thirty-five days of uninterrupted combat, 98 % of them suffered psychiatric disturbances of various degrees (1).

Modern understanding of PTSD situates in the 1970s, especially as a result of expe-

riencing with US veterans of the Vietnam War. The term PTSD was adopted in the mid 1970s and recognized in 1980. Earlier terms used for PTSD are: stress syndrome, shell syndrome, battle fatigue, railway spine, traumatic war neurosis.

NEUROENDOCRINE ASPECTS

To react appropriately to danger, one must rapidly trigger a complex set of behavioral responses. The locus ceruleus, located in the dorsal pons has a crucial importance in nervous system responses to threat. Its broad connections with cortical structures – hippocampus, hypothalamus, amygdala and spinal cord involve locus ceruleus in affective, cognitive and motor responses to acute stress. Activation of locus ceruleus neurons stimulates norepinephrine secretion, modulating behavioral responses to accute stressors (2, 3).

Besides activation of noradrenergic system, exposure to acute stress determines neuroendocrine changes, modulated by the HPA axis. Studies revealed low cortisol levels in PTSD patients (war veterans, holocaust survivors, rape victims, individuals exposed to a natural disaster) when compared to controls (4, 5).

Several studies reported that in response to dexamethasone, individuals with PTSD show a more intense suppression of ACTH and cortisol release than normal controls (6,7). Some studies showed changes in hippocampal size (reduction of hippocampal size in PTSD subjects, compared to controls) and function, which may be an important feature of chronic PTSD (8).

Sometimes is difficult to establish which of the biological responses are uniquely associated with PTSD, and which may be included in the range of normal stress responses.

SIGNS AND SYMPTOMS

Symptoms of PTSD usually develop in the first three months after the trauma, but sometimes they may not surface until months or even years have passed. The symptoms often continue for years after the trauma, or they may subside and return later in life, if an event triggers memories of the trauma (1). Usually, symptoms are easy to identify, resembling to stress, anxiety and depression.

Signs and symptoms, which are characteristic for PTSD, if they have lasted for a month or more after the event, include: re-experiencing the event, with recurrent and unwanted memories of the event, nightmares of it, flashbacks (acting/feeling as though the event is happening again), distress and fear when reminded of the event and psychological reactivity – feeling jumpy, startled or anxious, when reminded of the event; persistent avoidance of any reminders of the event, the subject is avoiding thinking or talking about the trauma, is avoiding activities, places or people reminding of the event, it has no memory of an important aspect of the event, is detached or estranged from others, has a limited range of emotions and the feeling that he will not live long; persistent feelings of anxiety and/or physical reactivity – difficulty of falling or staying asleep, is cranky, irritable or angry, has attention and concentration problems and an exaggerated startle response (2, 9).

Besides an increased risk of developing PTSD during adulthood, PTSD in children may show characteristic features (10).

TREATMENT

Besides psychotherapy, cognitive be-

havioral therapy and eye movement desensitization and reprocessing (EDMR), several classes of psychotropic drugs are used in the treatment of PTSD.

Studies on the US troops in Iraq, showed that the use of morphine in the early stages of treatment, resulted in a significantly lower rate of PTSD, compared to those who did not receive morphine (11).

Selective serotonin reuptake inhibitors (SSRIs) are considered a first-line drug treatment; fluoxetine, paroxetine, citalopran, fluvoxamine and sertraline have data to support their use (12).

Antipsychotics as Risperidone are used in dissociation, mood issues and aggression.

Beta blockers (Propranolol) are effective in reducing hyperarousal symptoms, including sleep problems.

Alpha-adrenergic agonists, Clonidine can be helpful in startle, hyperarousal and general autonomic hyperexcitability (13).

Monoamine-oxidase inhibitors, as Phenelzine (Nardil) are effective against hyperarousal and depression and are especially effective with nightmares.

Trycyclic anti-depressants (amitrptyline and imipramine) are used for the treatment of avoidance and intrusive symptoms.

Anti-convulsants, mood stabilizers, such as Carbamazepine is reducing arousal symptoms related to noxious affect and aggression. Topiramate (Topamax) is strongly effective in flashbacks and nightmares, with no reduction of effect over time (14).

Atypical antidepressants as Nefazodone (Serzone) are effective against secondary depression, anxiety, sexual dysfunction symptoms and sleep disturbances (15).

MDMA (3, 4-Methylenedioxymetamphetamine), commonly known as ecstasy is a drug of the phenetylamine and amphetamine families. Studies in the U.S., Switzerland and Israel are evaluating the efficacy of MDMA – assisted psychotherapy for treating PTSD (17).

Cannabis was also used in the treatment of PTSD; there are studies which demonstrate that cannabis provides control and amelioration of chronic stressors, without adverse side effects (16).

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NOUTĂȚI

FOXO1 JOACĂ UN ROL CHEIE ÎN REGLAREA METABOLISMULUI ENERGETIC

Factorul de transcripție FOXO1 joacă un rol deosebit în reglarea metabolismului prin modularea activității insulinei şi leptinei în numeroase celule, inclusiv la nivel neuronal. Nucleul ventromedial hipotalamic (VMH) reprezintă un situs cheie la nivelul căruia acționează FOXO1. Expresia FOXO1 în neuronii VMH mediază homeostazia glucidică şi balanța energetică. A fost demonstrată creşterea consumului energetic la modele animale la care lipseşte FOXO1 în neuronii SF-1 (steroidogenic factor 1) din VMH. De asemenea, şoarecii au prezentat toleranță crescută la glucoză prin creşterea sensibilității la insulină în mușchii scheletici. Receptorul nuclear SF-1 a fost identificat drept țintă directă a FOXO1 la nivelul VMH. Datele sugerează că rețelele transcripționale modulate de FOXO1 în neuronii VMH reprezintă componente cheie în reglarea homeostaziei glucidice şi a metabolismului energetic (Kim KW, Donato J Jr, Berglund ED et al. FOXO1 in the ventromedial hypothalamus regulates energy balance. *J Clin Invest*. 2012. pii: 62848. doi: 10.1172/JCI62848).

Teodora Vremeră

OPTIMIZATION OF CULTURE CONDITIONS FOR BONE MARROW STROMAL CELLS IN RPMI-1640 MEDIUM

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OPTIMIZATION OF CULTURE CONDITIONS FOR BONE MARROW STROMAL CELLS IN RPMI-1640 MEDIUM (Abstract): Bone marrow mesenchymal stem cells are important for both research and clinical purpose. A number of culture methods for these cells are available on the market, many of them consisting of specialized growing media in combination with growth factors. Our goal was to optimize a less expensive culture method for bone marrow mesenchymal cells. **Material and methods**: Eight samples of bone marrow aspirates from patients were used. Out these 8 samples 2 were from healthy people, 3 from chronic granulocytic leukemia patients, 2 from multiple myeloma patients and 2 from patients with myelodysplastic syndrome. Bone aspirates from healthy people were used to optimize the culture method and the rest were used for testing the optimized method. Two methods were tried: 1. Cell culture starting from whole bone marrow, 2) cell culture after bone marrow separation in density gradient with Histopaque. **Results**: Cell culture starting from whole bone marrow gives better yields for mesenchymal stem cells than methods which include gradient density separation of mononuclear cells with Ficoll-Histopaque. **Conclusions:** We have optimised a less expensive cell culture method for bone marrow mesenchymal cells. **Key words**: BONE MARROW STROMAL CELLS, CELL CULTURE, OPTIMIZATION METHOD.

Stem cells zygotic, embryonic or adult have the ability of self-renewal and differentiation into at least one mature specialized cell (1). The ability of adult stem cells to differentiate into other cells is in relation with their origin (1). Bone marrow contains a heterogeneous population of cells which includes adipocytes, reticular cells, macrophages, vascular endothelial cells, smooth muscular cells and stroma cells (2). Within bone marrow we find adult stem cells with restrictive genetic programs like hematopoietic stem cells which differentiate into leukocytes, erythrocytes, or megakaryocytes and mesenchymal stem cells (MSCs) or of bone marrow stromal cells (BMSCs) that can differentiate to form bone, cartilage, or adipose tissue (3, 4). The presence of stem cells is not limited to bone marrow, these cells have been observed in liver, periosteum, blood and support tissues in muscles (5).

Unlike embryonic stem cells, adult stem cells have a limited potential of differentiation, a trait that could be useful in many therapeutic applications (6, 7). Adult stem cells are an attractive source for cell therapy, gene therapy and tissue engineering. As clinical application, MSCs demonstrates great therapeutic characteristics in wounds and ischemic situation (8) and promise new solutions for bone repair (9-13) cartilage repair (14, 15) and neural repair (16, 17). Since MSCs play a significant role in tissue repair and homeostasis, new strategies in aesthetic surgery could be elaborated with these cells for tissue augmentation and regeneration (18).

With so many clinical applications for BMSCs, it is obvious how important they become for medicine. There are many specialized culture medium for BMSCs, but in general they are very expensive. We present here a cost effective optimized method for BMSCs culture using a less expensive culture medium.

MATERIAL AND METHODS

We have selected 8 samples of bone marrow aspirates from patients of Haematology Clinic in „St Spiridon" Clinical University Hospital Iasi. Samples have been received for FACS diagnostic tests and a small quantity has been used for our research experiment which was conducted according to institutionally approved protocols and legal form of informed consent. Out these 8 samples 2 were from healthy people, 3 from chronic granulocytic leukemia patients, 2 from multiple myeloma patients and 2 from patients with myelodysplastic syndrome. Bone aspirates from healthy people were used to optimize the culture method and the rest were used for testing the optimized method. We have tried two methods: 1. Cell culture starting from whole bone marrow, 2) cell culture of BMSCs after bone marrow separation in density gradient with Histopaque®-1077 (Sigma Aldrich).

Whole bone marrow method:

Three mL of bone marrow aspirate from one source is mixed with 7 mL of total medium which is composed by RPMI-1640 (Sigma Aldrich) supplemented with 10% FBS Gibco® (fetal bovine serum), 2% fresh L-glutamine 200 mM (Sigma Aldrich) and 1% penicillin-streptomycin Gibco®. PH of RPMI-1640 medium was adjusted to 7.35 prior using. This mixture with a total volume of 10 mL was plated in one 25 cm^2 cell culture flask (Greiner Bio-One) with filter cap. This was done for both samples from healthy people. Flasks were incubated at 37°C in a 5% CO2 humidified atmosphere. Medium was changed after 4 days first and every 3 days next.

Density gradient separation method:

This method is based on the fact that Ficoll solution or Histopaque solution, mononuclear cells and other marrow components have different densities. If centrifuged, it can be observed that mononuclear cells including BMSCs have a lower density than Histopaque so they form a ring on top of Histopaque, erythrocytes and granulocytes have a higher density than Histopaque so they settle under it.

In a 15 ml conic tube (Falcon), on top of one volume Histopaque, 1.5 volume of bone marrow from one source is added very slowly. Tube is centrifuged at 600 x g for 30 minutes at room temperature with brake and acceleration set to the lowest. After centrifugation, the mononuclear ring containing BMSCs is collected with the help of a sterile pipette. Total culture medium identical to the one described in pre-

vious method is added to BMSCs solution to a total volume of 10 ml. This final BMSCs solution is plated in a 25 cm^2 cell culture flask as in the first method with the same incubation conditions.

RESULTS AND DISCUSSION

During the optimization step for method one (whole bone marrow) after first 4 days from plating it was observed that culture medium was rich in diverse components of bone marrow: precursors for red and white lines of blood, adipocytes, and small dispersed colonies of BMSCs (fig.1a,1b).

Thanks to their adherence property, BMSCs adhere to the flask and separate themselves from the other cell types creating a halo around; they are much bigger, elongated, fibroblast like cells, and have a translucent look (fig. 1a, 1b). BMSCs from whole bone marrow method have reached 100% confluency after 3 weeks (fig.1c) while BMSCs separated with Histopaque (the second method) have reached 90% confluency after 4 weeks (Fig. 1e). This happened for both samples from healthy volunteers.

Since the whole bone marrow method gave better results, the other samples from patients with various pathologies were used next to test this method.

BMSCs from multiple myeloma patients have formed colonies with halo around within the first 4 days in a similar manner (Fig. 2a). Colonies were able to reach confluency within 3 weeks (fig. 2c).

BMSCs from patients with chronic granulocytic leukemia did not pose particular problems; they have reached 80% confluency after 3 weeks (fig. 3c).

For patients with myelodysplastic syndrome, cell culture was slower (fig. 4). Colonies couldn't reach more than 60% confluency after 3 weeks.

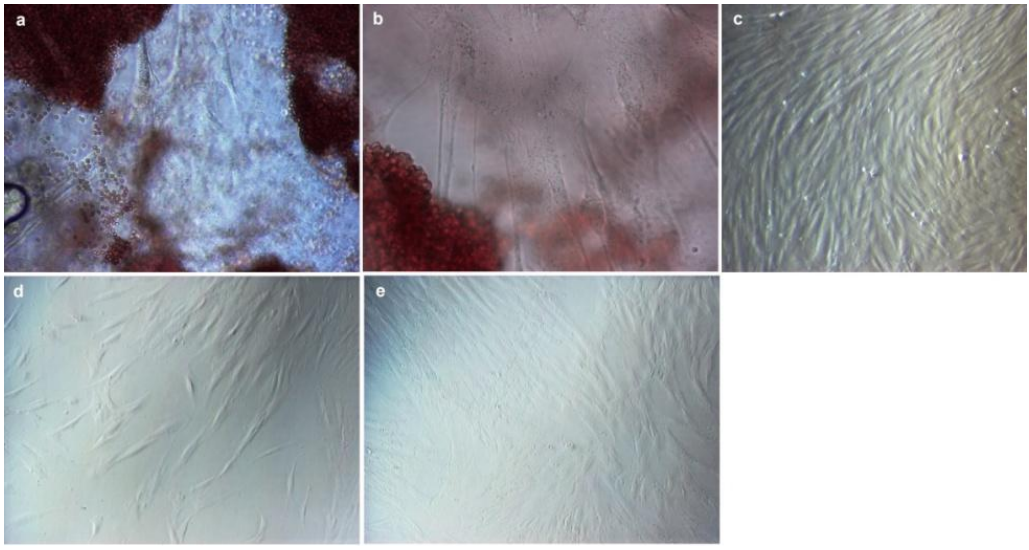

Fig. 1. Healthy volunteer BMSCs (bone marrow stromal cells) culture, phase contrast microscopy, objective 20X: a), c), d), e). Objective 40X: b) method one (from whole bone marrow) : a), b), c). Method 2 (Histopaque): d), e). Time in culture: a) and b): 4 days. c): 21 days. d): 21 days e): 28 days.

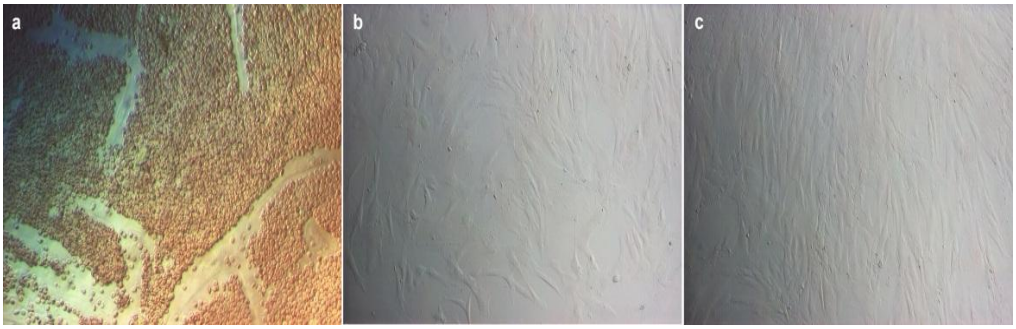

Fig. 2. Multiple myeloma BMSCs (bone marrow stromal cells) cultures, phase contrast microscopy, objective 20X. Time in culture: a) 4 days, b) 14 days, c) 21 days.

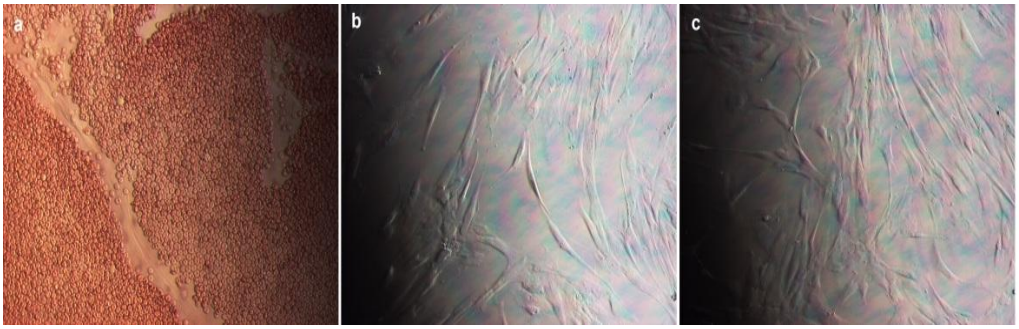

Fig. 3. Chronic granulocytic leukemia BMSCs (bone marrow stromal cells) culture, phase contrast microscopy, objective 20x.
Time in culture: a) 4 days, b) 14 days, c) 21 days.

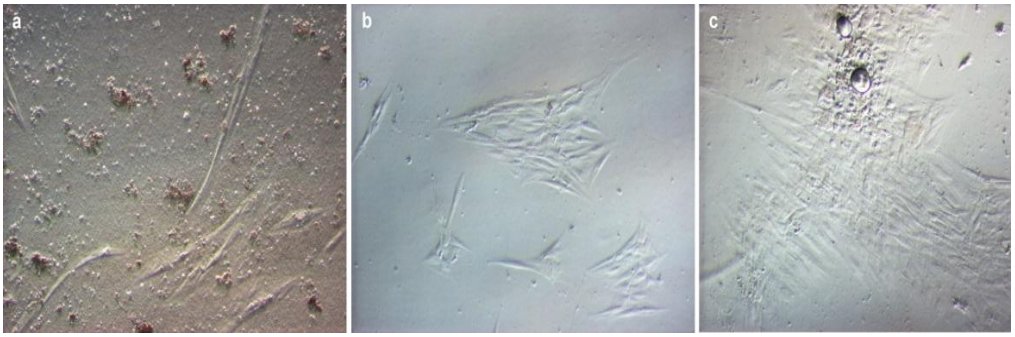

Fig. 4. Myelodysplastic syndrome BMSCs (bone marrow stromal cells) culture, phase contrast microscopy, objective 20x. Time in culture: a) 4 days, b) 14 days, c) 21 days.

BMSCs from culture were tested for specific stromal markers: endogline (CD105), Thy1 (CD90), integrin β1 (CD29), ICAM1 (CD54). All have proven to be positive, ensuring thus that our cell culture experiment was successful.

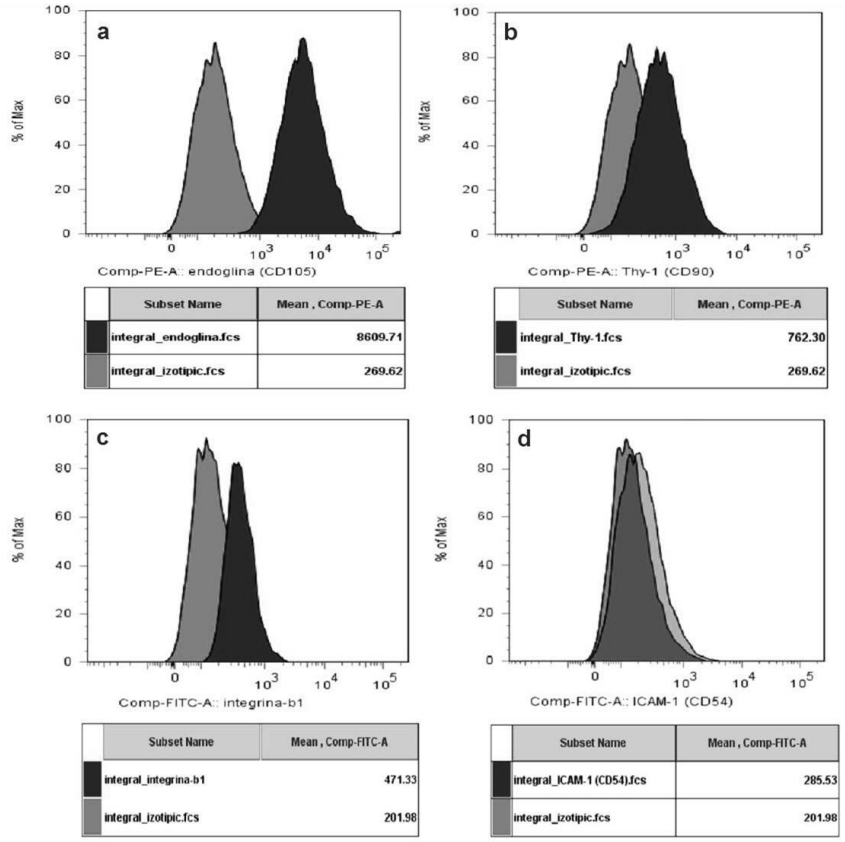

Fig. 5. Image shows phenotypic expression pattern for: a) endogline (CD105), b) Thy1 (CD90), c) integrin β1 (CD29), d) ICAM1 (CD54). Histograms overlay display mean fluorescence value obtained by surface staining BMSCs with mouse antibodies specific for human CD105, CD90, CD29 and CD54. Antibodies where conjugated with phycoerythin (Santa Cruz). In light grey: isotopic control. (Data acquisition was performed with FACSCantoII machine utilizing FACSDiva software, BD Biosciences and data analysis with FlowJo software, Tree Star, Inc.)

CONCLUSIONS

We have presented here an optimized method for BMSCs culture using RPMI-1640 medium without growth factors. This method is reliable and less expensive compared to other methods which require specialized stem cell media.

Our experiments have shown that method involving platting a mixture of whole bone marrow with growth medium gives better BMSCs yields than method which starts from Histopaque separated mononuclear cells. We believe there are two reasons for this: bone marrow present in culture mimics the physiological medium in organism and has numerous support ingredients; the second reason would be that a number of BMSCs are lost during Histopaque separation, this is important because BMSCs concentration in bone marrow is low.

This optimized method has proven to be successful for bone marrow samples com-

ing from healthy donors as well as for samples coming from patients with different pathologies.

ACKNOWLEDGEMENTS

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THE ROLE OF DNA REPAIR BY HOMOLOGOUS RECOMBINATION IN ONCOGENESIS

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THE ROLE OF DNA REPAIR BY HOMOLOGOUS RECOMBINATION IN ONCOGENESIS (Abstract): DNA repair by homologous recombination (HR) may be regarded as the two faces of a coin: lowering the oncogenetic potential (precise repair with no consequences on genomic status) or increasing it (deleterious action which may determine chromosome rearrangements such as loss of heterozigosity). Inherited mutations in genes involved in HR are associated with gene rearrangement and may be a prerequisite for tumor development in some cancer-prone hereditary diseases like Bloom, Werner and Rothmund-Thomson syndromes. Normal eukaryotic cells show some degree of balance between various mechanisms of repair. This review presents the main mechanisms and pathways of homologous recombination repair *(*synthesis-dependent single strand annealing, constitution of Holliday junctions with their resolution mechanisms and repair by break induced replication), the proteins involved in it and their contribution to oncogenesis. **Key words:** LOH, SDSA, BIR, HOLLIDAY JUNCTION, CROSSING OVER

Genome mutations in somatic cells are the main cause of any form of cancer. Mutated genes have to code for proteins that are involved in proliferation, differentiation, apoptosis or adhesiveness. There are two types of genes involved in oncogenesis: oncogenes and tumor suppressor genes. Oncogenes are gain-of-function mutated alleles of the proto-oncogenes (promoting cells proliferation or invasiveness). They have a dominant action at molecular level, because they present a gain of function. Thus, oncogenes can promote proliferation even in heterozygous conditions. Tumor suppressor genes encode proteins which are inhibitors of cell proliferation. Their loss-of-function by a recessive mutation promotes cell proliferation only in homozygotes. In heterozygous they do not promote proliferation because they are recessive. Genomic reshaping caused by homologous recombination (HR) can lead to loss of heterozogosity (LOH) (1). LOH is not the only oncogenetic mechanism; defects in others repair processes such as mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ) can lead to oncogenesis. This review only refers to HR as an oncogenetic mechanism; excellent recent reviews on the other mechanisms are available (2). Broken chromatids which are repaired by HR arise during the physiological processes such as: normal

replication, cutting of DNA strands by topoisomerases to facilitate the access of transcription factors etc. Also, broken chromatids are due to environmental influences such as: chemicals-pesticides, industrial pollutants, tobacco smoke, electromagnetic radiation -UV light, X-rays, radioactivity. Although these errors may be considered detrimental to the organisms housing them, they may also be regarded as the evolution engine (3).

HR is a multistep mechanism of repair that restores both continuity and correctness of broken chromatids using the redundant information of a sister or homologous chromatid with the aid of many proteins which are conserved in different organisms. These proteins are usually homologous in amino-acid sequence and similar in function (orthologs see tab. I). A description of double strand breaks repair by HR is presented in the following paragraph and in figure 1.

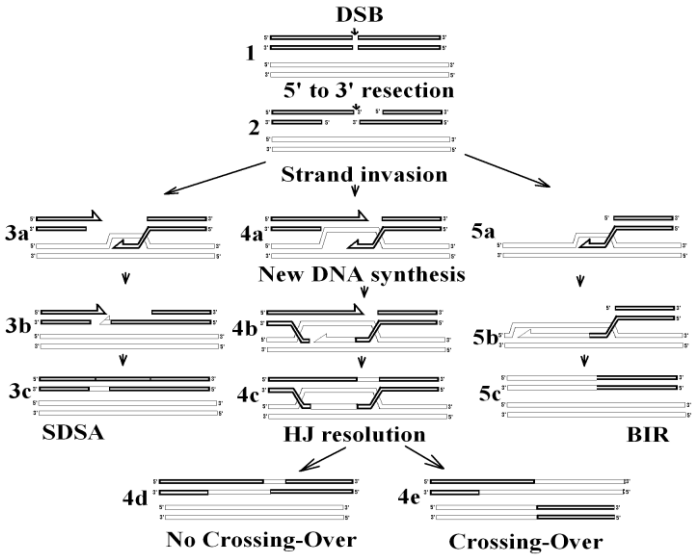

Fig.1. The mechanism of homologous recombination (HR) - data from ref. (12,13).

First, the broken duplex aligns to one of a sister chromatid or a chromatid of the homologous chromosome. The event is facilitated by protein Rad50 (1 in the fig.1). The two ends of the double-strand break are trimmed by shortening one strand from its 5' end so that a longer strand with a 3' end results (overhang). The operation is dependent on the nucleasic activity of Mre11, associated in a complex with Rad50 and Nbs1 (MRN complex). This event is named *end processing* (4). Sae2/CtIP functions with MRX/MRN complex either to activate the Mre 11 nuclease or via the intrinsic endonuclease, in the initial step to trim the duble-strand ends (2 in fig.1) (5). The single-stranded overhang is coated by Replicating Protein A (RPA). The single-stranded overhang invades the homologous duplex and anneals with its complementary strand. This process named *strand exchange* is mainly catalyzed by Rad51, a protein functionally similar with RecA in E.coli (3a, 4a, 5a of the fig.1, see also the tab. I) (6). Rad51 is a

homopolymer that wraps single-stranded invading strand after it is detached from the RPA. The process is mediated by the Rad51 paralogs (the Rad55/Rad59 complex in yeast, Rad51B, Rad51C, Rad51D, Xrcc2 and Xrcc3 in mammalians) and by Rad52. In humans the Rad52 function may be overtaken by BRCA2 (7) (tab. I).

TABLE I

Types of proteins implied in HR and their function

Name of orthologs			**Essential of the protein's role in DSB repair**	**References**
Mammals	***S. cerevisiae***	***S. pombe***		
Mre11	Mre11	Rad32	Form a complex that shortens one strand at its 5' end.	(13, 14)
Rad50	Rad50	Rad50		
Nbs1	Xrs2	Nbs1		
RPA	RPA	RPA	Heterotrimeric single-strand binding protein.	(15, 16)
Rad51	Rad51	Rhp51	Recombinase functionally similar to RecA from *E. coli*.	(13, 16)
Rad52	Rad52	Rad22	Mediates forming of the DNA-Rad51 filament, the strand exchange and the second-end capture.	(7, 13, 17)
Brca2	?	?	A protein mediating checkpoint responses and DNA repair.	(18, 19)
?	Rad55	Rad55	The orthologs in mammalians are unknown.	(7)
?	Rad57	Rhp57		
Rad54	Rad54	Rad54	Facilitates the strand exchange and the branch migration.	(20, 21)
BLM	Sgs1	Rqh1	RecQ type helicases. Resolve D-loops and Holliday junctions;	(22)
WRN				
Fbh1	Srs2	Fbh1	F-box helicase; disrupts the DNA-Rad51 filaments.	(10)
Mus81	Mus81	Mus81	Resolve Holliday junctions	(11, 12)
Eme1	Mms4	Eme1		

Also important for this process is a helicase function, which may be provided by Rad54, which locally denatures the intact duplex. The result of the strand exchange is called a *D loop* (fig. 1, 3a, 4a, 5a). The events described so far are common to any HR. Further, there are three alternative pathways along which HR can continue:

- *Synthesis-dependent Single Strand Annealing (SDSA)*; a replicative DNA pol-

ymerase adds nucleotides on the 3' end of the invading strand, according to the template complementary strand of the intact duplex. A possible evolution of the D-loop may be that its lengthened invading strand is unwinded from its complementary strand of the intact duplex (this may be catalysed by helicases such as RecQ class helicases (Sgs in yeast, Wrn or Blm in insects and humans) (8-9) or F-box helicases (10). The former invading strand may recapture the second end, and reanneal with its original complementary strand (3a, 3b, 3c).

- *Break Induced Replication (BIR*); at the level of D-loop a new replication fork is formed and the replication continues to the end of the chromatid causing the loss of all genetic information of the broken chromatid from the break down to its end. This can lead to the loss of heterozygosity when the model chromatid belong to the homologous chromosome (5a, 5b, 5c).

- *Holliday junctions (HJ) formation, migration and resolution*; the D loop may migrate (process called *branch migration*) due to the helicase function of Rad54 making the strand complementary to the invading strand available as a template for the synthesis by addition of nucleotides to the 3'end of the invading strand. Then, with a minimal unwinding, the 3' end of the invading strand can recapture its second end. This results in a *Hollyday junction (HJ)*. The symmetrical reciprocal invasion of the 3' ended overhang of the other end can lead to a double Hollyday junction (4b). HJs are resolved by endonucleolytic breakage and ligase rejoining and have one of two alternative endpoints (depending on which strands are endonucleolytically broken): crossover chromatids or non-crossover chromatids (4d, 4e). Four pathways are considered to be implied in this process: resolution by MUS81-EME1 (11,12); dissolution by the helicase-topoisomerase complex BLM/TOPIII/RIM1 (7, 11) resolution by GEN1 (11,13) and the endonuclease SLX1-SLX4 (11).

Repair by SDSA forms non-crossover products with minimal consequences compared with Holliday junctions which can be dissolved in a non-crossover or resolved in cross-over products (reciprocal exchange of genetic information and possible LOH). Moreover, the repair by break-induced replication (BIR) can also lead to the loss of heterosigosity (LOH). For a correct strand displacement of invading strand helicases are necessary. The enzymes are required for prevention and/or resolution of aberrant DNA structures that usually lead to the loss of heterozigosity. RecQ family helicase are encoded by BLM genes and, in human there are four paralogues: WRN, RECQ1, RECQ4 and RECQ5. Germinal mutation in these genes can lead to genetic diseases with cancer predisposition such as Bloom's syndrome (BLM), Werners's syndrome (WRN), Rothmund–Thomson syndrome, Baller–Gerold syndrome and RAPADILINO syndrome (RECQ4) (19).

Summarizing, homologous recombination can be involved in oncogenesis in two ways: 1) its insufficiency may lead to oncogenetic mutations or to alternative repair mechanisms promoting gross genomic rearrangements that also lead to oncogenesis and 2) its presence may lead to the loss of heterozygosity, an important oncogenetic process. In principle, homozygosity is the result in any kind of the homologous repair because of replacing the lost information in one chromosome with the information of the homologous chromosome. However, among the different of its alternative pathways: SDSA, crossing over and

BIR (see previous paragraphs) some have a greater oncogenetic potential than others. SDSA leads to LOH only in the neighborhood of the lesion repaired and a heterozygous mutation in a tumor suppressor gene becomes homozygous only when the lesion is located in it or near it. The probability of such a lesion is very small and so SDSA has a negligible oncogenetic potential. Crossing over and BIR, on the other hand, result in very long sequence replacements (from the lesion to the telomere) and the probability of turning a heterozygous tumor suppressor mutant allele into a homozygous is considerably greater and their oncogenetic potential is correspondingly greater. Therefore it is not surprising that genetic diseases such as Bloom, Werner and Rothmund-Thomson syndromes with loss-of-function mutations in helicases predispose to cancer. The absence of helicases function reduces the possibility of repair by SDSA and channels the repair to BIR or HJ resolved by crossing over. It is also of interest to evaluate the oncogenetic potential of HR with respect to other repair mechanisms. The fact that genetic diseases with mutations in genes encoding proteins involved in the first stages of HR (Mre11 mutated in the Ataxia telangiectasia-like syndrome and Nbs1 mutated in the Nijmengen breakage syndrome) predispose to cancer suggests that it is smaller than other repair mechanisms such as non-homologous end joining (NHEJ). This suggestion has been recently confirmed: BRCA1 (with its loss-of-function mutations predisposing to breast and ovarian cancer) inhibits the function of p53BP, a protein that blocks the MRN complex. When p53BP escapes from the control of BRCA, MRN does not function, homologous recombination cannot be started and repair by recombination is channeled to NHEJ (23).

CONCLUSIONS

Although of a smaller oncogenetic potential than other DNA repair mechanisms, homologous recombination with LOH remains an important cause that has to be taken into account in explaining and mastering of cancer.

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REEDUCAREA FUNCŢIONALĂ A TENDOANELOR FLEXOARE CU LEZIUNI ASOCIATE ÎN ZONA II – STUDIU COMPARATIV

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FUNCTIONAL REEDUCATION OF FLEXOR TENDONS WITH ASSOCIATED LESSIONS IN ZONE II – COMPARATIVE STUDY (Abstract) : Regaining satisfactory digital function after flexor tendon laceration and repair has long been one of the most important problems in hand surgery. But optimal therapy is often difficult to ascertain, given the plethora of immediat postoperative protocols published in the scientific literature. **Aim**: The purpose of this study is to compare the functional results between Kleinert, Silfverkiöld, Gratton and Strickland protocol towards evaluate them by interconnection and with the literature. **Material and methods**: The prospective study enorolled 75 patients who presented in our cabinet consecutives during 1.05.2008-1.12.2010. The subjects were evaluated regarding Total active motion test and Grip strenght test at 12 weeks postoperative. **Results**: We found the best results to Gratton group, followed by Strickland, Silfverkiöld-May and Kleinert groups. **Conclusions**: We conclude that, for the tendinous lessions with associated injury (nervs and vessels) in zone II, our first therapeutical indication is to use the Gratton protocol. **Key words**: FUNCTIONAL REHABILITATION, FLEXOR TENDONS, ZONE II

Reeducarea precoce postoperatorie a tendoanelor flexoare reprezintă un factor important pentru buna funcţionalitate a mâinii, reducând formarea de aderenţe în canalul sinovial, stimulând refacerea suprafeţelor de glisare şi facilitând formarea unui calus tendinos mai rezistent. Dar protocolul terapeutic optim este greu de ales, datorită numărului mare de lucrări din literatura de specialitate. Prin acest studiu, analizând rezultatele privind mobilitatea articulară şi forţa musculară, am încercat să găsim o posibilă ierarhizare a unor protocoale des folosite, servind ca ghid pentru terapeuţii specializaţi în terapia mâinii, care îşi vor putea alege protocolul adecvat.

MATERIAL ŞI METODE

Au fost luaţi în studiu 75 pacienţi care au prezentat leziuni ale tendoanelor flexoare cu leziuni asociate, care au fost trataţi chirurgical în Secţia de Chirurgie Plastică şi Reconstructivă a Spitalului Clinic de Urgenţe ’’Sf. Ioan’’ din Iaşi şi secţiile din

teritoriul Moldovei, care s-au adresat pentru tratament de reeducare funcţională la Cabinetul Medical Individual ,,Ţopa Ionuţ" în intervalul mai 2008 – decembrie 2010, unde le-au fost aplicate ortezele şi programele de reeducare funcţională. Evaluările au fost efectuate atât în spital cât şi la cabinet. Au fost selecţionate cazurile pacienţilor ce s-au prezentat consecutiv, care au putut fi urmăriţi până la 12 săptămâni postoperator (4 săptămâni la domiciliu sub protecţia ortezei şi 4 săptămâni la cabinet sub supravegherea personală) şi care s-au prezentat pentru evaluarea funcţională de la 12 săptămâni postoperator. Dintre aceştia 57 au fost bărbaţi (76%) şi 18 femei (34%) cu vârste cuprinse între 21 şi 69 de ani, 33 (44%) de pacienţi provenind din mediul urban şi 42 (56%) din mediul rural. Lotul de pacienţi a fost împărţit după momentul prezentării în tratament (pe perioade de 8 luni) în 4 grupuri la care s-au aplicat 4 protocoale terapeutice diferite:

– mai 2008-decembrie 2008 - 17 pacienţi – protocol Kleinert modificat (grupul Kleinert);
– -ianuarie 2009-august 2009 - 20 pacienţi - protocolul - Silfverkiöld şi May (grupul Silfverkiöld şi May);
– septembrie 2009-aprilie 2010 - 18 pacienţi - protocolul Strickland (grupul Strickland);
– mai 2010-decembrie 2010 - 20 pacienţi - protocolul Gratton (grupul Gratton).

Programul de reeducare funcţională

- *Protocol Kleinert modificat* (1,2,3). Orteze tip Kleinert modificate cu pumnul în 30° de flexie, articulaţiile metacarpofalangiene (AMF) în flexie 70°, articulaţiile interfalangiene (AIF) în poziţie neutră. Sub pliul palmar distal de flexie a fost aplicat pe orteză un scripete pentru direcţionarea elasticelor ataşate pe degetele operate pe direcţia scafoidului (fig. 1,2). În fiecare oră pacientul efectuează 10 repetări de extensie activă contra rezistenţei elastice şi flexie pasivă a degetului operat.

Fig. 1. Flexie pasivă AIF

Fig. 2. Flexie activă AIF

- *Protocolul - Silfverkiöld* (fig. 3,4). Orteză cu pumnul în neutru, flexie AMF 50-70° şi cu rezistenţe elastice ataşate pe toate cele 4 degete (four finger program)(4,5). În fiecare oră 10 repetări extensie activă, flexie dinamică şi flexie cu ,,place-hold" 2-3 secunde (se duc cu mâna sănătoasă degetele în diferite poziţii de flexie şi se menţin 2-3 secunde).

- *Protocol Strickland* (fig. 5,6). Orteză articulată (6,7) pentru exerciţii şi una dorsală de protecţie cu pumnul în flexie 20° şi AMF în flexie 50°. Orteza articulată permite flexia maximă a pumnului dar limitează extensia la 30°. În fiecare oră va efectua 15 repetări de flexie pasivă a AIFD; AIFP;

AMF în orteza statică urmate de 25 de repetări de plasare-menţinere a flexiei degetului pentru 5 secunde în orteza de tenodeză.

Fig. 3. Flexie pasivă AIF

Fig. 4. Flexie activă AIF

Fig. 5. Flexie pumn-extensie degete

Fig. 6. Extensie pumn 30°-flexie cu menţinere 5 s

- *Protocolul Gratton*. Orteză dorsală ce menţine pumnul în flexie 20°, AMF în flexie 80-90° iar AIF în extensie (fig. 7,8). Orteza este mai lungă cu 2 cm pentru a împiedica folosirea mâinii. În fiecare 4 ore cu toate degetele 2 repetări de flexie pasivă, flexie activă, extensie activă. Scopul primei săptămâni este obţinerea flexiei pasive complete, extensiei active complete, flexiei active de 30° pentru AIFP şi 5-10° pentru AIFD, cu o creştere graduală a flexiei active în următoarele săptămâni ajungând la 80-90° flexie AIFP şi 50-60° AIFD la sfârşitul celei de-a patra săptămâni (8).

Fig. 7. Orteză Gratton cu protecţie 20° AIFP

Fig. 8. Flexie activă 2 săptămâni

Evaluarea pacienţilor s-a făcut după următoarele teste :

- *Testul TAM al lui Strickland – versiunea a-2-a* (9). Este considerat testul cel mai bine adaptat leziunilor tendoanelor flexoare în zona a-II-a (1985). Constă în suma flexiei active a articulaţiilor interfalangiene minus deficitul de extensie şi se compară în procentaje cu valoare standard de 175°(tab. I).

TABELUL I

Strickland a-2-a versiune

Rezultate	Valoare comparativă	Procentaj
Excelent	>132°	75 – 100%
Bun	88 - 131°	50 – 74%
Mediu	44° - 87°	25 – 49%
Slab	<44°	< 25%

-Testul pentru măsurarea forţei prizei digito-palmare cu ajutorul dinamometrului Jamar. Această evaluare trebuie făcută bilateral şi care acordă mâinii dominante un procent de 100-120% faţă de cea nedominantă (10). Poziţia pacientului trebuie cu umărul în abducţie 30°, cotul flectat la 90°, antebraţul în poziţie neutră, pumnul în extensie 30° şi înclinare cubitală de 15° (tab. II).

TABELUL II

Testul JAMAR

Rezultat	Mâna dominantă	Mâna nedominantă
Bun	≥80% faţă de cea neafectată	≥60% faţă de cea neafectată
Slab	<80% faţă de cea neafectată	<60% faţă de cea neafectată

REZULTATE

Distribuţia cazurilor în funcţie de sexul pacientului, mediul de provenienţă şi vârstă nu prezintă diferenţe semnificative între grupurile luate în studiu (tab. III), ceea ce demonstrează omogenitatea grupurilor investigate după protocolul aplicat.

TABELUL III

Semnificaţia statistică a diferenţelor pe sex, mediu de provenienţă şi vârstă

Subgrupuri comparate		Sex		Mediu de provenienţă		Vârstă	
		χ^2	p	χ^2	p	t-Student	p
Kleinert	Silfverkiöld	0,06	0,811	0,32	0,571	1,18	>0,05
	Strickland	0,45	0,500	0,04	0,836	1,68	>0,05
	Gratton	0,16	0,686	0,07	0,792	1,46	>0,05
Silfverkiöld	Strickland	0,32	0,573	0,12	0,732	0,37	>0,05
	Gratton	0,14	0,705	0,10	0,752	0,26	>0,05
Strickland	Gratton	0,04	0,836	0,09	0,770	0,12	>0,05

Rezultatele excelente şi bune ale scorului TAM au variat ca frecvenţă de la 80%, care clasează protocolul Gratton pe locul 1, până la 70,6% clasând protocolul Kleinert modificat pe locul 4 (tab. IV).

Rezultatele bune ale scorului JAMAR au variat ca frecvenţă de la 85%, care clasează tot protocolul Gratton pe locul 1, până la 76,5% clasând protocolul Kleinert modificat pe locul 4 (tab. V).

TABELUL IV

Rezultatele Total active motion

Clasament	Protocol	E + B	M + S
1	Gratton	80,0%	20,0%
2	Strickland	77,8%	22,2%
3	Silfverkiöld	75,0%	25,0%
4	Kleinert	70,6%	29,4%

TABELUL V

Rezultatele la JAMAR

Clasament	Protocol	Bun	Slab
1	Gratton	85,0%	15,0%
2	Strickland	83,3%	16,7%
3	Silfverkiöld	80,0%	20,0%
4	Kleinert	76,5%	23,5%

Pentru a studia eficienţa aplicării scorului JAMAR comparativ cu scorul TAM se analizează comparativ pe tipuri de protocoale sensibilitatea şi acurateţea metodei de evaluare (tab. VI).

Se evidenţiază cea mai bună acurateţe la pacienţii din grupul la care s-a aplicat protocolul Gratton, urmat de subgrupul Strickland, Silfverkiöld şi May şi pe ultimul loc Kleinert, ceea ce confirmă ierarhia anterior stabilită (tab. IV,V, VI, VII).

TABELUL VI

Analiza comparativă a acurateţei testelor TAM şi JAMAR

Lot A2	VPP (%)	VPN (%)	Sensibilitate (%)	Specificitate (%)	Acurateţe	p
Kleinert	91,7	40,0	78,6	66,7	72,7	0,022
Silfverkiöld	93,3	40,0	82,4	66,7	74,6	0,009
Strickland	92,9	50,0	86,7	66,7	76,7	0,043
Gratton	93,8	50,0	88,2	66,7	77,5	0,028

TABELUL VII

Ierarhizarea protocoalelor la 12 săptămâni postoperator

Ierarhie	TAM	JAMAR	TAM + JAMAR
1	Gratton	Gratton	Gratton
2	Strickland	Strickland	Strickland
3	SMay	SMay	SMay
4	Kleinert	Kleinert	Kleinert

DISCUŢII

Testarea după *Total Active Motion (TAM)* la 12 săptămâni postoperator a evidenţiat rezultate excelente şi bune cu o variaţie între 71 şi 80%, clasând protocoalele terapeutice astfel: Gratton (80%), Strickland (78%), Silfverkiöld şi May (75%) şi Kleinert modificat (71%). Valorile excelente/bune obţinute la testul TAM în acest studiu sunt comparabile cu cele din literatura de specialitate: 75% Chambon(11), 84% Peck (12), 71% Hung(13), 81% Silfverkiöld şi May (14), 75% Riaz (15), 72% Evcik (16), 81% Graf (17).

Testarea după *Grip strenght test (dinamometrul JAMAR)* a găsit o variaţie ca frecvenţă a rezultatelor bune de la 92% până la 75% şi a condus la următoarea clasificare a protocoalelor folosite : Gratton (85%), Strickland (83%), Silfverkiöld şi May (80%) şi Kleinert modificat (77%). Forţa de prehensiune reprezintă un factor important în funcţionalitatea mâinii dar

numărul de lucrări publicate la care ne putem raporta privind rezultatele obţinute la Jamar este mic : 71% Bal (18), 94% Riaz (15), 67% Evcik(16), 72% Chan (19), 81% Kitis (20).

Testarea acurateţii celor două teste ne-a indicat aceeaşi ordine de utilizare a protocoalelor terapeutice.

CONCLUZII

Coroborând rezultatele testelor aplicate pe loturile de pacienţi împreună cu interpretările statistice concluzionăm că, în cazul leziunilor tendoanelor flexoare cu leziuni asociate în zona II, indicaţia noastră de utilizare a protocoalelor este Gratton, Strickland, Silfverkiöld şi Kleinert.

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NOUTĂȚI

LEZIUNEA ACUTĂ RENALĂ POATE FI MOTIV DE ÎNGRIJORARE PENTRU PROGRESIA CĂTRE BOALĂ RENALĂ CRONICĂ?

În timp, cât mai multe dovezi indică faptul ce atât incidenţa leziunii renale acute (AKI) cât şi a bolii renale cronice (CKD) la copii este în creştere, iar etiologia s- schimbat dramatic, sunt relativ puţine cunoştinţe cu privire la potenţialul tranziţiei de la AKI la CKD. Barierele majore în evaluarea potenţialului trecerii de la AKI la CKD sunt legate de lipsa de standarde pediatrice pentru definiţia AKI, atenţia fiind centrată numai pe copiii cu AKI ce primesc terapie de supleere renală, şi se recurge la nivelul seric al creatininei ca biomarker principal pentru a depista şi diagnostica AKI şi CKD. Date recente au validat un sistem multidimensional de clasificare al AKI la copii şi a sugerat sechele renale cronice în populaţia pediatrică cu AKI sau ca risc pentru AKI. În plus, un număr de noi biomarkeri au fost riguros validaţi ca indicatori precoci a CKD inicpientă. Autorii îşi stabilesc drept scopuri de a revedea modificările recente survenite în epidemiologia AKI şi CKD pediatrice, să exploreze potenţialul AKi de a progresa către CKD şi propunerea de noi paradigme clinice şi de cercetare pentru a elucida mai bine progresia AKI către CKD. (Goldstein SL, Devarajan P, Acute kidney injury in childhood: should we be worried about progression to CKD? *Pediatr Nephrol*, 2011; 4(26): 509-522).

Mihaela Munteanu

ASPECTE LEZIONALE MACROSCOPICE ŞI MICROSCOPICE ÎN ASTMUL BRONŞIC

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MACROSCOPIC AND MICROSCOPIC LESIONS IN BRONCHIAL ASTHMA (Abstract): Bronchial asthma is a chronic inflammatory disease characterized by reversible airways obstruction, inflammation, and increased bronchial hyperresponsivennes. The aim of this study was to evaluate the macroscopic and microscopic airways lesions, using bronhoscopy followed by biopsy in 38 cases of Outpatient Department from Clinic of Pulmonary Diseases Iaşi, investigated between January 2005 - June 2008. Patients distribution according to the degree of severity was performed, using the international recommendations for asthma management of the Global Initiative for Asthma (GINA): intermittent (n=7 patients), mild persistent (n= 10 patients), moderate persistent (n= 16 patients), and severe persistent (n= 5 patients). 23,68% of patients had a chronic disease of more than 15 years. The endoscopic changes of the peripheral airways were represented by edema, congestion, bronchial stenosis and mucus hypersecretion. Microscopic routine exam showed epithelial lesions (epithelial desquamation, goblet cell hyperplasia, squamous metaplasia), basement membrane thickening, edema, increased vascularisation, glandular and muscular hypertrophy and bronchial inflammatory infiltrate. Although bronhoscopy exam offers important information regarding changes of the airway mucosa in bronchial asthma, it concomitantly provide bioptic control, as microscopic examination still represents the „gold standard" assessment of bronchial wall remodeling process. **Key words:** BRONCHIAL ASTHMA, BRONHOSCOPIC EXAM, MICROSCOPIC LESIONS

Astmul bronşic este o boală inflamatorie cronică a căilor respiratorii caracterizată prin obstrucţie bronşică difuză şi variabilă reversibilă în mod spontan sau sub tratament.

Evaluarea adecvată a modificărilor căilor aeriene în astmul bronşic se poate realiza prin fibrobronhoscopie, metodă invazivă care permite atât vizualizarea directă a căilor aeriene mari, cât şi practicarea lavajului bronhoalveolar şi/sau a biopsiei bronşice. Această metodă este frecvent greu efectuată şi acceptată de către unii pacienţi astmatici cu insuficienţă respiratorie. Fibrobronhoscopia permite caracterizarea macroscopică a leziunilor, oferind astfel o primă orientare asupra substratului morfologic asociat astmului bronşic (1,2). Suplimentar, lavajul bronhoalveolar aduce informaţii asupra leziunilor prezente la nivelul căilor aeriene mici şi a spaţiilor

alveolare (3,4). Biopsia bronşică constituie însă investigaţia relevantă pentru analiza mucoasei căilor aeriene, modificările instalate în evoluţia astmului bronşic fiind astfel certificate şi cuantificate prin examen microscopic (2,5).

Cu toate avantajele sale de necontestat, examenul fibrobronhoscopic – însoţit sau nu de biopsie bronşică nu este o metodă de rutină în evaluarea pacienţiilor cu astm bronşic (2). În contextul patologiei astmatice, investigarea fibrobronhoscopică este practicată în principal în scop de cercetare, fapt susţinut şi de numărul relativ mic de publicaţii din literatura de specialitate care au ca subiect examenul bronhoscopic la pacienţii astmatici (aproximativ 200 în 20 ani) (6,7). Cu toate acestea, paşii hotărâtori în descifrarea aspectelor fiziopatologice au fost realizaţi odată cu posibilitatea studierii, prin diferite metode, a fragmentelor bronşice recoltate pe cale fibrobronhoscopică, la pacienţii cu astm în stadii evolutive (8). Punctul de plecare este reprezentat de încadrarea pacienţilor după examinarea clinică şi în raport cu rezultatele testelor de explorare funcţională pulmonară, în raport de cele 4 trepte de severitate conform clasificării GINA 2006: astm intermitent, astm uşor persistent, astm moderat persistent şi astm sever persistent (9).

În contextul datelor raportate în literatură, studiul de faţă îşi propune să evalueze tipul şi amploarea leziunilor macroscopice ale peretelui bronşic la pacienţii astmatici, în corelaţie cu leziunile microscopice.

MATERIAL ŞI METODĂ

Lotul de studiu a fost reprezentat de 38 de pacienţi astmatici, sub tratament steroidian inhalator şi în stadii evolutive progresive, dispensarizaţi prin Ambulatoriul de Specialitate al Spitalului Clinic de Pneumologie Iaşi, în perioada ianuarie 2005 – iunie 2008.

Studiul s-a efectuat după consimţământul informat, conform ,,Ghidului informativ" care a realizat familiarizarea cu procedura şi obţinerea unei cooperări optime din partea pacienţilor. Metodologia de lucru a urmărit protocolul standard de pregătire (premedicaţie cu bronhodilatatoare cu acţiune scurtă pe cale inhalatorie (Salbutamol 2,5 mg în nebulizare sau 2 pufuri Ventolin) (10), benzodiazepine anxiolitice şi antitusive. Examinarea a fost efectuată sub administrare continuă de oxigen prin canulă nazală tip „ochelari", cu un debit de 1-2 l/min, dupa anestezia locală progresivă a zonelor reflexogene (faringe, laringe, trahee).

Toţi cei 38 de pacienţi cu astm bronşic au fost monitorizaţi atât pe parcursul procedurii, cât şi ulterior acesteia prin pulsoximetrie şi evaluarea semnelor vitale, timp de minimum 2 ore post-examinare.

Pentru efectuarea fibrobronhoscopiei s-au folosit videoendoscopul Olympus BFType 160, care a permis şi înregistrarea video a examenului, cu posibilitatea efectuării unor fotografii digitale, sau fibroscopul clasic Olympus BF1T60, cu canal peste 2,8 mm.

Pentru examenul microscopic, fragmentele de mucoasă bronşică recoltate bioptic au fost prelucrate conform protocolului standard şi colorate prin metoda Hematoxilină-Eozină.

Prelucrarea statistică a datelor a fost efectuată cu programul specializat SPSS, varianta 17 (11).

REZULTATE

Caracterizarea clinică în raport de severitate

În raport cu clasificarea GINA 2006 de încadrare pe trepte de severitate pacienţii au prezentat următoarea distribuţie: 7 cazuri cu astm bronşic intermitent, 10 cazuri cu astm bronşic uşor persistent, 16 cazuri cu astm bronşic moderat persistent şi 5 cazuri

cu astm bronşic sever persistent. Media VEMS (din valoarea sa prezisa) în cadrul lotului a fost de 78,3 +/- 22,6 % (tab. I).

În ceea ce priveşte vechimea bolii astmatice s-a constat un procent mai mare de pacienți (23,68%) care au prezentat o vechime a afecțiunii cuprinsă între 15 şi 20 ani (fig. 1).

TABELUL I

Mediile VEMS (% din prezis) în raport de severitatea astmului

VEMS (% din ideal)	**78.3±22.6**	**25.3**	**62.0**	**76.2**	**93.9**	**122.0**		
Astm intermitent (n=7)	106.3±14	88.0	92.8	111.0	119.0	122.0	0.010819	ss
Astm uşor (n=10)	94.5±6.3	82.0	92.0	93.7	100.0	103.0	0.000125	ss
Astm moderat (n=16)	66.7±6.2	59.2	62.0	64.5	70.3	79.8	0.000162	ss
Astm sever (n=5)	43.6±10.8	25.3	45.0	45.2	48.8	53.7	0.000126	ss

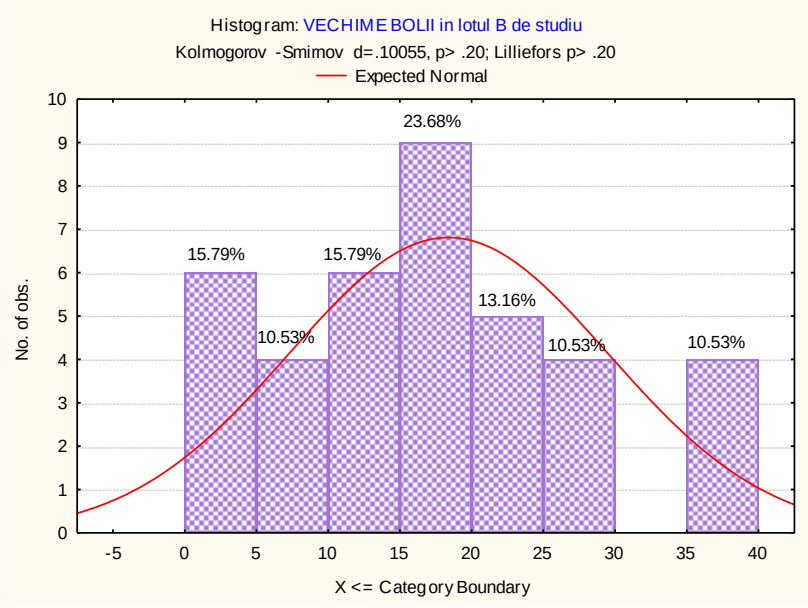

Fig. 1. Histograma valorilor vechimii afecțiunii în lotul de studiu

Profilul macroscopic al leziunilor

În timpul examenului endoscopic realizat la pacienții incluşi în studiu s-au putut observa următoarele leziuni macroscopice ale peretelui bronşiilor şi ale mucoasei acestora:

a) modificări ale calibrului bronşiilor:

- îngustarea orificiilor bronşiilor lobare (n= 1);
- diminuarea diametrului bronşiilor principale secundară îngroşării mucoasei bronşice (n= 9) (fig. 2);
- diminuarea până la ştergere a reliefului cartilaginos (n= 7);
- rotunjirea pintenilor bronşiilor lobare şi segmentare, cu aspect lucios (n=8);
- diminuarea diametrului bronşiilor principale secundară îngroşării mucoasei bronşice, cu aspect fascicular longitudinal la nivelul peretelui posterior (n=1) (fig. 3).

b) leziuni ale mucoasei bronşice:

- hiperemie, de aceeaşi intensitate pe toată suprafața examinată (n=18) (fig. 4);

Fig. 2. Îngroşări neregulate, difuze ale mucoasei bronşice – astm bronşic moderat persistent

Fig. 3. Îngroşarea mucoasei bronşice, cu aspect fascicular longitudinal, la nivelul peretelui posterior – astm bronşic moderat persistent

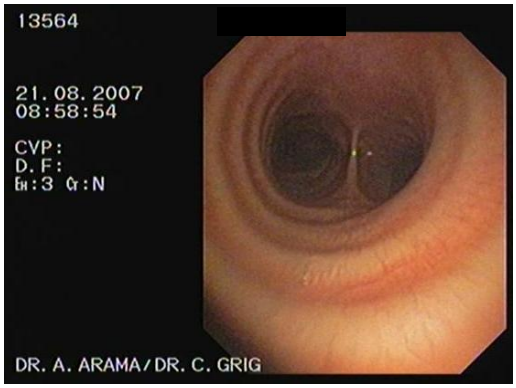

Fig. 4. Mucoasă bronşică cu hiperemie cu aceeaşi intensitate pe întreaga suprafaţă – astm bronşic uşor persistent

Fig. 5. Atrofie mucoasă bronşică – astm bronşic uşor persistent

- aspect lucios şi neted a mucoasei peretelui bronşic secundar edemului acesteia (n=13);
- atrofia mucoasei bronşice (n=3) (fig. 5);

c) alte modificări endoscopice:

- prezenţa în căile aeriene a unui mucus vâscos în cantitate variabilă (n=19) (fig. 6);
- diskinezie hipotonă (colabare în expir) a pereţilor bronşici (n=2).

La un număr de 7 pacienţi, mucoasa bronşică a prezentat un aspect macroscopic cvasinormal.

Fig. 6. *Mucus vâscos, edem şi congestia mucoasei – astm bronşic moderat persistent*

Profilul microscopic al leziunilor

Examenului microscopic al fragmentelor de mucoasă bronşică realizat la pacienţii incluşi în studiu a permis observarea următoarelor leziuni microscopice:

a) leziuni ale epiteliului mucoasei bronşice:

- denudare epitelială totală (n=21);
- denudare epitelială parţială (n=15) (fig. 7);
- hiperplazia celulelor caliciforme (n= 36) (fig. 8);
- leziuni de metaplazie scuamoasă (n= 16).

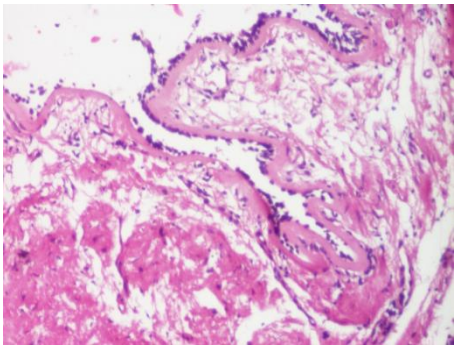

Fig. 7. *Denudare epitelială parţială – astm bronşic sever persistent, H.E. x100*

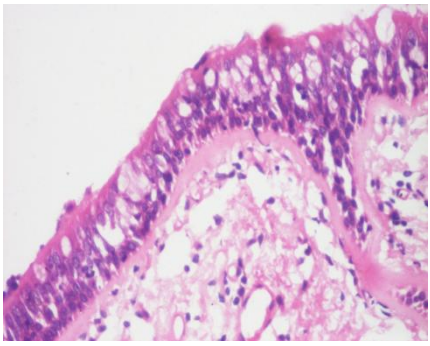

Fig. 8. *Hiperplazie a celulelor caliciforme – astm bronşic uşor persistent, H.E. x 200*

b) leziuni ale membranei bazale a epiteliului mucoasei bronşice

- îngroşarea membranei bazale a epiteliului respirator (fig. 9), mai importantă la pacienţii cu forme sever persistente de astm - comparativ cu pacienţii care au fost încadraţi în stadiile intermitente şi uşoare de boală.

c) leziuni ale laminei propria (corionului):

- edemul mucoasei bronşice (n=38);
- creşterea numărului de vase la nivelul laminei propria la toţi pacienţii incluşi în lot (n=38);
- hipertrofie musculară (n=20) (fig. 10);
- hipertrofie glandulară (n=20);
- prezenţa unui infiltrat inflamator polimorf în cantitate variabilă (n=38) în raport cu severitatea bolii astmatice.

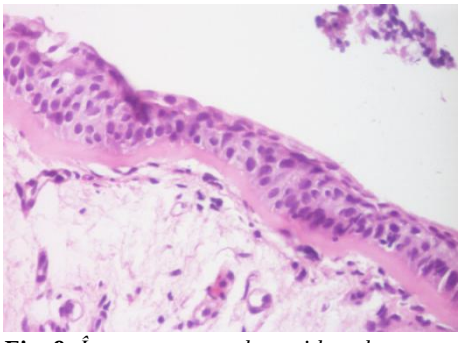

Fig. 9. *Îngroşarea membranei bazale – astm bronşic uşor persistent, H.E. x 200*

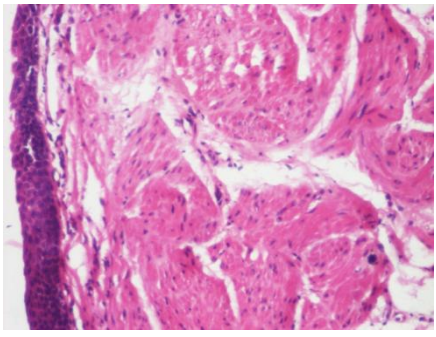

Fig. 10. *Hipertrofie musculară - astm bronşic sever persistent, H.E. x 100*

DISCUŢII

Prevalenţa bolii astmatice a crescut semnificativ din anii 70, în 2009 raportându-se, la nivel mondial, aproximativ 300 milioane de astmatici. Mai mult, deşi metodele de diagnostic şi de tratament s-au îmbunătăţit semnificativ în ultimii ani, în cursul anului 2009 astmul bronşic a determinat peste 250 000 de decese în întreaga lume (12,13).

Examenul fibrobronhoscopic nu constituie o metoda curentă de investigaţie in astmul bronhic. Utilitatea metodei este restrânsă la diagnosticul diferenţial al obstrucţiilor bronşice determinate de neoplazii sau de corpi străini endobronşici (2,8).

Prima etapă în cuantificarea leziunilor peretelui bronşic este examinarea directă a suprafeţei mucoasei bronşice, în cursul examenului fibrobronhoscopic, permiţând vizualizarea bronşiilor de calibru mare şi mediu (în general până la nivelul bronşiilor subsegmentare) (2). Teritoriile distale, aflate dincolo de limita abordului endoscopic direct, sunt explorate prin lavaj bronhoalveolar care permite analiza compoziţiei celulare totale şi/sau parţiale precum şi a mediatorilor inflamaţiei (14).

Examenul macroscopic realizat în cursul fibrobronhoscopiei aduce date importante în aprecierea gradului inflamaţiei bronşice, care se corelează macroscopic cu edemul şi congestia mucoasei (1). În lotul studiat, hiperemia şi edemul mucoasei au fost modificări frecvent observate şi anume la 18 pacienţi (47,36%) şi respectiv la 13 pacienţi (34,21%) din totalul celor examinaţi. La apariţia acestor modificări macroscopice participă congestia şi angiogeneza prezente la nivelul mucoasei bronşice (6,15). Edemul mucoasei bronşice determină şi ştergerea reliefurilor cartilaginoase, aspect remarcat şi la 7 pacienţi din lotul examinat (18,42%).

Îngroşarea mucoasei bronşice conduce la reducerea diametrului acesteia, leziune observată la 9 pacienţi din cazuistică (23,68%), fiind constantă, comparativ cu leziunile de atrofie a mucoasei bronşice, semnalate doar în 3 cazuri (7,89%). În determinismul atrofiei mucoasei intervine în mod adjuvant terapia cronică steroidiană (6).

Hipersecreţia de mucus observată endoscopic se corelează cu hiperplazia celulelor caliciforme din structura epiteliului respirator şi a glandelor din submucoasă, cu intensitate variabilă în funcţie de cu severitatea bolii astmatice (16, 17). Macroscopic, în cazuistica de faţă, s-a constatat prezenţa în căile aeriene a unui mucus vâscos, în cantitate variabilă la 19 pacienţii (50%). Reducerea diametrului bronşiilor se datorează în parte edemului care duce la îngroşarea mucoasei şi fenomenului de ,,remodelare a peretelui bronşic" care apare în evoluţia bolii astmatice (18). Acest proces ireversibil este caracterizat şi prin leziuni de hiperplazie/hipertrofie musculară şi hiperplazie glandulară (18).

Se consideră astăzi că apariţia leziunilor epiteliale stau la baza dezvoltării unui proces inflamator cronic al mucoasei, element care contribuie suplimentar la întreţinerea modificărilor epiteliale (19,20). Denudarea epitelială apare ca o reacţie epitelială la agresivitatea inflamaţie de la nivelul mucoasei, deşi există studii în literatura de specialitate care sugerează ideea că aspectele de denudare epitelială remarcate la examenul microscopic în astmul bronşic s-ar datora, în parte, şi tehnicilor de recoltare fibrobronho-scopică (21). În studiul de faţă au fost observate leziuni de denudare epitelială parţială (15 cazuri - 39,47%), precum şi leziuni de denudare epitelială totală, acestea din urmă fiind notate la 21 de cazuri (55,26%) din totalul celor examinate.

O altă modalitate de răspuns a epiteliului respirator faţă de procesul inflamator cronic este şi apariţia hiperplaziei de celule

caliciforme, precum şi a unor arii mai mult sau mai puțin extinse de metaplazie scuamoasă. Hiperplazia celulelor caliciforme are grade diferite de severitate, acesta stând la baza hipersecreție de mucus care caracterizează boala astmatică (22). În cadrul lotului studiat, hiperplazia caliciformă a fost o leziune epitelială frecvent notată (36 de cazuri – 94,73%), în timp ce metaplazia scuamoasă a fost mai rar observată (la 16 de pacienți - 42,10%%).

Îngroşarea membranei bazele a epiteliului respirator este leziunea microscopică ce caracterizează boala astmatică în cadrul bronhopatiilor obstructive. Studiile din literatura de specialitate descriu îngroşarea membranei bazale ca şi modificare constantă la toți pacienții astmatici (23,24). Mai mult, grosimea membranei bazale este în concordanță cu treapta de severitate a bolii (25). Această leziune a fost notată la toți pacienții (38 de cazuri – 100%) incluşi în lot, o îngroşare importantă a membranei bazale fiind observată la persoanele cu forme sever persistente de boală.

Leziunile epiteliale descrise mai sus sunt acompaniate de existența unui infiltrat inflamator polimorf în lamina propria. Intensitatea şi stabilirea compoziției celulare pentru infiltratul inflamator a constituit obiectul de studiu a numeroase cercetări de specialitate (26,27). Prezența acestuia la nivelul mucoasei bronşice determină apariția edemului şi a hipervascularizației, elemente care contribuie la îngroşarea mucoasei bronşice (28,29). În acest proces intervine şi hipertrofia musculară şi glandulară - leziuni remarcate mai ales în formele severe de boală (astm moderat şi sever persistent) (30,31).

Rezultatele examenului microscopic pe lotul evaluat concordă cu datele din literatură, leziuni precum edemul, hiper-vascularizația şi infiltrarea inflamatorie a mucoasei bronşice fiind observate la toți pacienții (38 de cazuri – 100%) din cazuistică. Hipertrofia musculară şi cea glandulară au fost remarcate la 20 dintre pacienți (52,63%), aceştia fiind încadrați în stadiile moderat persistent şi respectiv sever persistent de boală.

CONCLUZII

Fibrobronhoscopia reprezintă cea mai importantă metodă de explorare endoscopică a căilor aeriene. Deoarece aceasta metodă invazivă necesită doar anestezie locală, toleranța pacientului este bună şi riscurile minime, fibrobronhoscopia a devenit astăzi metoda de elecție pentru susținerea diagnosticului în patologia pulmonară. Examenul macroscopic fibrobronhoscopic aduce date importante în ceea ce priveşte modificările mucoasei căilor respiratorii din astmul bronşic, permițând de asemenea recoltarea de fragmente de mucoasă bronşică prin biopsie şi realizarea unui diagnostic diferențial corect. Totuşi, biopsia bronşică rămâne „gold standard''- ul în ceea ce priveşte evaluarea inflamației mucoasei şi a procesului de ,,remodelare" a peretelui bronşic din evoluția bolii astmatice.

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PYRIDINE-DERIVED SCHIFF BASE-ANALYTICAL REAGENT FOR IRON (II) IONS

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PYRIDINE-DERIVED SCHIFF BASE-ANALYTICAL REAGENT FOR IRON (II) IONS (Abstract): **Aim**: Development of a new spectrophotometric method for quantitative determination of Fe (II) ions in pharmaceutical products based on the complexation reaction of the cations with an original Schiff base-2-salicylidene-aminopyridine, when a red complex, extractable in chloroform, with a maximum absorption at 525 nm has formed. **Material and methods:** A UV-VIS Hewlett-Packard 8453 Spectrophotometer has been used for all determinations. The optimum working conditions of the complexation reaction have been established by studying the influence of pH, ionic strength, and combining ratio on the complexation reaction. **Results:** The calibration curve had been plotted, and the detection limit was determined (L_D=1 μg). The proposed method has a good linearity in the 5-50 μg/mL range with a correlation coefficient r = 0.9946. The stability constant and the molar extinction coefficient were calculated ($\varepsilon = 4.16 \cdot 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$). The cation-ligand ratio was 1:2. The complexation reaction of Fe (II) ions with the Schiff base had no cation interferers, not even Fe (III) ions. **Conclusions:** The proposed method has been applied for the determination of Fe (II) in pharmaceutical products with very good results. **Keywords:** SCHIFF BASE, IRON (II) IONS, SPECTROPHOTOMETRY.

Iron is an essential element for living organisms, as it is involved in the transport of oxygen and in cellular oxidative processes. It is found in amounts of 5 mg/kg bodyweight for men and 30 mg/kg bodyweight for women. Approximately 2/3 of all iron ions are found in circulating red blood cells in the composition of hemoglobin, while smaller percentage may be found in myoglobin (4%), hemic enzymes (0.2%), transferrin (0.12%) and about 25% is deposited as ferritin and hemosiderin. Depending on the way iron they are bound, iron-protein complexes can be classified into three groups: hemoproteins, iron-sulfur proteins and iron-proteins. In hemoproteins, iron is incorporated into a system of four tetrapyrrole rings linked to proteins, such as myoglobin, hemoglobin, cytochrome c and enzymes such as cytochromoxidase, catalase and peroxidases. The binding of Fe to proteins occurs at certain sites of amino acids (1-4).

Schiff bases are a sub-class of imines. They are common enzymatic intermediates. During physiological processes involving pyridoxal-5'-phosphate, intermediate Schiff bases form between the α-NH_2 group of L-

valine, the ε-NH_2 group of lysine and the protein chains (5). In aqueous medium at pH = 4.5-8.5, fluorescent Schiff bases of 5'-dioxipyridoxal with n-hexylamines, are formed (6). In the synthesis of temperature-dependent tryptophan and the pH of the medium, an intermediate ligand is formed which is a Schiff base, pyridoxal-5'-phosphate-L-serine (7). There are also Schiff bases that exhibit electrical and magnetic properties (8).

The Schiff bases can be used as analytical reagents for the quantitative determination of important cations in the body (9). We have performed an analytical study on a Schiff base: 2-salicylidene-amino pyridine (fig. 1). It is an organic ligand obtained through the reaction of salicylaldehyde with 2-aminopyridine (10, 11).

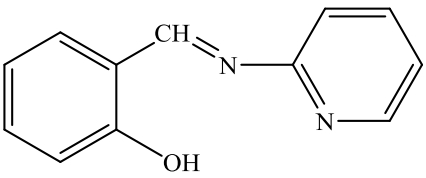

Fig. 1. 2-salicylidene-aminopyridine

The paper presents the development and validation of a new spectrophotometric method for the quantitative determination of Fe (II) ions and its application for the analysis of pharmaceutical products.

MATERIAL AND METHODS

A UV-VIS Hewlett-Packard 8453 spectrophotometer has been used for all determinations. All reagents and solvents were analytical grade.

A 0.1 mg/mL Fe (II) stock solution was prepared in distilled water, and then by suitable dilutions a 5-50 μg/mL Fe (II) standard solution was obtained. Reagent solution 0.1% (w/v) was prepared by dissolving 2-salicylidene-amino pyridine (SB) in methanol. A 0.2M sodium acetate and acetic acid buffer solution with pH = 5.6 has been used.

The Fe (II) ions react with 2-salicylidene-amino pyridine (SB) at pH = 6.0, and a complex combination is formed. It is extracted in chloroform and its absorbance measured at 525 nm is proportional to the concentration of the cations.

The optimum wavelength for detection was selected and the optimum working conditions were established by studying the influence of pH, formation time, and the stability of the complex (12, 13).

The cation/ligand combination ratio, the stability conditional constant (βn), and the limit of detection were calculated, and the potential interferers have been evaluated.

The complexation reaction was influenced by the ionic strength of the solution. Thus, the concentration and volume of the KCl solution that provided the ionic strength that maximized the absorption must be established. 1 mL of 30 μg/mL Fe(II) standard solution was treated at pH = 5.6 (0.2M sodium acetate and acetic acid buffer solution)with 0.5 mL solution with various concentration levels of KCl, 1 mL 1% (w/v) SB reagent solution. After extraction in 5 mL $CHCl_3$ and separation, anhydrous sodium sulfate was used to remove any traces of water, and the absorbance of the organic layer was measured at λ = 525 nm against a blank sample.

The complexation reaction was influenced also by the pH of the solution. So 1 mL of 30 μg/mL Fe(II) standard solution was mixed with 1 mL sodium acetate and acetic acid buffer solutions with a pH that varied in the range 3.5-7.0. 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1 mL 1% (w/v) SB reagent solution. After the extraction of the

red complex in 5 mL $CHCl_3$ and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured at λ = 525 nm against a blank sample.

The influence of the number of extractions in chloroform of the complex combination on its absorbance was evaluated by comparing the results obtained when using 5 mL of organic solvent once versus using 2.5 mL of chloroform twice.

The metal-ligand combination ratio was determined using various volumes of Fe (II) standard solution and mixing then with SB reagent solution with various concentration levels ($7 \cdot 10^{-3}$ M; $5 \cdot 10^{-3}$ M; 10^{-2} M), thus achieving the following combination ratios: 0.2, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, and 3.0. The mixtures were processed as previously described.

The stability constant (K_s) - expressed as $L \cdot cm^{-1} \cdot mol^{-1}$ - of the complex combination was determined using Harvey-Manning dissociation method based on the instability constant (K_i), according to the following equations (14):

$$K_s = 1/K_i$$

$$K_i = \frac{\alpha^2 C}{1-\alpha} \quad \text{and} \quad \alpha = \frac{A_m - A}{A_m}$$

where: α = degree of dissociation, A_m = maximum absorbance, A = equilibrium absorbance, c = molar concentration of Fe (II).

A second method for calculating the stability constant (β_n) was based on the following equation (15):

$$\beta_n = \frac{A/A_m}{(1-A/A_m)n^2 C_L^n}$$

where: Am = maximum absorbance,
A = equilibrium absorbance,
n = the coordination number of the ligand, and C_L = molar concentration of the ligand.

The new spectrophotometric method was validated and applied for the quantitative determination of Fe (II) (16-21).

RESULTS AND DISCUSSION

The optimum pH value for the complexation was established to be 5.6 (fig. 2). It was achieved using a 0.2 M acetic acid-sodium acetate buffer solution.

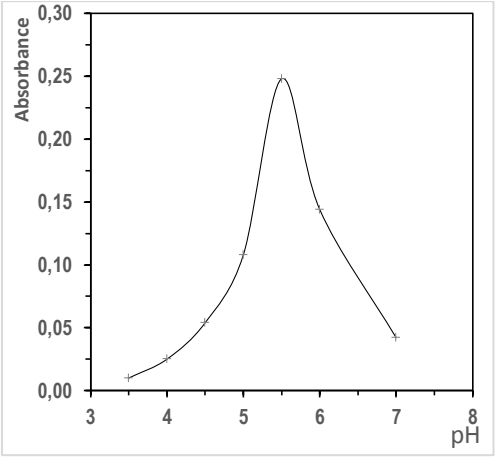

Fig. 2. Influence of pH on the absorbance of the SB-Fe (II) complex

The concentration and volume of the KCl solution providing the ionic strength for maximum absorbance of the complex was established based on the results shown in table I. It was established that a volume of 0.5 mL of KCl $2.5 \cdot 10^{-2}$ M solution provided a μ = 0.005526 ionic strength and maximum absorbance.

The results of the study on the variation of absorbance according to the number of extractions are shown in table II, and they prove that one extraction was found to be effective.

TABLE I

Influence of ionic strength on absorbance

Fe(II) (μg/mL)	KCl concentration	1% SB reagent solution (mL)	$CHCl_3$ (mL)	A 525 nm
30	$7.5 \cdot 10^{-1}$ M	1	5	0.140
30	$5 \cdot 10^{-1}$ M	1	5	0.147
30	$2.5 \cdot 10^{-1}$ M	1	5	0.155
30	10^{-1} M	1	5	0.167
30	$2.5 \cdot 10^{-2}$ M	1	5	0.248

TABLE II

Absorbance variation depending on the number of extractions

Fe(II) (μg/mL)	Buffer solution pH = 5.6 (mL)	$2.5 \cdot 10^{-2}$M KCl (mL)	1% SB reagent solution (mL)	$CHCl_3$ (mL)	A 525 nm
30	2.5	0.5	1	5.0	0.250
30	2.5	0.5	1	2.5 × 2	0.248

TABLE III

Absorbance variation depending on the number of extractions

Fe (II):SB Combination ratio	A 525 nm		
	$5 \cdot 10^{-3}$ M Fe (II)	$7.5 \cdot 10^{-3}$ M Fe (II)	$5 \cdot 10^{-2}$ M Fe (II)
1:5	0.169	0.294	0.578
1:3	0.200	0.323	0.702
1:2	0.212	0.391	0.869
1:1.4	0.177	0.315	0.777
1:1	0.092	0.285	0.653
1:0.66	0.069	0.219	0.546

Based on the data obtained (tab. III) the ion: ligand combination ratio was plotted, and its value was 1:2.

Determination of complex stability constant was done using the graph method in order to determine the values for the maximum absorbance (Am = 0.890) and the equilibrium absorbance (A = 0.292), and then to calculate the instability constant of the complex $K_i = 7.3 \cdot 10^{-5}$ and the stability constants $K_s = 1.4 \cdot 10^4$, considering that n = 2, and the concentration of the Fe (II) was 10^{-2} M. The calculated value of the extinction molar coefficient was $4.16 \cdot 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$. The second method of calculating the stability constant produced results ($\beta_n = 1.5 \cdot 10^4$ and $1.45 \cdot 10^4$) which were very close to those obtained using the Harvey-Manning dissociation method.

The optimized procedure was: 1 mL Fe(II) standard solutions (5-50 μg/mL) were mixed with 2.5 mL sodium acetate and acetic acid 0.2M pH = 5.6 buffer solution, 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1

mL 1% (w/v) SB reagent solution. After one extraction of the red complex in 5 mL $CHCl_3$ and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured after 10 minutes at 525 nm against a blank sample in a 1 cm cuvette.

During the validation of the method, it was established that the absorbance was proportional to the concentration in Fe (II) and the Lambert-Beer Law was obeyed in the 5-50 μg/mL range, with a correlation coefficient of 0.9946. The calibration curve is presented in fig. 3 and the linear equation was: $A = 0.008877 \cdot c - 0.02547$ (correlation coefficient = 0.9946, intercept = 0.008877, slope = 0.02547).

Table IV includes the results of the statistical data processed during the validation of the VIS spectrophotometric method for the quantitative determination of Fe (II).

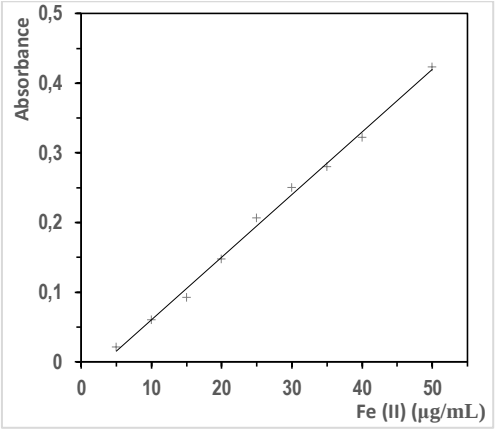

Fig. 3. Calibration curve

TABLE IV

Validation parameters

Fe (II) (μg/mL)	Analyzed Fe (II) (μg/mL)	Recovery (%)	Statistical parameters
5	5.18	98.95	Linearity range: 5.18-50.74 μg/mL Fe(II) Intercept = 0.008877·and slope = 0.02547 r = 0.9972; SD = 0.91 Accuracy: 98.94±1.009 n = 18; t_α = 2.11; α = 0.95 Repeatability: CV_r = 0.92 (n = 9) Reproducibility: CV_R = 2.05 (n = 18)
10	9.62		
15	13.23		
20	19.42		
25	26.00		
30	31.19		
35	34.21		
40	39.14		
50	50.74		

There were studies conducted that proved that the complexation reaction of Fe (II) using the Schiff base was not interfered by any other cation. The interference of Fe (III) on the complexation reaction at pH = 5.6 in the presence of KCl ($2.5 \cdot 10^{-2}$ M) has also been ruled out. The method could still be used for the indirect quantitative determination of Fe (III) after the reduction reaction to Fe (II) using reduction agents such as hydroxylamine or ascorbic acid.

The spectrophotometric determination method for Fe (II) using the Schiff base as reagent has been applied with very good results for the analysis of an antianemic pharmaceutical product formulated as syrup (tab. V).

The following procedure had been used: 2.5 mL syrup sample (7.2 mg/mL Fe (II)) was diluted with distilled water into a 100 mL graduated flask. 1 mL diluted sample solution was diluted even further with dis-

tilled water using a 10 mL graduated flask. 1 mL of solution was mixed with 0.5 mL sodium acetate and acetic acid 0.2M pH = 5.6 buffer solution, 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1 mL 1% (w/v) SB reagent solution. After one extraction of the red complex in 5 mL $CHCl_3$ and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured after 10 minutes at 525 nm against a blank sample in a 1 cm cuvette.

TABLE V

Applications of the spectrophotometric method for Fe (II)

Labelled Fe(II) (mg/5mL)	Analyzed Fe(II) (mg/5mL)	Recovery (%)	Statistical parameters	
			Repeatability	Reproducibility
36	36.3794	101.05	n = 5 M = 36.3342 S = 0.1883 S_x = 0.0842 A = 36.3342±0.2341 CV_r = 0.5184%	n = 15 t_α = 2.15 α = 0.95 M = 36.31 S = 0.34 A = 36.31±0.188 CV_R = 0.93
36	36.6046	101.67		
36	36.1541	100.42		
36	36.3794	101.05		
36	36.1541	100.42		
FRX Criteria: 34.92-37.12 mg/5mL				

The method proved to be as accurate if not more as other spectrophotometric methods, while using a simple procedure (22-23).

CONCLUSIONS

We proposed a new spectrophotometric method for the determination of Fe (II) in antianemia pharmaceutical products, based on our study on the complexation reaction of those cations with 2-salicylidene aminopyridine. The Schiff base that was used as reagent, formed with Fe (II) at pH = 5.6 a red complex extractible in chloroform with a maximum absorption at λ = 525 nm. The Lambert-Beer Law was obeyed in the concentration range 5-50 μg/mL Fe (II), and the linearity coefficient was r = 0.9946. The statistical parameters highlighted the good precision of the spectrophotometric method. The optimization of the method included: a study of pH and ionic strength influence on the complexation reaction, calculus of the combination ratio, stability constant and molar extinction coefficient, plotting the calibration curve, establishing the detection limit, and the evaluation of interferers. The statistical parameters highlighted the accuracy of the method, with relative errors well within the limit of criteria for VIS spectrophotometric methods. The method has been applied successfully for the analysis of iron (II) ions in antianemia pharmaceutical products, and it could be also used for the indirect analysis of iron (III) ions.

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SĂNĂTATEA PUBLICĂ ÎN RAIONUL ORHEI: REALITĂŢI ŞI OPORTUNITĂŢI

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PUBLIC HEALTH IN ORHEI COUNTY – REALITIES AND OPPORTUNITIES (Abstract): Orhei District Hospital has 420 beds located in 17 wards. Providing beds in 2010 is 39.2 to 10 000 people compared with 47.7 in 2008, but it is still more than the regional average for the country- 32.6. Rotation bed, the lethality rate of hospitalization of people have not changed in the previous year. The average duration of treatment and average bed occupancy rose in 2010 compared with 2009, but the bed occupancy rate is still small - about 60%.Share divergence diagnostic is lower than the previous year - this is a good indicator of quality. Enough old equipment is still an issue for the District Hospital Orhei. Making an analysis of the distinctive features of health care evaluations, where resources are expressed by the cost and the results are expressed as effects on health, we conclude that it is necessary to change the conceptual aspects regarding health care financing. For redress the economic and financial situation, is proposed following strategy: 1. Reduced costs for hotel accommodation, by introducing "a day hospital". This system implies investigation and medical advice, and writing medication without the patient to remain hospitalized for a long time (for cases that do not require prolonged hospitalization) 2. Reducing costs the necessary material resources with, the introduction of electronic auction. 3. Introducing the concept of health management, need for hospital management, better management the four types of resources available (human resources, material resources, financial resources, time). 4. Increasing managerial capacity, through a competitive selection manager, will be combine short-term planning and the long-term strategy (more flexible) to raise the efficiency of medical care. In this respect spaces allocated section of Pulmonology, will be reassembled in geriatric beds, and the space available, will be outsourced of other persons (individuals or corporate person), under the law where they will earn additional income. It is noted that the actual development costs are increasing. **Key words**: PUBLIC HEALTH, HOSPITAL, QUALITY OF MEDICAL CARE.

Conform legii, una din activităţile importante ale administraţiei raionului Orhei este asigurarea sănătăţii în conformitate cu necesităţile populaţiei şi prestarea serviciilor pentru maladiile prioritare în limita resurselor disponibile (2,5). Primul pas în atigerea acestui scop este evaluarea situaţiei referitor la starea sănătăţii populaţiei şi serviciile medicale existente la nivel local (raion Orhei).

SCOPUL LUCRĂRII

Sistemul public de servicii de sănătate existent în Raionul Orhei, Republica Moldova este necesar de a fi îmbunătăţit şi de asemenea se impune implementarea de noi servicii

axate pe problemele prioritare de sănătate ale populaţiei deservite. Pacienţilor trebuie să li se ofere o posibilităţi de alegere mai mari astfel încât fiecare pacient să aibă acces de fiecare dată la cea mai bună îngrijire. Calitatea înaltă a serviciilor de sănătate este posibilă doar atunci când fiecare membru al instituţiei şi fiecare parte a sistemului de sănătate lucrează eficient şi în armonie cu restul. Deasemenea trebuie să ne asigurăm că acţiunile întreprinse să fie orientate spre pacient şi să vedem serviciile oferite din perspectiva pacientului.

Identificare problemelor şi neajunsurilor activităţii instituţiilor prestatoare de servicii de sănătate este primul pas în demersul de creştere a calităţii acestora.

MATERIAL ŞI METODĂ

Acest studiu s-a realizat pe baza rezultatelor analizei activităţii medicale şi economice a instituţiilor medicale publice din raionul Orhei în perioada 2009-2010. Aceste rezultate au fost coroborate cu datele statistice oficiale oferite de Centrul de Statistică a raionului Orhei, indicatorii demografici calculaţi de Biroul Stării Civile Orhei, datele activităţii financiare oferite de CNAM, indicatorii stării de sănătate a populaţiei prezentaţi de Centrul Medicilor de Familie (CMF) Orhei, planurile de afaceri ale instituţiilor medicale, rapoartele rulajului materialelor şi produselor farmaceutice, statistica şi politica de resurse umane.

Datele au fost sistematizate şi analizate după cum urmează : indicatorii demografici (1) şi socio-economici ; indicatorii stării de sănătate ; indicatorii sistemului de sănătate ; finanţarea serviciilor medicale la nivel regional.

REZULTATE

Raionul Orhei are o suprafaţă de circa 120 mii hectare. Populaţia raionului este de 127,824 mii locuitori (dintre care 30 536 copii şi 97 288 adulţi).

Ponderea populaţiei 0-14 ani este mai mică decât ponderea populaţiei în vârstă (50 ani şi peste), respectiv 17,6% şi 25,3%.

Situaţia din perioada anilor 1995–2009 a arătat o reducere a ratei natalităţii în regiune de la 19,9‰ în 1995 la 10,3‰ în 2009. Numărul total al femeilor de vârstă fertilă este 36 247, rata generală a fertilităţii fiind de 26,7 la 1000 femei de vârstă reproductivă.

Din numărul total de persoane decedate, 15,3‰ constitue populaţia aptă de muncă. Mortalitatea infantilă în raionul Orhei este mai mică decât cea la nivel naţional (respectiv 10,8‰ şi 12,4‰).

Morbiditatea generală a populaţiei este prezentată sintetic în fig. 1.

În structura prevalenţei pe primul loc se situează Maladiile aparatului circulator– 102,7 la 10 000 populaţie ; II loc– Maladiile aparatului digestiv– 882,8 şi locul III– Tulburările mentale şi de comportament– 579,2 la 10 000 populaţie (tab. 1).

Prevalenţa alcoolismului în raionul Orhei este mult mai mare decât cea de la nivel naţional (2046,8 şi respectiv 1292,0 la 100 000 populaţie).

În acelaşi timp narcomania a înregistrat o creştere în rata de prevalenţă (circa de 2 ori), dar incidenţa (cazurile noi) a scăzut.

Pe parcursul anului 2010 au fost încadraţi în grad de invaliditate 361 persoane, ceea ce este cu 3 persoane mai puţin decât în 2009, mai mult de jumătate dintre acestea– 51,5% au primit gradul II de invaliditate şi fiecare a treia persoană– gradul III.

Invaliditatea copiilor sub 16 ani a scăzut în 2010 comparativ cu anul 2009 (19,42 şi respectiv 23,03 la 1 000 copii) : gradul I– 33%, gradul II– 59,6% şi gradul III– 8,9%). Pe locul întâi sunt tulburările mentale şi de comportament– 29,9%, majoritatea fiind reprezentată de cazurile de retard mental şi epilepsie. Pe locul doi sunt malformaţiile congenitale– 23%, iar pe locul trei sunt maladiile sistemului nervos– 18,0%.

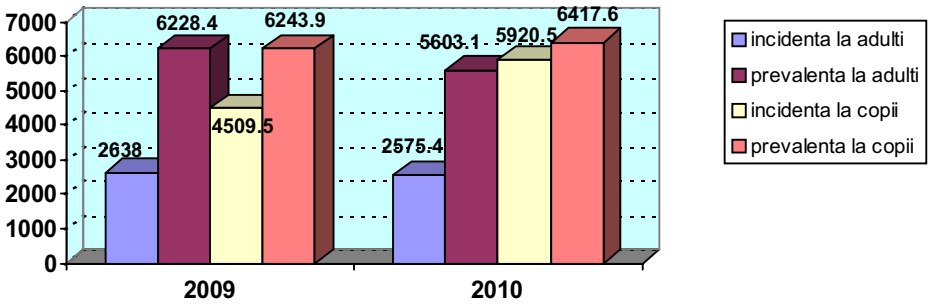

Fig. 1. Morbiditatea populaţiei (la 10 000 populaţie)

TABELUL 1

Incidenţa şi prevalenţa pentru primele cinci cauze de îmbolnăvire *(adulţi)*

	Prevalența					
Anul	**2009**			**2010**		
Locul în structură	Nosologia	la 10 000 populație	% din total	Nosologia	la 10 000 populație	% din total
I	Maladiile aparatului circulator	1041,46	16,7	Maladiile aparatului circulator	1102,7	19,6
II	Maladiile aparatului digestiv	874,18	14,0	Maladiile aparatului digestiv	888,8	15,8
III	Maladiile aparatului respirator	589,2	9,5	Tulburări mentale şi de comportament	579,2	10,3
IV	Maladiile aparatului genito-urinar	584,05	8,8	Maladiile aparatului respirator	569,2	10,2
V	Tulburări mentale şi de comportament	546,19	8,7	Maladiile aparatului genito-urinar	506,9	9,0
	Incidența					
	Nosologia	la 10 000 populație	% din total	Nosologia	la 10 000 populație	% din total
I	Maladiile aparatului respirator	440,12	16,7	Maladiile aparatului respirator	426,8	16,5
II	Maladiile aparatului genito-urinar	286,93	10,8	Maladiile aparatului circulator	255,2	9,9
III	Maladiile aparatului circulator	213,17	8,0	Maladiile aparatului genito-urinar	253,8	9,3
IV	Maladiile aparatului osteo-muscular şi ale ţesutului conjunctiv	210,39	7,9	Graviditate, naştere şi puerperium	227,5	8,8

În raionul Orhei, personalul care activează în sistemul public de sănătate constă în 1139 angajaţi : 185 medici, 581 asistente medicale, 237 infermiere, 136 angajaţi auxiliari.

Asigurarea cu medici de familie a populaţiei din raionul Orhei este mai mică decât la nivel naţional (4,8 comparativ cu 5,7 la 10 000 populaţie) (tab. 2).

Centrul Medicilor de Familie (CMF) Orhei, parte componentă a Spitalului Raional Orhei, deserveşte populaţia prin intermediul a 18 Centre de Sănătate, 30 Oficii ale Medicilor de Familie şi 17 filiale ale Oficiilor Me-

dicilor de Familie. Din totalul de 64 de centre medicale– 46 sunt amplasate în clădiri corespunzătoare (71,8%); 27 (42%) necesită reparaţii curente; 4 (6%) necesită reparaţii capitale.

Numărul total de consultaţii efectuate de medicii de familie a crescut de la 272 895 în 2009 la 333 625 în 2010, iar valoarea medie a numărului de consultaţii/locuitor de la 2,09 în 2009 la 2,57 în 2010. Cea mai mare pondere revine populaţiei de până la 18 ani de la 2,83 în anul 2009 la 3,91 consultaţii/locuitor în 2010.

În anul 2010 au fost luate la evidenţă 1 252 gravide. Ponderea gravidelor luate la evidenţă până la 12 săptămâni de sarcină are o tendinţă de creştere de la 71,1% în 2009 la 78,8% în 2010. În acelaşi timp, ponderea anemiei la gravide este încă mare– 56%, cu toate că conform Programului Unic sunt prescrise gratuit preparate de fier pentru gravide. Ponderea patologiilor extragenitale la gravide este încă mare (114,2 la 1 000 gravide în Orhei comparativ cu 65,5% la nivel naţional). 75% dintre gravide au fost examinate USG la 18-21 săptămâni, mai mult decât în 2009– 71,6% şi în 2008– 66,6%. 99% din gravide au fost consultate de ginecolog ceea ce se consideră un indicator bun. 96,5% naşteri au fost la termen şi 3,5%- prematur. Ponderea avorturilor a constituit 60,4/ 100 născuţi vii în 2009, ceea ce este mai mult decât în 2008– 55,0 şi mai mult decât la nivel naţional– 40,8. În anul 2010 au fost înregistrate 20 cazuri de naşteri la domiciliu. *Stillbirth rate* este mai mică decât la nivel naţional (respectiv 4,6‰ comparativ cu 5,8‰) şi a scăzut în 2010 comparativ cu anul precedent. Mortalitatea perinatală este mai mică decât la nivel naţional (8,1‰ şi respectiv 11,5‰), dar este puţin mai mare decât în anul 2008 (7,5‰).

Asistenţa Medicală Urgentă în raionul Orhei este asigurată de 7 echipe medicale: 4 cu profil general, 1 specializată pentru copii, 1 cardiologică şi o echipă de asistenţi medicali. Rata solicitărilor/ 1 000 populaţie rurală a crescut de la 232,3 în 2009 la 313,3 în 2010.

Asistenţa Medicală Specializată de Ambulator este asigurată de 29 medici şi 33 asistente medicale. Numărul total de vizite a crescut – 110 301 în 2008, 160 685 în 2009 şi 181 077 în 2010.

În 2010, din cheltuielile totale reale ale asistenţei medicale, 34,9% au fost pentru Sectorul primar (faţă de 31,7% în 2009), 6,9% pentru Asistenţa Medicală Specializată de Ambulator (2009–5,2%) şi 58,2% pentru Sectorul spitalicesc (2009–58,8%). S-a observat o tendinţă mică pentru redirecţionarea veniturilor şi cheltuielilor între sectoare, dar oricum Sectorul spitalicesc rămâne pe primul plan şi este încă privilegiat.

Analiza structurii cheltuielilor a relevat că în cadrul sectorului de Asistenţă Medicală Primară, 82,7% din cheltuieli au fost reprezentate de salarii şi alte sume pentru remunerarea muncii personalului medical şi plata contribuţiilor de stat (2009– 86,9%), iar pentru activităţi de investigaţii şi prestarea serviciilor de sănătate numai 5,0% (2009– 2,3%). În 2010 volumul cheltuielilor reale "per-capita"a fost 68,17 lei şi pentru populaţia asigurată– 90,47 lei, dar în acelaşi timp conform Contractului au fost stipulaţi 107,0 lei pentru persoane asigurate şi 6,0 lei pentru persoane neasigurate. Costul unei vizite este respectiv 25,79 lei şi 28,67 lei.

În Sectorul Specializat de Ambulator în 2010 remunerarea muncii şi plata contribuţiilor de stat au fost folosite la nivelul de 77,8% (2009– 75,6%) din totalul cheltuielilor. Pentru activitatea de menţinere (cheltuieli de menţinere şi gestiune)– 4,2% (2009–19,7%). Pentru consultaţiile pacienţilor şi investigaţii numai 5,0% (2009 –4,7%). Costul real al unei consultaţii pentru persoane asigurate– 12,5 lei.

Cheltuielile fixe pentru 2010 sunt în descreştere şi cheltuielile variabile sunt în creştere cu aceeaşi valoare de 8,9% com-

TABELUL 2
Numărul medicilor din Raionul Orhei

Medici	2009	2010	Asigurarea cu resurse umane
CMF/ medici de familie, a/med	69/61	72/62	72,4/70,1
Staţionar	92	84	87,1
Secţia Consultativă	26	29	87,1
TOTAL	187	185	80,6

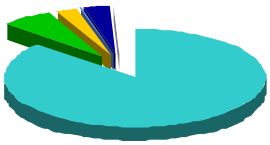

Fig. 2. Structura bugetului consolidat al IMSP spitalul Orhei

parativ cu anul 2009.

Ca şi celelalte două sectoare, Sectorul spitalicesc în 2010 a folosit majoritatea cheltuielilor pentru retribuirea muncii şi contribuţiile de stat 60,3% din cheltuielile totale (2009–56,6%), cheltuieli de menţinere şi gestiune– 15,1% (2009–18,3%), pentru hrana pacienţilor– 6,7% (2009–7,8%) şi pentru tratament- 15,8% (2009– 17,4%). Structura bugetului consolidat al IM SP spitalul Orhei este redată în fig. 2.

Costul real zi/pat este 130,33 lei (2009 – 125,80 lei) şi costul real al unui caz tratat– 1058,3 lei (2009–917,50 lei). Conform Contractului în 2010 pentru caz tratat iniţial au fost stipulaţi 990,72 lei.

Cheltuielile fixe pentru 2010 au crescut şi cheltuielile variabile au scăzut cu aceeaşi valoare de 5,0% comparativ cu anul 2009.

DISCUŢII

Raionul Orhei este cel mai mare raion din Republica Moldova. Densitatea populaţiei este de 96 persoane/m^2 ; 22% din populaţia raionului locuieşte în oraş, iar 78% în zona rurală. Oraşul Orhei este centrul administrativ şi cultural al raionului. Este situat la circa 40 km de capitală–municipiul Chişinău, şi are 29 700 locuitori. Raionul cuprinde 75 localităţi rurale, organizate în 38 primării. Ca şi majoritatea regiunilor ţării, raionul Orhei este o regiune multi-etnică. În 3 localităţi rurale majoritatea locuitorilor sunt ucraineni (3).

Structura populaţiei este mai mult de tip regresiv, deoarece ponderea populaţiei tinere (0-14 ani) este mai mică decât ponderea populaţiei în vârstă (50 ani şi peste), respectiv 17,6% şi 25,3%.

Schimbările demografice din anii precedenţi în raion, ca şi în toată ţara, sunt caracterizate prin următoarele tendinţe : scăderea natalităţii ; creşterea mortalităţii ; sporul natural negativ al populaţiei.

Principale cauzele ale mortalităţii infantile (0-1 an) sunt : unele afecţiuni din perioada perinatală–35,7% ; malformaţii congenitale, anormalii cromosomice– 21,4% şi bolile aparatului respirator– 21,4% din cazurile de deces.

Incidenţa şi prevalenţa la adulţi au scăzut comparativ cu anul 2009. În acelaşi timp, datele nu arată situaţia reală a morbidităţii generale a populaţiei, pentru că se bazează numai pe datele despre adresare.

Structura morbidităţii pe principalele cauze de îmbolnăvire a cuprins : maladiile cardiace (21,3%) ; maladiile respiratorii (20,9%) ; maladiile aparatului digestiv (11,8%) precum şi maladiile neurologice şi endocrine (11,7%). În cazul maladiilor sistemului

cardio-vascular, accidentul cerebral ocupă primul loc ca şi cauză a decesului, în aparatul digestiv– ciroza, în maldiile aparatului respirator– pneumonia.

Structura mortalităţii generale în raionul Orhei diferă de cea la nivel naţional (la nivel naţional maladiile oncologice sunt pe locul II, iar maladiile aparatului digestiv sunt pe locul III).

Analiza structurii invalidităţii a arătat că pe primul loc în structură se situează aceleaşi nosologii ca şi în structura mortalităţii şi morbidităţii. Fiecare al patrulea caz de invaliditate este cauzat de maladiile aparatului circulator, majoritatea acestor cazuri constituie maladiile hipertensive. Pe primele locuri în structura invalidităţii sunt şi maladiile aparatului digestiv, maladiile sistemului nervos, tumorile.

Consiliul Raional Orhei este fondatorul IMSP „Spitalul Raional Orhei" care prestează servicii medicale înalt calificate şi specializate pentru populaţia raionului şi raioanele adiacente: Rezina, Teleneşti şi Şoldăneşti (în special pentru asistenţa medicală specializată şi spitalicească).

Autorităţile publice locale sunt informate despre activitatea spitalului; aceasta a fost analizată trimestrial la Consiliul de Administrare. Autorităţile locale au susţinut financiar spitalul în vederea efectuării reparaţiilor şi procurării echipamentului costisitor (4).

Dacă în anul 2009 Agenţia Teritorială şi administraţia spitalului şi-au focalizat atenţia pe creştrea numărului cazurilor tratate, în 2010 atenţie deosebită s-a acordat calităţii prestaţiilor medicale, respectării standardelor, asigurării continuităţii între Asistenţa Medicală Urgentă, Asistenţa Medicală Primară, Secţia Consultativă şi Staţionar.

În anul 2010 CNAM a introdus diferenţierea în salarizarea cazurilor chirurgicale tratate operator şi medicamentos. Administraţia spitalului a propus în scris să fie luate în consideraţie cazurile tratate operator în traumatologie, obstetrică şi ginecologie cu diferenţiere de cost, însă s-a refuzat. Au fost făcute propuneri şi pentru serviciile specializate, inclusiv Centrul Perinatal, pentru raioanele alăturate ca fiind succesorul Spitalului Judeţean, dar încă nu s-a acceptat. CNAM propune reluarea acestor negocieri în primul semestru al anului 2011.

Conform *business plan*-urilor pe anii precedenţi, au crescut veniturile de la CNAM, dar conform opiniei administraţiei spitalului încă nu este suficient. De exemplu, costul medicamentelor şi costul investigaţiilor pentru o zi de spitalizare este de 22 lei. O problemă mare rămân reparaţiile curente şi procurarea echipamentului, deoarece echipamentul este foarte vechi (aproape 80% fiind uzate fizic şi moral) şi pentru creşterea calităţii serviciilor de sănătate este necesar un echipament performant şi tehnologii noi.

Analizând cheltuielile reale în 2010, observăm că ponderea cea mai mare în structura cheltuielilor în Sectorul primar şi în Sectoru specializat de ambulator revine remunerării muncii şi contribuţiilor obligatorii de stat, precum şi pentru plata serviciilor de mentenanţă, şi limitarea excesivă a cheltuielilor pentru procesul curativ şi procurarea de echipamente medicale.

CONCLUZII

1. Schimbările demografice în ultimii ani în raionul Orhei, ca şi în toată ţara, se caracterizează prin scăderea natalităţii, creşterea mortalităţii şi un spor natural negativ al populaţiei.

2. Principale cauzele ale mortalităţii generale a populaţiei în raionul Orhei sunt maladiile sistemului cardiovascular, maladiile digestive, tumorile, traumele, otrăvirile şi anumite consecinţe externe. Această structură diferă de cea la nivel naţional.

3. În 2010 s-a înregistrat o scădere a ponderii deceselor populaţiei în vârstă aptă de muncă, dar ponderea cazurilor de deces la domiciliu la această categorie de populaţie

este încă mare (65,7%).

4. Cu toate că în 2010 în raionul Orhei, mortalitatea infantilă este mai redusă decât cea la nivel naţional (10,8‰ şi respectiv 12,4‰) este totuşi mai mare decât cea din 2009. Mortalitatea infantilă are aceeaşi structură (după cauza de deces) ca şi la nivel naţional. Este necesar de menţionat că 20% dintre copiii mai mici de un an au decedat la domiciliu, iar 28,5% au decedat în primele 24 ore după spitalizare. Aceste valori relevă faptul că se impun mai multe eforturi pentru prevenirea mortalităţii infantile.

5. Incidenţa şi prevalenţa la adulţi au scăzut în 2010 comparativ cu 2009. Menţionăm că aceste date nu arată situaţia reală despre morbiditatea generală a populaţiei din raionul Orhei deoarece se bazează numai pe datele de adresare. Cauzele principale ale morbidităţii populaţiei sunt: maladiile aparatului circulator, maladiile digestive. Este important de menţionat că pe parcursul ultimilor cinci ani incidenţa şi prevalenţa prin alcoolism şi narcomanie au crescut în raionul Orhei. Incidenţa tuberculozei în ultimii ani a crescut de la 92,5 la 100.000 populaţie la 121,3 în 2010.

6. Incidenţa la copii a crescut, ceea ce poate fi datorită faptului că atenţia acordată copiilor a crescut. Maladiile aparatului respirator şi maladiile infecţioase încă sunt principalele cauze de îmbolnăvire la copii, dar traumele, otrăvirile şi anumite alte consecinţe externe rămân pe primul loc în incidenţa (cazuri noi de îmbolnăvire) la copii, cu toate că acestea sunt cauze care pot fi prevenite în morbiditatea şi mortalitate la copii. Rezultatele examinării profilactice a copiilor au arătat că în raionul Orhei ponderea copiilor cu probleme de sănătate (probleme auditive, de vedere, scolioză, retard în dezvoltarea fizică) este mai mare decât la nivel naţional.

7. Pe primele locuri în structura invalidităţii primare se situează nosologiile care sunt primele în structura pe cauze de îmbolnăvire şi deces. Fiecare al patrulea caz este datorat maladiilor sistemului circulator, prima cauză fiind reprezentată de maladia hipertensivă. Pe primele locuri în structura invalidităţii sunt şi maladiile digestive, maladiile sistemului nervos şi tumorile.

8. În baza rezultatelor prezentate putem concluziona că există relaţii importante de management epidemiologic şi clinic între principalele cauze ale morbidităţii şi mortalităţii. Pentru multe din maladiile cronice (ca hipertensiunea în maladia cardiologică şi cerebro-vasculară, maladiile respiratorii, digestive) complicaţiile sunt legate de morbiditatea principală. Sublinem că majoritatea acestor maladii pot fi depistate şi aproape în totalitate administrate de un practician generalist. Cu atât mai mult că, incidenţa poate fi mult redusă prin educaţie pentru sănătate şi profilaxie primară adecvate.

9. În Asistenţa Medicală Primară cea mai importantă problemă este lipsa medicilor de familie. La acest moment în raionul Orhei sunt vacante 27 de posturi de medic de familie. Condiţiile instituţiilor de sănătate, în special în zona rurală, şi echipamentul insuficient rămân prioritare. Numărul de vizite la domiciliu efectuate de medicii de familie a crescut de la 272 895 în 2009 la 333 625 în 2010, iar valoarea medie per capita a crescut de la 2,09 în 2009 la 2,57 în 2010. Cel mai mare număr revine copiilor sub 18 ani (2,83 în 2009 respectiv 3,91 în 2010).

10. Sectorul de Asistenţă Medicală Primară a îndeplinit indicatorii de calitate în baza criteriilor CNAM: imunizare (97,6%), monitorizarea TA (97,2%), evidenţa gravidelor (78,8%) şi examenul citologic (68,7%). În acelaşi timp, ponderea anemiei la gravide este încă mare–56%, deşi Programul Unic stipulează că gravidele luate în evidenţă beneficiază gratuit de preparate de fier şi6ţ. Ponderea avorturilor este mai mare decât la nivel naţional, iar educaţia pentru sănătate, inclusiv cu gravidele, şi planificarea familiei

trebuie să fie îndeplinite pentru a reduce problemele menţionate.

11. Analiza Asistenţei Medicale Specializate de Ambulator a arătat o creştere a numărului de vizite la specialişti. În acelaşi timp, numărul vizitelor specialiştilor în teritoriu (în mediul rural) a scăzut în comparaţie cu anul 2009: 89 în 2009 respectiv 64 în 2010. Numărul pacienţilor consultaţi în timpul acestor vizite a scăzut: 9 816 în 2009 şi respectiv 2 252 în 2010. Organizarea vizitelor a fost deficitară din cauza cooperării insuficiente cu Asistenţa Medicală Primară. Cooperarea cu Asistenţa Medicală Urgentă, Asistenţa Medicală Primară, Secţiile Spitaliceşti şi Secţia Consultativă trebuie să fie îmbunătăţite.

12. În baza analizei financiare, putem concluziona că sectorul spitalicesc necesită cea mai mare rată de mijloace financiare pentru mentenanţa mijloacelor fixe, procurarea echipamentului şi a mijloacelor de transport sanitar, ceea ce reprezintă 73,3% din total. Sectorul primar necesită 20,8% şi Sectorul Specializat de Ambulator–5,85%. Costul unei vizite în Sectorul primar pentru persoanele asigurate este stabilit în sumă de 32,20 lei. În structura costului unei vizite, reparaţia mijloacelor fixe constituie 1,60 lei sau 5,1% din volumul estimat de mijloace financiare, şi pentru procurarea echipamentului (mijloace fixe) respectiv–2,80 lei (8,7%). În Sectorul Specializat de Ambulator ponderea acestor cheltuieli în structura costului este de 0,90 lei (11,7%) şi respectiv 0,60 lei (8,4%0. Costul unei zile/pat în sectorul spitalicesc este estimat la 197,90 lei. Creşterea costului unei zile/pat este de 51,9%. În structura costului unei zile/pat, procurarea de mijloace fixe este de 9,0 lei (4,5%) din mijloacele financiare estimate şi pentru reparaţia mijloacelor fixe 24,6 lei (12,4%).

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FUMATUL, CONSUMUL DE ALCOOL ŞI SARCINA LA POPULAŢII DIN SUD-VESTUL ROMÂNIEI

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SMOCHING, ALCOHOL CONSUMPTION AND PREGNANCY IN A POPULATION OF SOUTH-WESTERN ROMANAI (Abstract): The purpose of the study was to evaluate the amplitude of tobacco and alcohol consumption among two populations living in urban, respectively rural areas in the South-Western part of the country. The presence of the two risky behaviours during the pregnancy was also analysed. **Material and method**: A number of 1,200 people, men and women, aged 18-75 years, with different educational levels, living in urban and rural communities were asked to answer a questionnaire with 111 items regarding their knowledge and attitudes about the sexual-reproductive health; 11 items referred to tobacco smoking and alcohol drinking. **Results**: About half of the subjects have never smoked. The mean age of starting smoking was 16.97 years. A number of 295 subjects (24.6%) were daily active smokers, men more frequently than women ($p < 0.001$). A percentage of 77.3% of the subjects used to drink alcohol, 16.4% of them daily. The daily alcohol consumption was more frequent among men, low educated people and rural inhabitants comparing to women, respectively high educated or urban inhabitants ($p < 0.001$); 11.2% of the respondents got frequently drunk. During the last pregnancy in the couple, women and high educated people stopped smoking and drinking more frequently then men, respectively low educated people. **Conclusions**: The above findings indicate the need to start in school effective health promotion campaigns to reduce tobacco and alcohol consumption and the need for support of population-based educational interventions aimed at smoking cessation in both parents. **Key words**: SMOKING, ALCOHOL DRINKING, PREGNANCY

Este unanim acceptată ideea că sănătatea este o condiţie indispensabilă pentru desfăşurarea la parametri optimi a întregii activităţi umane, pentru atingerea unor nivele superioare de competenţă şi performanţă în plan personal, familial, socio-economic şi profesional. Obţinerea şi menţinerea unei stări optime de sănătate sunt incontestabil dictate de atitudinea pe care fiecare persoană o are faţă de factorii de risc şi de protecţie prezenţi în mediu (1).

Principalii factori ce influenţează starea de sănătate sunt acele comportamente ce intră în alcătuirea stilului de viaţă: consumul de

alcool şi de tutun, alimentaţia, activitatea fizică, regimul de odihnă. Organizaţia Mondială a Sănătăţii (OMS) apreciază că jumătate din decesele înregistrate în lume se datorează unui stil de viaţă nesănătos în care fumatul, consumul excesiv de alcool, alimentaţia neraţională şi sedentarismul joacă un rol esenţial (2). Astfel, se consideră că aproximativ 50% dintre fumătorii adulţi (estimaţi la circa 1,3 miliarde în anul 2003) vor deceda ca urmare a unor afecţiuni cauzate de fumat - accidente vasculare coronariene şi cerebrale, boli pulmonare (inclusiv cancer bronho-pulmonar) şi alte afecţiuni maligne ; peste jumătate dintre aceste decese vor surveni la vârste medii (între 45 şi 54 de ani) (3). Problemele sanitare şi sociale generate de consumul abuziv de alcool sunt tot mai acute în numeroase ţări, ca urmare fie a creşterii cantităţilor de alcool consumate, fie a modificării grupelor populaţionale de consumatori (cu referire directă la femei şi tineri) (4). Alimentaţia nesănătoasă şi inactivitatea fizică sunt printre cauzele majore ale unor afecţiuni cronice precum bolile cardio-vasculare, diabetul zaharat de tip 2 şi unele forme de cancer, contribuind esenţial la creşterea numărului global de cazuri de îmbolnăviri şi decese (5). Prin urmare, promovarea stilului sănătos de viaţă apare ca cea mai eficientă metodă de prevenire a îmbolnăvirilor şi de menţinere a stării optimale de sănătate (1, 2).

În acest context, prezenta cercetare şi-a propus evaluarea extinderii a două componente ale stilului de viaţă (consumul de tutun şi de alcool) în rândul a două populaţii provenite din medii de rezidenţă diferite. Am investigat atât un grup populaţional din mediul urban, cât şi un altul din mediul rural – segment populaţional de cele mai multe ori „uitat" de către investigatori. Am urmărit cele două comportamente atât în evoluţia lor cotidiană, cât şi în perioada ultimei sarcini survenite în cuplurile în care cel puţin un partener a fost respondent în studiul nostru.

MATERIAL ŞI METODĂ

Studiul de faţă face parte dintr-o cercetare mai amplă vizând sănătatea sexual-reproductivă a populaţiei din zona de sud-vest a României (judeţul Dolj). În cercetare au fost incluşi 1.200 de subiecţi, bărbaţi şi femei, 600 (50%) domiciliaţi în municipiul Craiova şi 600 (50%) – în comune din judeţul Dolj. Selecţia participanţilor a fost aleatorie, urmărindu-se însă crearea unei structuri cât mai echilibrate a lotului din punct de vedere al nivelului de instruire (elementar, liceal şi superior) şi al grupelor de vârstă (18-25, 26-35, 36-45, 46-55, 56-65, 66-75 ani).

Subiecţii au fost rugaţi să răspundă unui chestionar conţinând 111 itemi (pentru ambele sexe), plus alţi opt itemi specifici pentru persoanele de sex feminin ; chestionarul a cuprins preponderent itemi precodificaţi, dar şi itemi cu răspuns liber. Subiecţii au fost informaţi asupra obiectivelor şi modalităţii de aplicare a chestionarelor. Participarea lor la cercetare s-a făcut în urma consimţământului lor liber exprimat, cu respectarea permanentă a principiilor anonimatului şi confidenţialităţii. În prezentul studiu sunt analizate răspunsurile oferite de subiecţi la 11 întrebări referitoare la consumul de tutun (vârsta şi motivele de debut al fumatului, frecvenţa şi numărul de ţigări fumate) şi alcool (frecvenţa consumului – incluzând episoadele de stare de ebrietate -, tipul şi cantitatea de băuturi alcoolice consumate, situaţia consumului de alcool în familie) ; subiecţii au fost, de asemenea, întrebaţi dacă au consumat tutun şi/sau alcool în timpul ultimei sarcini (subiecţii de sex feminin), respectiv în timpul ultimei sarcini a partenerei (subiecţii de sex masculin).

Rezultatele studiului au fost prelucrate statistic folosind programul SPSS. Evaluarea semnificaţiei statistice a diferenţelor dintre subloturi s-a realizat prin intermediul testului Pearson (testul χ^2) (6,7,8).

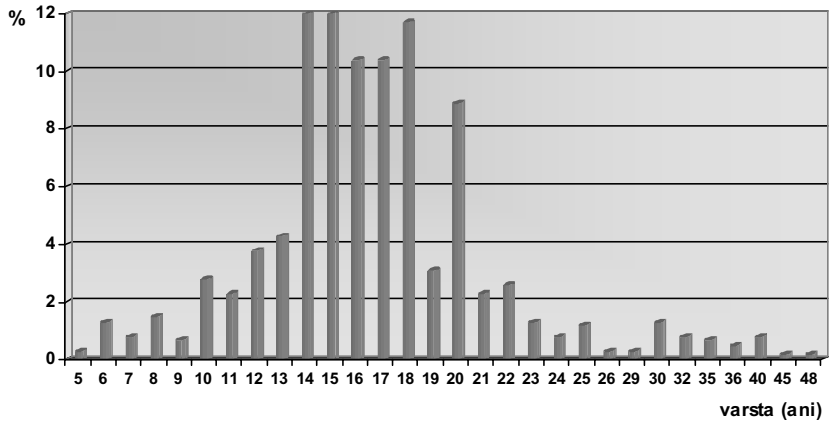

Fig. 1. Distribuţia statistică a fumătorilor (%) în funcţie de vârsta de debut a fumatului

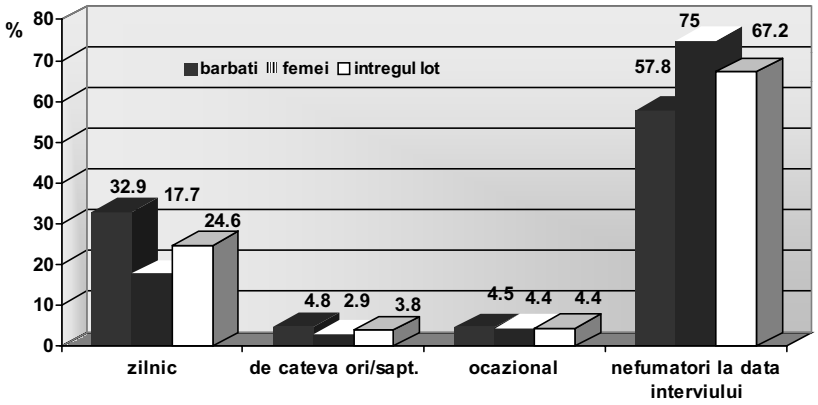

Fig. 2. Distribuţia statistică a subiecţilor (%) în funcţie de frecvenţa fumatului la data interviului

REZULTATE

FUMATUL. Prelucrarea statică a răspunsurilor oferite la întrebările din chestionar a indicat faptul că 594 de subiecţi (49,5%) nu au fumat niciodată. În cadrul lotului de 606 subiecţi (50,5%) care fumaseră cândva de-a lungul vieţii şi fumau sau nu la momentul interviului, vârsta medie de debut a fumatului a fost de 16,97 $\pm$ 5,68 ani; mai mult de jumătate dintre ei (52,3 %, N=317) erau deja fumători la împlinirea vârstei de 17 ani (fig. 1).

Cei mai mulţi dintre foştii şi actualii fumători au indicat influenţa anturajului (44,4%, N=269), curiozitatea (42%, N=255) şi necazurile (2,1%, N=13) ca fiind principalele motivele care i-au determinat să înceapă să fumeze,

La data interviului, mai mult de două treimi din subiecţi (67,2%, N=806) erau nefumători, iar ceilalţi 394 (32,8%) - fumători (fig. 2); 24,6% subiecţi din întregul eşantion (N=295, reprezentând 74,9% din numărul fumătorilor) fumau zilnic. Prevalenţa fumatului a fost mai mare în rândul bărbaţilor decât în rândul femeilor ($p<0.001$).

Cei mai mulţi dintre fumători (61%, N=240) fumau între 10 şi 20 de ţigări pe zi; un procent de 9,1% din numărul fumătorilor (N=36) consumau mai mult de 20 de ţigări pe zi, în câteva cazuri fiind menţionat chiar consumul a 40-50 de ţigări pe zi. Ceilalţi 19 subiecţi care fumau zilnic (4,8% din numărul fumătorilor) utilizau mai puţin

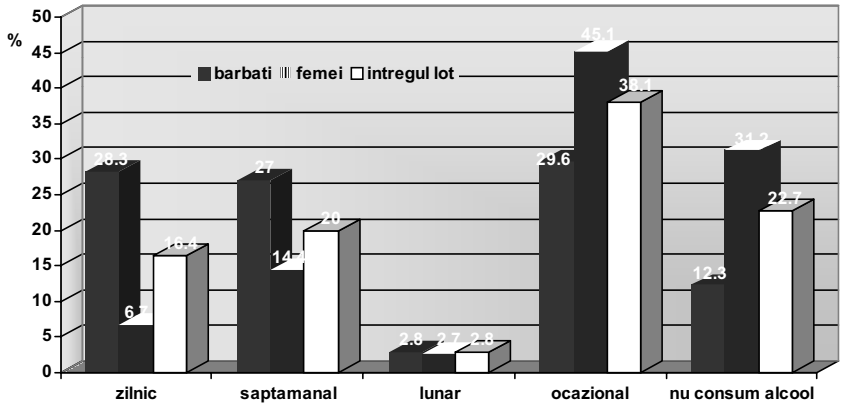

Fig. 3. Distribuţia statistică a subiecţilor (%) în funcţie de gen şi de frecvenţa consumului de alcool

de 10 ţigări pe zi.

În lotul nostru, nivelul de instruire al subiecţilor nu a determinat diferenţe statistic semnificative în ceea ce priveşte prevalenţa fumatului.

CONSUMUL DE ALCOOL. La nivel individual, rezultatele cercetării indică faptul că 197 de subiecţi (16,4%) consumau zilnic alcool la data interviului (fig. 3). Cei mai mulţi dintre subiecţi (N=457, 38,1%) s-au declarat doar consumatori ocazionali, în timp ce alţi 272 (22,7%) nu consumau deloc băuturi alcoolice. Consumul de alcool este mult mai frecvent în rândul bărbaţilor decât în rândul femeilor ($p<0.001$), aceeaşi situaţie constatându-se şi în ceea ce priveşte frecvenţa episoadelor de beţie acută (bărbaţii au fost „frecvent" în stare de ebrietate în proporţie de 23,2%, în timp ce femeile - numai în proporţie de 1,4%).

În această cercetare, atât nivelul de instruire, cât şi mediul de rezidenţă au apărut ca factori de discriminare între subloturi în privinţa consumului de alcool (tab. I), diferenţele fiind statistic semnificative pentru ambele variabile ($p<0,001$).

Astfel, s-a constatat faptul că procentul consumatorilor zilnici de alcool reprezenta 23,8% din subiecţii cu studii elementare şi doar 5,6% din respondenţii cu studii universitare, cei din urmă declarându-se, în proporţie de 49,8%, doar consumatori ocazionali de alcool.

În mediul rural, 23,1% din respondenţi au indicat consumul zilnic de alcool, în timp ce populaţia din mediul urban s-a declarat consumatoare de băuturi alcoolice ocazional (47,8%) sau la sfârşit de săptămână (16,7%).

La nivel familial, prevalenţa consumu-

TABELUL I

Distribuţia statistică a subiecţilor (%) în funcţie de nivelul de instruire şi mediul de rezidenţă, respectiv frecvenţa consumului de alcool

	FRECVENŢA CONSUMULUI DE ALCOOL				
	zilnic (% din lot)	**săptămânal (% din lot)**	**lunar (% din lot)**	**ocazional (% din lot)**	**nu consum alcool (% din lot)**
NIVEL DE INSTRUIRE					
elementar	23,8	20,6	1,7	30,2	23,7
liceal	13,2	17,9	2,9	41,4	24,6
superior	5,6	22,9	4,8	49,8	16,9
MEDIU DE REZIDENŢĂ					
urban	9,7	16,7	3,3	47,8	22,5
rural	23,1	23,4	2,2	28,4	22,9

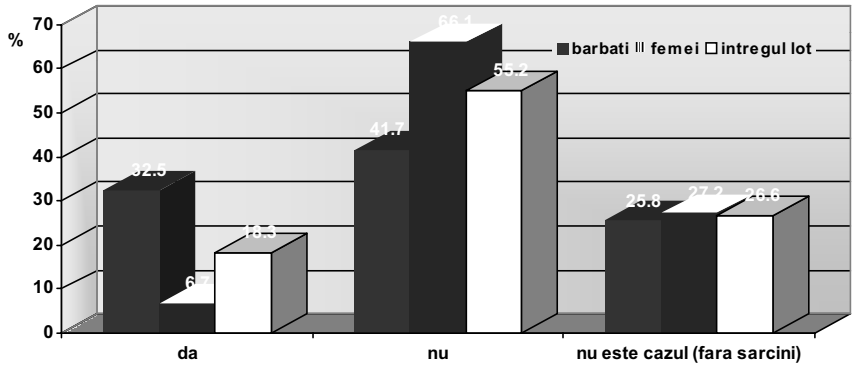

Fig. 4. Distribuţia statistică a subiecţilor (%) în funcţie de gen şi de consumul de tutun în timpul sarcinii/în timpul sarcinii partenerei

lui de alcool a indicat valori mai mari decât cele constatate în cazul consumului individual. Astfel, băuturile alcoolice se consumau zilnic în familiile a 288 de subiecţi intervievaţi (24%); numai 184 de subiecţi (15,3%) proveneau din familii în care nu se consuma alcool. Am constatat diferenţe statistic semnificative ($p < 0,001$) în funcţie de nivelul de instruire al respondenţilor, consumul zilnic de alcool fiind întâlnit în 33,6% din familiile ai căror membri prezentau un nivel elementar de instruire şi în 9,1% din cele alcătuite din persoane cu pregătire universitară.

CONSUMUL DE TUTUN ŞI ALCOOL ASOCIAT SARCINII. Rezultatele prezentate în figura 4 indică procentul redus de subiecţi (18,3%) care au continuat să fumeze atunci când în cuplu a apărut o sarcină. Remarcăm, totodată, proporţia semnificativ mai mică ($p < 0,001$) de femei care au continuat să fumeze în perioada ultimei sarcini (6,7%), comparativ cu proporţia bărbaţilor ce nu au renunţat la acest comportament (32,5%) pe parcursul sarcinii partenerei.

Prelucrarea statistica a datelor a indicat faptul că numai 7,3% din numărul total de femei au consumat băuturi alcoolice în timpul ultimei sarcini.

În tabelul II este redată distribuţia statistică a subiecţilor în funcţie de nivelul de instruire şi de consumul de tutun şi alcool în timpul sarcinii/în timpul sarcinii partenerei. Am constatat faptul că procentele persoanelor cu studii superioare care au menţinut cele două comportamente de risc în timpul sarcinii (17,3% pentru fumat, 23,8% pentru alcool) au fost mai reduse compa-

TABELUL II

Distribuţia statistică a subiecţilor (%) în funcţie de nivelul de instruire şi de consumul de tutun şi alcool în timpul sarcinii/în timpul sarcinii partenerei

NIVEL DE INSTRUIRE			
	elementar	**elementar**	**elementar**
FUMATUL ASOCIAT SARCINII			
Nu am fumat (% din lot)	57,3	55,9	48,9
Am fumat (% din lot)	20,1	16,6	17,3
Nu este cazul – fără sarcini (% din lot)	22,6	27,5	33,8
CONSUMUL DE ALCOOL ASOCIAT SARCINII			
Nu am consumat (% din lot)	50,8	53,5	42,4
Am consumat (% din lot)	26,6	19	23,8
Nu este cazul – fără sarcini (% din lot)	22,6	27,5	33,8

rativ cu persoanele cu instruire elementară (20,1%, respectiv 26,6%), diferenţele fiind semnificative statistic (p=0,02, respectiv p=0,002).

DISCUŢII

În lotul analizat, procentul persoanelor fumătoare la momentul interviului a fost similar celui întâlnit în studiul naţional referitor la consumul de tutun, derulat de Centrul pentru Politici şi Servicii de Sănătate în anul 2007 (32,8% vs. 30%). Am constatat însă că subiecţii din cercetarea de faţă au început să fumeze la o vârstă mai mică (16,97 ani vs. 19,55 ani), astfel încât, în acest lot, mediana a fost 16 ani, iar în studiul naţional - 19 ani. Această constatare ar putea sugera unele deficienţe în educaţia sanitară a copiilor şi tinerilor privind efectele nocive ale (debutului precoce al) fumatului asupra sănătăţii în judeţul nostru. În ambele studii, cea mai mare parte a fumătorilor (procente situate în jurul valorii de 61%) consumau 10-20 ţigări pe zi şi aproximativ fiecare al zecelea fumător consuma mai mult de 20 de ţigări pe zi (9).

Procentul consumatorilor de alcool în lotul studiat (77,3%) a înregistrat valori crescute, în condiţiile în care cercetările arată că 64% dintre persoanele cu vârsta de peste 12 ani consumă alcool (1) Prevalenţa crescută a consumului de alcool în rândul populaţiei se datorează faptului că, pentru majoritatea oamenilor, acest comportament reprezintă o modalitate de petrecere a timpului liber, de creare a unei stări de bine, de sărbătorire a unor evenimente. Pe lângă această explicaţie, procentul mare de consumatori zilnici de alcool întâlnit (în prezentul studiu) în mediul rural a avut la bază consumul de băuturi alcoolice obţinute în gospodăria proprie; în cazul bărbaţilor, acest consum a fost estimat la 2-3 l de vin şi 300-400 ml băuturi spirtoase (ţuică) pe zi. În schimb, locuitorii din mediul urban au declarat cu precădere consumul de vin sau bere, fie ocazional, fie săptămânal, în zilele libere, cu prilejul servirii mesei alături de ceilalţi membri ai familiei – situaţie mai puţin probabilă în zilele lucrătoare.

Efectele nocive ale consumului de alcool şi tutun în timpul sarcinii sunt numeroase şi bine argumentate în literatură. Astfel, expunerea intrauterină a fătului la fum de ţigară poate genera numeroase efecte adverse asupra evoluţiei sarcinii, naşterii şi lehuziei, supune fătul la acţiunea unui puternic stres oxidativ, favorizează apariţia bolilor alergice (mai ales respiratorii) şi poate influenţa negativ creşterea în greutate a fătului, dezvoltarea sistemului său nervos şi a aparatului cardio-vascular (3,10,11,12,13). Aceste efecte pot apare atât atunci când mama este fumătoare activă, cât şi pasivă. În lotul analizat, rezultatele sugerează expunerea cu precădere pasivă a femeilor însărcinate la fumul de ţigară: procentul femeilor fumătoare active a scăzut de la 17,7% la 6,7% după apariţia sarcinii, în timp ce partenerii lor au continuat să fumeze (32,5% vs. 32,9%). Studiile arată ca majoritatea fumătorilor obişnuiesc să fumeze acasă, situaţie în care domiciliul fumătorului (alături de locul de muncă) reprezintă locul unde expunerea la fumul de ţigară a altor persoane se face în proporţiile cele mai mari. Cei mai mulţi fumători fumează în spaţiile închise de la domiciliu şi chiar în prezenţa membrilor nefumători ai familiei (9).

Expunerea prenatală la alcool poate afecta dezvoltarea fătului, generând *spectrul tulburărilor alcoolice fetale*. Cea mai severă formă de manifestare a acestuia este *sindromul alcoolic fetal,* caracterizat prin malformaţii faciale, tulburări de creştere şi afectarea sistemului nervos central (13,14,15, 16,17). În lotul analizat, numai 44 de femei (7,3%) au declarat că au consumat alcool în timpul sarcinii, procentul fiind inferior celor întâlnite în studii similare: într-un studiu coreean, 16,4% dintre femei au declarat că au consumat alcool în timpul sarcinii

(17), în timp ce 19,8% dintre femeile australiene participante la o cercetare similară au fost de acord cu consumul de alcool asociat sarcinii (18).

CONCLUZII

Prevalenţa crescută a consumului de tutun şi alcool printre participanţii la prezenta cercetare, adoptarea acestor comportamente de risc încă din copilărie sau adolescenţă şi situarea frecventă a subiecţilor în postura de „fumători activi" atrag atenţia asupra necesităţii declanşării intervenţiilor educaţionale încă din primii ani de şcoală şi intensificării lor la adolescenţi şî adulţi tineri, înainte de apariţia sarcinii în cuplu. Intervenţiile bazate pe un model teoretic al comportamentului sanogen şi care iau in considerare experienţele anterioare, influenţele sociale, percepţia riscului şi identificarea cunoştinţelor şi a concepţiilor eronate pot avea mai mult succes în rândul tinerilor decât o abordare educaţională tradiţională.

MULŢUMIRI

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GHRELIN AND ANG 1-7 HAVE VASODILATATORY CUMULATIVE EFFECTS ON PULMONARY VESSELS

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GHRELIN AND ANG 1-7 HAVE VASODILATATORY CUMULATIVE EFFECTS ON PULMONARY VESSELS (Abstract): Ghrelin is an orexigenic hormone that has been shown to have vasodilator effects. Angiotensin 1-7 (Ang 1-7) is a bioactive component of the renin-angiotensin system and may play an important role in the regulation of cardiovascular homeostasis. **Aim**: The aim of this study was to investigate the interaction between ghrelin and a better known vasodilatator, Ang 1-7. **Material and method**: The dose – dependent relaxing effects of ghrelin were assesed on pulmonary artery rings of male Wistar rats with intact endothelium in the absence or the presence of Ang 1-7. Ang 1-7 was added in the organ bath with 5 minutes before N^G-nitro L-arginine methyl ester (L-NAME, 1 μM) and indomethacin administration in the presence of cumulative doses of ghrelin. **Results**: Our results showed that Ang 1-7 could improve and restore vasorelaxant effects of ghrelin on rat pulmonary rings with intact endothelium. **Conclusion**: Vasodilatatory actions of ghrelin are potentiated by Ang 1-7 and mediated by local synthesis of prostaglandins and nitric oxide. **Key words**: GHRELIN, ANGIOTENSIN 1-7, PULMONARY RAT ARTERY, NITRIC OXIDE

Ghrelin was identified in 1999 (1) as the endogenous ligand of growth hormone secretagogue receptor 1a (GHSR-1a) described by Howard et al. in 1996 (2). Ghrelin has been viewed by many researchers as a central modulator of energy homeostasis and a regulator of growth hormone secretion (3). Some studies revealed cardiovascular effects of ghrelin, including lowering of peripheral resistance, possible improvement of contractility and cardioprotective effects both *in vivo* and *in vitro* (4).

The aim of this study was to investigate the interaction between ghrelin and a better known vasodilatator, angiotensin 1-7 (Ang 1-7) (5). In addition both the cyclo-oxygenase inhibitor indomethacin and the nitric oxide synthetase inhibitor N(G)-nitro L-arginine methyl ester (L-NAME) were investigated.

MATERIAL AND METHOD

The experiments were conducted in age-matched Wistar male rats (Bucharest Cantacuzino Institute) with body weights of 150-200 g housed under standard laboratory conditions, with free access to standard rodent chow and tap water. This study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi and it was performed according to the Helsinki convention for the use and care of animals, and to the European Communities Council Directive 609 of 24 November 1986. Rats were decapitated and exsanguinated. The rat pulmonary artery was rapidly removed,

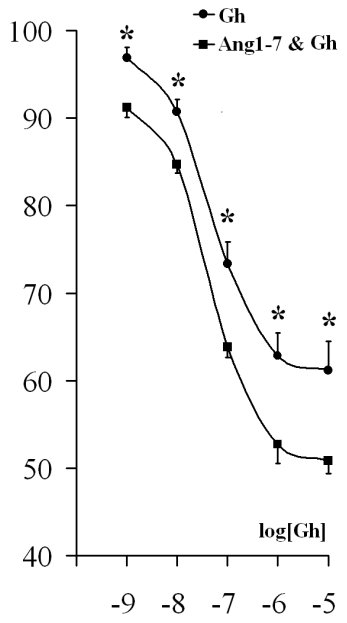

Fig. 1. Effects of ghrelin (Gh, 10 nM – 10 μM) on rat pulmonary artery ring preparations with intact endothelium precontracted with 1 μM phenilephrine in the absence and the presence of 10 μM Angiotensin (Ang1-7) (n=6). Data are presented as percentage of pre-contraction induced by 1 μM phenilephrine. *: $p<0.05$ as compared with ghrelin alone

cleaned and cut into rings, 2 mm wide. Individual rings were then mounted between wires in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed by ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). Changes in isometric tension were monitored using force transducers connected to a PC via the PC–LPM–16 multifunctional I/O board (National Instruments Corp., Austin TX), driven by the respective standard software (NiDaqWin). The tissue organ bath contained the Krebs–Henseleit solution containing (mM): NaCl 118, KCl 4.8, CaCl2 2.5, MgSO4 1.6, KH2PO4 1.2, NaHCO3 25, glucose 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O2 and 5% CO2 (pH=7.2–7.4). A resting tension of 0.5 g for rat pulmonary artery was applied to each ring and then allowed to equilibrate for 90 minutes before the start of the experiment. The bathing medium was renewed from 15 to 15 minutes.

Nitric oxide (NO) releasing was measured using an APOLLO 4000 system (World Precision Instruments - WPI). The method was previous described (6). Calibration of 30 microns diameter L-shaped electrodes (ISO--NOP30-L) using S-Nitroso-N-acetyl-D, L-penicillamine (SNAP) was made accordingly to WPI technical indication.

After the equilibration period, vessel rings were initially stimulated with 40 mM KCl as a standard stimulus. After re-equilibration in Krebs solution for 45 min, the 10 μM phenylephrine (Phe) was used to generate a sustained contraction; the relaxant responses to cumulative concentration of ghrelin (10 nM – 10 μM) were subsequently studied. Angiotensin 1-7 (10 μM) was added in the organ bath with 5 minutes before L-NAME (10 μM) and indomethacin (1 μM) administration in the presence of cumulative doses of ghrelin (10 nM – 10 μM).

Expression of results and statistical analysis : The results are expressed as the percentage of the control contraction (KCl, 40 mM; mean ± S.E.M.). Statistical significance was tested using one-way analysis of variance (ANOVA), completed with Bonferroni method (SigmaStat software, Jandel Corporation). $p<0.05$ was considered statistically significant.

Phenilephrine, angiotensin 1-7, L-NAME, indomethacin, recombinant rat ghrelin were all obtained from Sigma (Sigma-Aldrich Inc., St. Louis MO). All the other compounds used were of analytical grade.

RESULTS

The relaxing effects of ghrelin cumulative doses are presented in fig. 1. Admi-

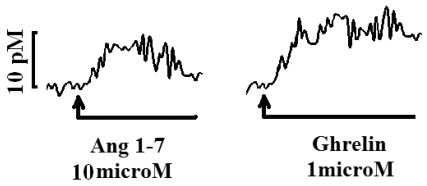

Fig. 2. Nitric oxide releasing on rat pulmonary rings by 10 μM Ang 1-7 and 1 μM ghrelin.

nistration of 10 μM Ang 1-7 had significant amplificatory effect on ghrelin – induced vasorelaxation: the Emax became 50.86$\pm$1.42 in the presence as 61.21$\pm$3.30 in the absence of of Ang 1-7 ($p<0.05$). The EC50 was not significantly different (48.61 nM without Ang 1-7 *vs*. 47.14 nM with Ang1-7).

The NO – releasing effects of 10μM Ang 1-7 and 1 μM ghrelin (Gh) on rat pulmonary arteries with intact endothelium are presented in fig. 2. The ghrelin – induced NO releasing effects were with at least 25% higher than Ang1-7.

The L-NAME pre-treatment (fig. 3) dramatically decreased the ghrelin -induced relaxation (E_{max} = 88.08$\pm$1.76). The Ang1--7 significantly increased the ghrelin effects just for 0.1 μM (83.71$\pm$2.03 *vs*. 91.22$\pm$1.73), 1 μM (80.42$\pm$2.55 *vs*. 88.08$\pm$1.38) and 10 μM (77.38$\pm$2.76 *vs*. 90.20$\pm$2.52).

Inhibition of prostaglandin synthesis (fig. 4) with 10 μM indomethacin (Indo) did not decrease the ghrelin effects with exception of low doses (100.94$\pm$0.95 *vs*. 96.88$\pm$1.22 for 10 nM ghrelin; 81.01$\pm$1.30 *vs*. 73.35$\pm$2.52 for 0.1 μM ghrelin). In this conditions the amplificatory Ang1-7 effects were still maintained, even if were lower as compared with results obtained without Indo (63.92$\pm$1.25 *vs*. 71.94$\pm$2.13 for 0.1 μM ghrelin; 52.76$\pm$2.17 *vs*. 59.82$\pm$1.17 for 1 μM ghrelin; 50.86$\pm$1.42 *vs*. 55.56$\pm$0.93 for 10 μM ghrelin).

Incubation of vascular rings (fig. 5) with both L-NAME and Indo blocked the relaxation and revealed moderate vasoconstrictory ghrelin effects (E_{max} = 117.99$\pm$3.97). Pre-treatment with Ang1-7 did not modify the ghrelin effects.

DISCUSSIONS

Ghrelin (10 nM – 10 μM) had dose dependent relaxant effects on the pulmonary rat ring preparations with intact endothelium pre-contracted with 1 μM phenilephrine (fig. 1). Ang 1-7 potentiated vasodilatatory effects of ghrelin (fig. 1). Taking into account that Ang (1-7) actions are dependent by both NO and vasodilatatory PGs we attempt to investigate the roles of these two mediators in the ghrelin effects.

Both, Ang 1-7 and ghrelin could stimulate nitric oxide (NO) releasing on rat pulmonary rings with intact endothelium (fig. 2).

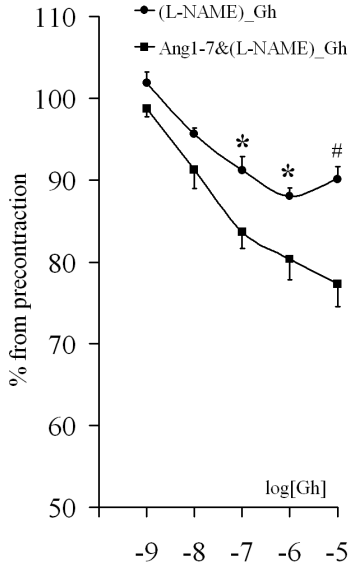

Fig. 3. Influences of 10 μM Angiotensin (Ang1-7) on ghrelin (Gh, 10 nM – 10 μM) effects on rat pulmonary artery ring preparations precontracted with 1 μM phenilephrine and incubated with 10 μM N(G)-nitro L-arginine methyl ester (L-NAME) (n=6). Data are presented as percentage of pre-contraction induced by 1 μM phenilephrine. * $p<0.05$ in absence as compared with presence of Ang1-7; # $p<0.01$ in absence as compared with presence of Ang1-7

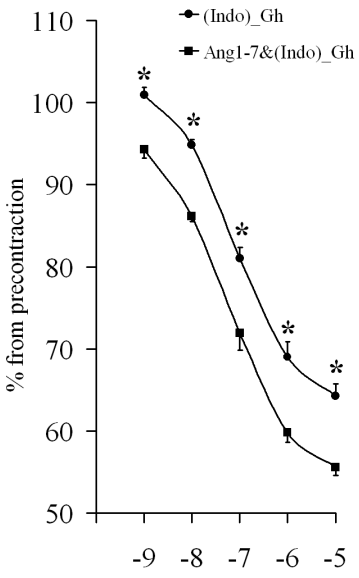

Fig. 4. Influences of 10 μM Angiotensin (Ang1-7) on ghrelin (Gh, 10 nM – 10 μM) effects on rat pulmonary artery ring preparations precontracted with 1 μM phenilephrine and incubated with 1 μM indomethacin (Indo) (n=6). Data are presented as percentage of pre-contraction induced by 1 μM phenilephrine. *: $p<0.005$ in absence as compared with presence of Ang1-7

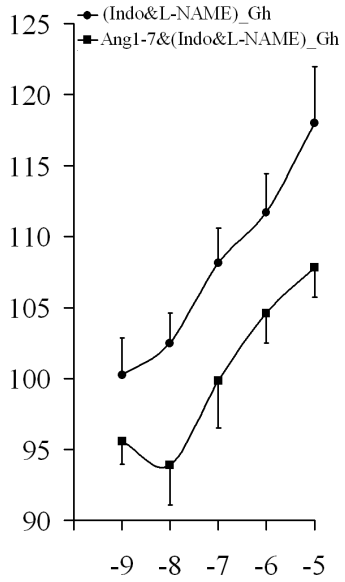

Fig. 5. Influences of 10 μM Angiotensin (Ang1-7) on ghrelin (Gh, 10 nM – 10 μM) effects on rat pulmonary artery ring preparations precontracted with 1 μM phenilephrine and incubated with 1 μM indomethacin (Indo) and 10 μM N(G)-nitro L-arginine methyl ester (L-NAME) (n=6). Data are presented as percentage of pre-contraction induced by 1 μM phenilephrine

It is generally accepted that NO is a potent vasodilator that derives from the oxidation of L-arginine by NO synthetases (NOSs): neuronal NOS (nNOS, brain NOS or type I NOS), inducible NOS (iNOS or type II NOS) and endothelial NOS (eNOS or type III). eNOS and nNOS are calcium – dependent constitutive enzymes that generate small amounts of NO involved in vasodilation / maintenance of vascular tone, antiplatelet aggregation and neurotransmission. iNOS is a calcium - independent inducible enzyme that produces great amounts of NO. Our results agree well with those from the studies on human vessels *in vivo* who showed that ghrelin could have vasodilatatory effects partially mediated by NO (7,8). The next step was to investigate the functional significance of this NO releasing effect of ghrelin. So, we assessed the vasodilatatory effects of ghrelin on pre-contracted pulmonary artery rings but in presence of L-NAME. The relaxant effects of ghrelin were markedly reduced, but not totally blocked on the presence of 1 μM L-NAME (fig. 3). Even more, the Ang1-7 presence effects was lower (two third from Ang1-7 effects in absence of L-NAME) amplificatory effect on ghrelin – induced vasorelaxation (fig. 4). So, both the ghrelin actions and the Ang1-7 effects on ghrelin--induce vasorelaxation were partially dependent by NO.

In order to verify if vasodilatatory effects of ghrelin are due to prostaglandin synthesis we repeated the experiments in the presence of 1 μM indomethacin. The relaxant effects of ghrelin were slightly reduced by indomethacin (fig. 4) and the Ang1-7

amplificatory effects were maintained. Nevertheless, the incubation of rat pulmonary rings with indomethacin reduced the vasodilatatory effects of 1 - 100μM ghrelin in the presence of Ang1-7.

Blocking of local synthesis of both nitric oxide and prostaglandins uncover the barely vasoconstrictor effects of ghrelin (fig. 5). In this case, the Ang1-7 did not modify the Gh effects. So, similar to other vascular territories (9), the vasodilatatory effects of Ang1-7 on pulmonary arteries were mediated by NO and prostaglandins.

CONCLUSIONS

Ghrelin induced dose-dependent and nitric oxide mediated vasodilatatory effects on phenylephrine pre-contracted rat pulmonary artery rings. Even more vasodilatatory actions of ghrelin are potentiated by Ang 1-7. The Ang1-7 actions are mediated by local synthesis of nitric oxide and prostaglandins with vasodilatatory effects.

ACKNOWLEDGEMENTS

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TESTAREA PE ANIMALE DE LABORATOR A RĂSPUNSULUI SISTEMULUI IMUNITAR, A REACTIVITĂŢII CUTANATE ŞI HEPATICE, DUPĂ ADMINISTRAREA TOPICĂ RETROAURICULARĂ A DOI COLORANŢI REACTIVI TEXTILI

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4. Disciplina de Fiziopatologie

RESEARCHES ON IMMUNE SYSTEM RESPONSE, SKIN AND HEPATIC REACTIVITY IN LABORATORY ANIMALS AFTER RETRO-AURICULAR TOPICAL ADMINISTRATION OF TWO REACTIVE TEXTILE DYES (Abstract): **Aim**: To evaluate immune system response, skin and hepatic reactivity in Swiss mice after retro auricular local administration of two textile dyes. **Material and methods**: On 3 groups of white Swiss mice: group I (Cibacron Yellow F-4G), group II (Cibacron Orange P-2R) and control group (DMSO - dimethylsulfoxide), substances were applied on left retro auricular ear, in dose of 25 μL solution 10% in DMSO (the dyes were not soluble in water), once a day, for 7 days. After euthanasia, blood samples were taken to assay differential cell count, peripheral neutrophils activity (NBT test), serum opsonic capacity, peritoneal macrophages activity (phagocytic and bactericidal capacity), and activity of spleenic T-lymphocytes with rossetting capacity, and spleen cells forming Jerne plaques. There were taken skin from local application of dyes, retro auricular and later cervical nodes from left side and hepatic samples. **Results**: Retro auricular and later cervical nodes mass increases after administration of textile dyes, compared to control group. Basophiles percentages were increased after administration of two dyes (stronger after yellow dye). Peripheral neutrophils activity was increased after administration of two dyes, especially after orange dye. Administration of the two dyes decreased serum opsonic capacity and peritoneal macrophages activity, but did not influenced the activity of spleenic T-lymphocytes with rossetting capacity and spleen cells forming Jerne plaques. There were significant skin and hepatic changes in microscopy. **Conclusion**: The studied dyes influenced the activity of immune system, increased skin and hepatic reactivity. **Key words**: TEXTILE DYES, IMMUNE SYSTEM RESPONSE, SKIN AND HEPATIC REACTIVITY

DEVELOPMENT AND VALIDATION OF A METHOD FOR QUANTITATIVE DETERMINATION OF AMIODARONE HYDROCHLORIDE IN BLOOD SERUM BY HPLC-MS-MS

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DEVELOPMENT AND VALIDATION OF A QUANTITATIVE METHOD FOR THE EVALUATION OF AMIODARONE HYDROCHLORIDE BY HPLC-MS-MS IN BLOOD SERUM (Abstract) **Aim**: The paper presents the development and validation stages of a new method using high performance liquid chromatography coupled with mass spectrometry for the determination of amiodarone hydrochloride in blood serum. **Material and methods**: The method used a TSQ Quantum^{TM} Access MAX Triple Quadrupole Mass Spectrometer coupled with a HPLC Transcend TLX1 system and octadecylsilyl silica gel C_{18} Cyclone MAX polymeric chromatographic columns (50 mm x 2.1 mm; 1.9 μm). Mass spectrometry detection was based on transition of ions from 645.9 to 200.23 m/z, while the other parameters were: 2400 V ionization potential, 10 psi pressure of gas ionization, 60 psi auxiliary gas pressure, 2.8 mTorr energy collision, 200°C ionization temperature and 330°C capillary temperature. A thorough validation study was conducted that included identifying the characteristic peaks of amiodarone hydrochloride, evaluating system compatibility, studying linearity and accuracy, establishing chromatographic retention times, limits of detection and quantification. **Results:** The retention time for amiodarone hydrochloride was 2.42 minutes, the method was linear over the concentration range 0.05-1.25 mg/mL, the limit of detection was 5.38 mg/mL, the limit of quantification was 17.68 mg/mL, and the regression coefficient was $r^2 = 0.9986$. **Conclusions:** The HPLC-MS-MS method developed and validated for the quantitative determination of amiodarone hydrochloride in serum is fast, specific and simple, and it can be used with good results during the *in vivo* studies required for the development of new therapeutic systems of amiodarone. **Keywords:** AMIODARONE, HPLC-MS-MS, BLOOD SERUM.

Amiodarone (AMD) is one of the most frequently prescribed therapeutic agents for the treatment of cardiac tachycardia worldwide due to its unanimously recognized efficiency. The mechanism of action includes the prolongation of action potential and effective refractory period of the myocardial fibers especially in the atria and His-Purkinje system, but also in the ventricular myocardium, and a the decrease in sinus node automatism. AMD determines atrioventricular conduction delay, de-

creased heart rate and significant lengthening of the Q-T interval on standard electrocardiogram (1, 2, 3).

The physicochemical properties of AMD were at the basis of this analytical study in order to develop new quantitative methods consistent with drug control regulations for bulk substance and solid oral dosage forms with modified and immediate release (4, 5).

For the quantitative analysis of AMD conditioned into modified release solid oral pharmaceutical formulations F1 and F10 or as amiodarone/hydroxyl-propyl-β-cyclodextrine inclusion complex (HP-β-CD/AMD), we have previously developed and validated a new HPLC method for the assay of AMD for *in vitro* studies. An exhaustive study on the quantitative determination methods of AMD from raw materials, formulations and biological fluids was at the basis of the method presented in this article. Most of the reported methods either do not include stress degradation studies, are validated incompletely, or are time consuming, cumbersome and expensive. For that reason a novel method using HPLC coupled with MS was developed and validated, method that is fast, specific and easy to use for the quantitative determination of AMD in blood serum (6-8).

MATERIAL AND METHODS

The reagents used were: methanol HPLC grade (Merck, Germany), formic acid, ammonium formate (Merck, Germany), acetonitrile, isopropanol, acetone HPLC grade (Merck, Germany), HP-β-CD - 99.70% purity (Roquette France), amiodarone hydrochloride (AMD·HCl) - 99.85% purity (Zhejlang Pharmaceutical Co. Ltd., China), HP-β-CD/AMD complex - 99.80% purity (”Petru Poni” Institute of Macromolecular Chemistry Iasi, Romania), Millipore-filtered water - 0.01 μS/cm conductivity.

The solution used were: sample solution (1 mL serum sample plus 2 mL of acetonitrile were sonicated for 5 minutes and then centrifuged for 10 minutes at 4000 rpm and decanted off), 0.01 mg/mL AMD standard solution (100 mg AMD·HCl were dissolved in water and diluted to 100 mL, and then 0.1 mL were diluted with mobile phase up to 10 mL).

The chromatographic parameters used were: chromatographic column 1 - C_{18} octadecylsilyl silica gel (0.05 m x 2.1 mm, 1.9 μm, 25°C), chromatographic column 2 - polymeric Cyclone MAX (0.05 m x 2.1 mm, 1.5 μm, 50°C), mobile phase A (for C1) 0.1 mM ammonium formiate and 0.001% formic acid, mobile phase B – water, mobile phase C - methanol, mobile phase D - acetonitrile: isopropanol: acetone (40:40:20) (v/v/v) (last 3 for C2). The volume of sample injected was 5 μL.

The quantitative determination of AMD was based on the external standard method. The method was based on separation of the analyte from the biological matrix through chromatographic techniques with mass spectrometry detection.

The chromatographic method of analysis included two phases. First, the distribution of the analyte was carried out through column 1 which provided a turbulent flow, and then the analyte was transferred to column 2 for separation and identification by MS. The column was prepped with the mobile phase at a flow rate of 0.5 mL/min for 30 minutes.

Validation procedure

The chromatographic peaks were identified by injecting a solution containing the active substance AMD in the biological matrix – blood serum.

Chromatographic system compatibility study was done by injecting 10 times con-

secutively 200 ng/mL of AMD solution. Injection repeatability, reflected in the invariability of both peak area and retention times, defined the accuracy of the system. Also peak asymmetry and number of theoretical plates in the column were two other factors that characterized system compatibility.

The linearity study of the signal corresponding to AMD·HCl was done by plotting the calibration curve over the linearity range 0.05-1.25 µg/mL. Three independent series of these solutions for each concentration level have been used (9,10).

In order to study the accuracy of the analysis method, spiked samples were prepared containing various amounts of AMD·HCl dissolved in a mixture of 500 mL plasma and 500 mL water, as follows: SP1 - 1250 ng AMD, SP2 - 1000 ng AMD, SP3 - 500 ng AMD, SP4 - 200 ng AMD, SP5 - 75 ng AMD, SP6 - 50 ng AMD. The recovered concentration ($C_{recovered}$), percentage recovery (R%), and percent deviation (Xd%) were calculated, while the obliquity of the experiment was evaluated.

For the calculation of the limit of detection (LOD) and limit of quantification (LOQ), the standard deviation and the slope of the regression curve were used according to the following equations: LOD = 3.3 × SD/slope and LOQ = 10×SD/slope (11-12).

RESULTS AND DISCUSSION

The results obtained for the identification of the corresponding peaks of AMD·HCl (fig. 1-4) are reported in table I.

The experimental results obtained in the linearity study are presented in table II.

Estimation of peak areas had been done using linear regression equation: Area = 4.142 x Concentration.

The fitting of peak areas based on the variation of residual values according to the concentration of AMD·HCl is shown in table III.

The experimental results of the accuracy study are presented in table IV.

The experimental results of the linearity study are presented in table V.

TABLE I

Identification of the characteristic peaks of AMD·HCl

Solution injected	AMD·HCl concentration (µg/mL)	Retention time (minutes)	Area
SI1	500	2.42	1984
Blank	-	-	-

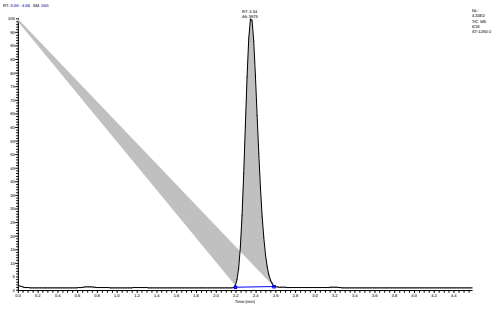

Fig. 1. Peak of 1250 ppb reference AMD

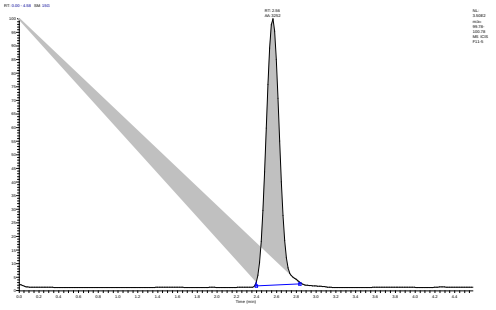

Fig. 2. Peak of AMD inclusion complex

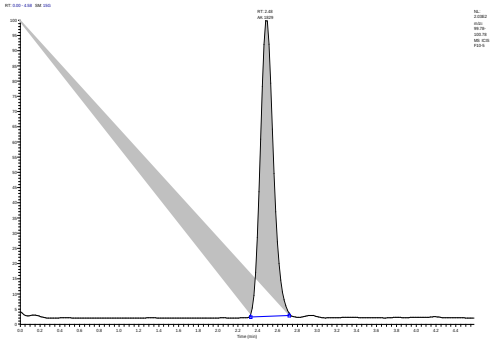

Fig. 3. Peak of AMD from F1

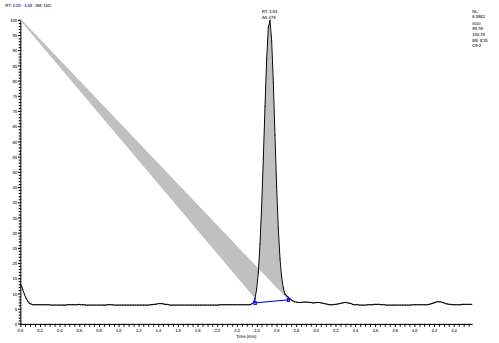

Fig. 4. Peak of AMD from F10

TABLE II

Linear variation of peak area with sample concentration

$C_{AMD \cdot HCl}$ (ppb)	50	75	200	500	1000	1250
Mean area	120.00	240.50	835.51	2088.77	4472.63	5535.44

TABLE III

Fitting residual values

No.	Predicted values	Residual values	Standard residual values
1	83.87788	-23.8779	-0.54378
2	192.2334	-7.23343	-0.16473
3	734.0112	59.72215	1.360064
4	2034.278	-49.9444	-1.13739
5	4201.389	47.61122	1.084259
6	5284.944	-26.2776	-0.59843

TABLE IV

Study on method accuracy

Sample	Theoretical concentration (ng/mL)	$C_{recovered}$ (ng/mL)	R%
SP1	50	48	96
SP2	75	72	96
SP3	200	196	98
SP4	500	490	98
SP5	1000	970	97
SP6	1250	1191	95

TABLE V
Linearity of the method

$C_{AMD \cdot HCl}$	50	75	200	500	1000	1250
Area(S1)	62.00	185.00	793.73	1984.33	4249.00	5258.67
Area(S2)	68.54	186.00	794.73	1984.22	4334.00	5345.00
Area(S3)	53.75	184.00	792.55	1953.40	4149.00	5144.00
Average	60.30	185.00	793.50	1973.98	4244.00	5249.22

The limits of detection and quantification were calculated based on the response to the lowest concentration for which the relative standard deviation has been determined, and it was then compared to the slope. The values of the limit of detection and limit of quantification were found to be 5.83µg/mL and 17.68 µg/mL, respectively.

The method was validated in accordance with international regulations (13,14). The representative chromatograms of AMD free and spiked plasma samples showed no secondary peaks of interfering substances such as endogenous plasma components, with similar retention time. The retention time of the analyte was 2.42 minutes.

All accuracy and precision values were within the recommended range. The degree of recovery ranged from 95% to 98% (15,16).

Injection repeatability, peak area and retention times defined system accuracy. Also peak symmetry and number of theoretical plates of the column were two other factors that characterized the compatibility of the system. Injection repeatability was proven by RSD $\leq$ 2.

Under the experimental conditions, it was observed that the confidence interval for the average area (793.67) was X = -362.87 ÷ 1950.20 and $RSD\%_{AMD}$ (area) was 1.59. Stretch factor was T $\leq$ 1.8, and T_{AMD} = 1.1.

The number of theoretical plates for AMD·HCl was N_{AMD} = 3757. Calibration curve showed a linear variation of the peak area with sample concentration. During accuracy study, the experimental data highlighted an 80.48% average recovery (17,18).

CONCLUSIONS

We validated the new method in a 0.05-1.25 µg/mL concentration range, covering the therapeutic plasma concentration of amiodarone hydrochloride.

The developed method developed is simple, fast, and accurate and it did not involve great cost. Compared to other published HPLC methods for the assay of amiodarone hydrochloride in animal plasma, the new method performed better in terms of speed and cost, important characteristics for the methods used routinely.

We have successfully used the newly validated method in a pharmacokinetic study, but it may also have widespread applications in monitoring AMD therapeutic concentration in blood serum during studies investigating drug interactions and in toxicological research.

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AUTOMATIC ANALYSIS OF CARDIOTOCOGRAPHIC RECORDINGS FOR FETAL ACIDOSIS STUDY

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AUTOMATIC ANALYSIS OF CARDIOTOCOGRAPHIC RECORDINGS FOR FETAL ACIDOSIS STUDY (Abstract) **Aim**: Fetal acidosis is a consequence of anaerobic metabolism due to lack of or a limited amount of oxygen delivered through the umbilical cord to the fetus. Direct determination of fetal blood pH remains the current method used in practice and needs fetal blood extraction. This is an elaborate procedure and cannot be performed or repeated anytime during labor. An alternative, non-invasive solution is to monitor continuously the fetus through cardiotocography (CTG). **Material and methods:** This paper presents an automatic analysis software tool designed and implemented in Matlab, which computes a fetal heart rate (FHR) baseline, identifies accelerations, decelerations of FHR signal and characterizes maternal uterine contractions. **Results and discussion**: The software was tested using a public cardiotocography database available from Physionet.org. We looked for results consistency in term of events per hour and if the software may differentiate between normal and metabolic acidosis (pH<7.2) group. We found in acidosis group, a significant increase in number of traces with prolonged decelerations. Also, there is a significant difference in the mean number of accelerations and late deceleration per hour of CTG trace. The mean number of uterine contractions, expressed per hour of CTG trace was, not significantly different. **Conclusions:** We developed and implemented a Matlab software used for CTG analysis, respectively identification of accelerations, decelerations and uterine contractions. The software was tested using a public database of intrapartum CTG recordings. CTG parameters analyzed by an in house developed software could reliable identify the characteristics related to fetal metabolic acidosis. **Keywords:** CARDIOTOCOGRAPHIC RECORDINGS, FETAL ACIDOSIS.

Fetal metabolic acidosis represents one of the factors that cause fetal distress. Unrecognized and untreated can lead to issues after birth such as neurological damage, intracranial hemorrhage, cerebral palsy and in worst case scenarios still birth (1). Metabolic acidosis is caused by anaerobic metabolism due to low concentrations of oxygen in the blood of the fetus. This is caused by different factors limiting the amount of oxygenated blood that is supplied to the fetus (2). If this type of metabolism is present for a longer period, the fetal buffers will be exhausted and the blood will

change its pH value from the normal level higher than 7.2 to an acidic level. This phenomenon occurs due to the production of organic acids such as lactic acid as a byproduct of anaerobic metabolism. In time, the concentration of organic acids will be higher due to the low natural rate of absorption (3).

The fetus has a series of defense mechanisms that activate when metabolic acidosis sets in, the most predominant being an increase of heart rate or accelerations. If these mechanisms do not lead to the normalization of pH values, in time the heart rate will change into late and prolonged decelerations that are considered pathological (4).

The purpose of this paper is to present the implementation of a software tool that can be used to analyze cardiotocographic (CTG) recordings to highlight the main effects of fetal metabolic acidosis. We analyzed fetal heart rate (FHR) and maternal uterine contractions (UC) to determine accelerations per hour, decelerations per hour and contractions per hour.

MATERIAL AND METHODS

1. Software tool

Available intrapartum CTG recordings contained the signals of fetal and maternal heart rate, uterine contractions / pressure and maternal blood pressure (5). We focused on the analysis of FHR and UC signals from these CTG recordings, as they are interrelated and offer the most information about the fetus wellbeing.

The software was developed in Matlab and the CTG traces were analyzed by two sub functions for FHR and UC, respectively. At the beginning of analysis, we used some preprocessing procedures, to improve signal quality.

The first preprocessing procedure used a smoothing filter of a moving window of 30 samples, centered on the current analyzed sample. This removes abrupt spike values in the signal, caused by maternal movement artifacts (6).

$$X_s(i) = \frac{1}{2N+1} \sum_{j=-N}^{N} x(i+j) \quad (1)$$

where $X_s(i)$ is, the value obtained after applying the smoothing filter, i is the index of the sample that is analyzed and N is the length of the filtering window, in this case 30 samples (7).

Second preprocessing procedure consisted in a curve fitting spline interpolation algorithm for the FHR signal to remove signal segments where the sample value is equal to zero. These segments correspond to artifacts caused by a loss of electrode contact to the fetus' scalp (8). Figure 1 presents an example of CTG signals after we applied the preprocessing procedures.

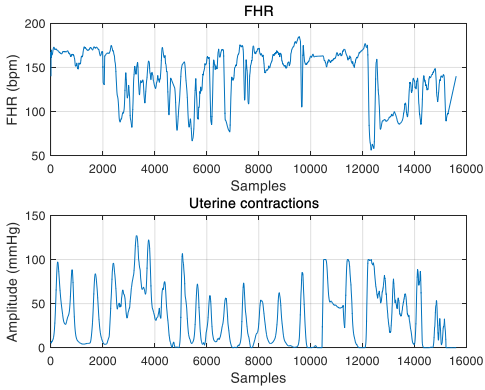

Fig. 1. Pre-processed FHR and UC signals. FHR, fetal heart rate; bpm, beats per minute.

Determination of FHR baseline allows characterization of accelerations and decelerations. We determined the baseline value (in beats per minute, bpm) by identifying segments of the signal where the sample

values, compared to an average baseline value, differ by less than 10 bpm (9). For this, we computed the first derivative of FHR signal (dFHR), which allows identification of the very high and very low amplitude samples that are not part of the baseline, as presented in figure 2. These values, identified as very high or low amplitude using the first derivative, were not used to compute the average baseline value. Samples used for baseline assessment must be part of segments with a length greater than 15 seconds (60 samples), with an amplitude between ±10 bpm, compared to the average baseline (10). We determined the segments that corresponded to FHR baseline and we computed the average value baseline from these segments, as presented in formula (2):

$$FHR_{Avg_baseline} = \frac{\sum_{n=i}^{i+N} FHR_{Bs}(n)}{N} \quad (2)$$

where $FHR_{Avg_baseline}$ is the average baseline value determined for all the identified baseline segments, i is the index of the starting sample of the segment in the FHR signal, N is the number of samples of that segment and $FHR_{Bs}(n)$ is the amplitude of the sample n that is part of a baseline segment.

After the average baseline value and the baseline segments determination, we combined segments into a baseline continuous signal using linear interpolation. In order to combine two consecutive segments, we used the last sample of the current baseline segment and the first sample of the next baseline segment (11). For the first segment identified we used the FHR signal origin, and for the last identified segment we used the FHR signal end sample. Thus, we defined the two samples $FHR_{seg(k)}(i+N)$ and $FHR_{seg(k+1)}(i)$, where k represents the total number of segments identified, to compute a linear interpolation between them, as presented in formula (3).

$$FHR_{Linear_interp}(j) = FHR_{seg(k)}(i+N) + (j-(i+N)) \times \frac{FHR_{seg(k+1)}(i) - FHR_{seg(k)}(i+N)}{i-(i+N)} \quad (3)$$

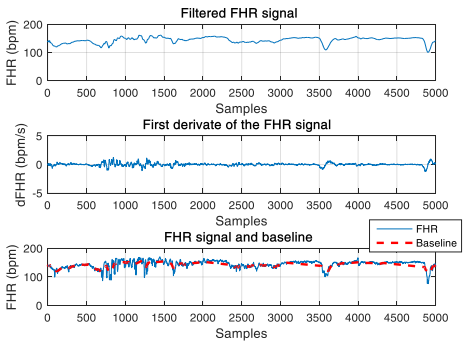

Fig. 2. Determined FHR baseline compared to the FHR signal.
FHR, fetal heart rate; bpm, beats per minute; dFHR, fetal heart rate first derivate.

Accelerations are considered normal events during pregnancy. For their detection, we defined a set of quantitative parameters that describe this type of event: the length of the acceleration has to be over 60 samples (15 seconds) and the amplitude has to be 15 bpm higher than the baseline in that signal segment (fig. 3) (12).

Other episodic events related to FHR and uterine activities are decelerations. They are divided into three types.

First type is represented by early decelerations. They are considered normal events that occur at the same time as a uterine contraction and don't differ by

more than 40 bpm compared to the baseline (13). The early deceleration returns normally to baseline value by the end of contraction.

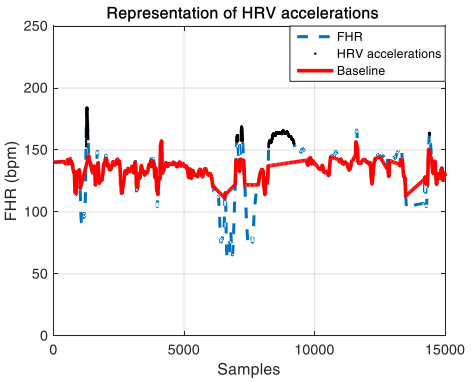

Fig. 3. Representation of heart rate accelerations. FHR, fetal heart rate.

The second type is the late decelerations which are considered pathological events (fig. 4). These appear after the initiation of contraction, and their minimum value occur at least 15 seconds after the contraction peak. To identify this type of decelerations, significantly associated with fetal acidosis, we imposed a set of quantitative parameters: the amplitude of the signal should be more than 40 bpm lower than the baseline value for that signal segment and the deceleration should last over 120 samples (30 seconds) (14).

The third type is represented by prolonged decelerations, long segments with a length between 2 and 10 minutes, with an amplitude 40 bpm lower than the baseline value in that signal segment. A prolonged deceleration over 10 minutes represents a changing in baseline. These decelerations decrease the oxygen transferred to the fetus and represents a high risk for fetal acidosis (fig. 5) (15).

To characterize and compare CTG tracings, we calculated the number of accelerations or decelerations per hour. The formulas used to determine these values are:

$$Acc/h = \frac{N_{Acc}}{L/F_s/3600} \quad (4)$$

where *Acc/h* is the accelerations per hour, N_{Acc} is the total number of identified HRV accelerations, L is the total signal length and F_s is the sampling frequency.

$$Ldcc/h = \frac{N_{Ldcc}}{L/F_s/3600} \quad (5)$$

where *Ldcc/h* is the value of late decelerations per hour, N_{Ldcc} is the total number of identified late decelerations, L and F_s have the same signification as formula (4).

We designed and implemented an algorithm that analyses uterine contractions, in terms of contractions/hour. To identify uterine contraction (UC) we implemented a peak finder algorithm, which computes the first derivative of the UC signal and thus identifies high amplitude samples. We identified the sample with the maximum amplitude. This is used to determine the threshold value to which all samples are compared.

The threshold value is set at 40% of the maximum value sample (16). We then compare the samples to the threshold value to obtain only high amplitude samples. This step allows us to identify signal segments of high amplitude. The maximum value sample of each of these segments is considered the peak of the contraction.

In case the contraction has two or more peaks we use a selection threshold of 20% difference in amplitude between two consecutive peak samples to identify false peak detections and keep only the value that corresponds to the real contraction peak (17).

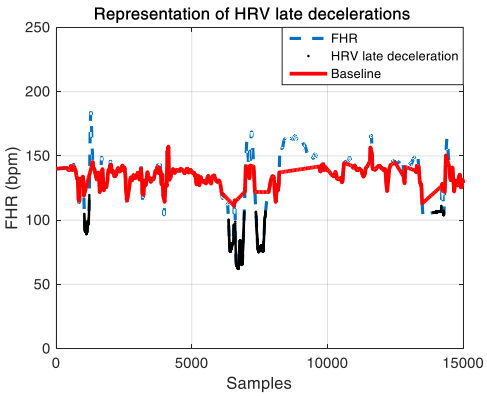

Fig. 4. Representation of late decelerations. FHR, fetal heart rate.

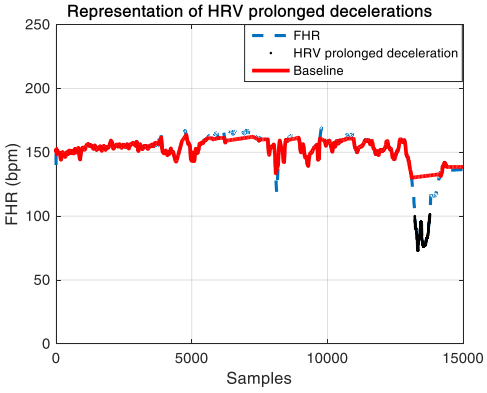

Fig. 5. Representation of prolonged decelerations. FHR, fetal heart rate.

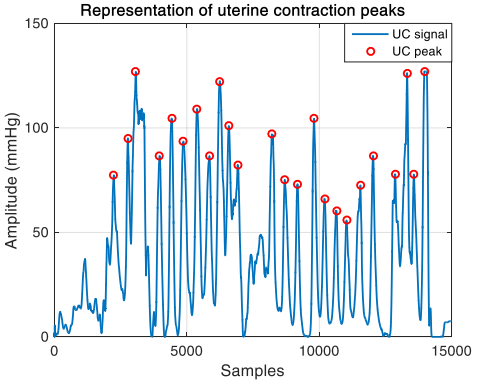

Fig. 6. Representation of the identified contraction peaks

We computed the contractions per hour parameter as a risk factor for fetal stress.

$$NC/h = \frac{NC}{L/F_s/3600} \quad (7)$$

where *NC/h* represents the uterine contractions per hour, *NC* is the total number of identified contractions, *L* is the signal length and F_s is the sampling frequency.

2. Database

We tested the software tool using CTU-UHB Intrapartum Cardiotoco-graphy Database, a public CTG recordings database available from Physionet.org. The database was collected between 2010 and 2012 by the combined efforts of the Czech Technical University of Prague and the University Hospital in Brno (Czech Republic) (18). The database contains 552 cardiotocographic recordings, each approximately 90 minutes in length, sampled at a frequency of 4 Hz. FHR and UC signals were acquired during labor, using intrauterine sensors. Each recording also has an annotation file that contains clinical and fetal outcome data such as delivery type, fetal gender and weight at birth, artery blood sample parameters (pH value, base excess or BDecf) and Apgar score.

We eliminated 24 recordings from the database because of a high signal loss ratio (the ratio between the number of samples equal to 0 and the total signal length) (19). The threshold value was set at 50%, meaning that recordings that had a signal loss ratio higher than the threshold value were eliminated from testing.

The recordings were divided into two groups, based on the pH value. Thus, re-

cordings that have a pH value below 7.2 represent the pathological group or the acidosis group and recordings with a pH value over or equal to 7.2 represent the normal group. The acidosis group contains 166 CTG recordings and the normal group contains 362 recordings.

EXPERIMENTAL RESULTS

The purpose of these tests is to establish if we can obtain consistent results, using previously described analysis software, for the identified parameters: Acc/h, Ldcc/h and NC/h that can allow us to identify similarities within the same recordings group (normal or acidosis) and differences between groups (normal and acidosis).

The first step was to determine the values for analyzed parameters for both groups. The results are presented in figure 7 for Acc/h, figure 8 for Ldcc/h, and figure 9 for NC/h.

For prolonged decelerations, given the significance of this pathological event and the rarity of its occurrence, we determined the number of recordings which presented prolonged decelerations. We found only 7 cases with prolonged decelerations in 362 normal recordings (1.7%), while in the acidosis group, from 166 pathological recordings we found 17 recordings that present prolonged decelerations (10.2%) (Fisher's exact test, $p<0.001$).

The second step is to determine the data dispersion within each group and to determine the value for each parameter.

Based on the values obtained for the parameters for both groups we compute an unpaired Student's t-test with a probability value $p<0.05$ to identify if the groups are statistically different (20) (fig.10-13).

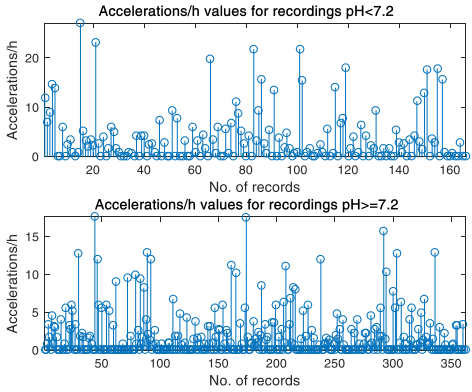

Fig. 7. Accelerations/h values for both recordings groups.

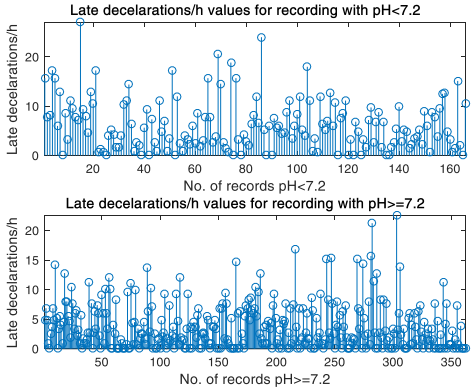

Fig. 8. Late decelerations/h for both groups (normal and acidosis)

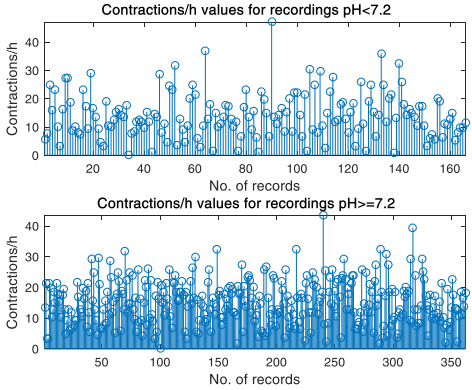

Fig. 9. Number of contractions/h for both groups

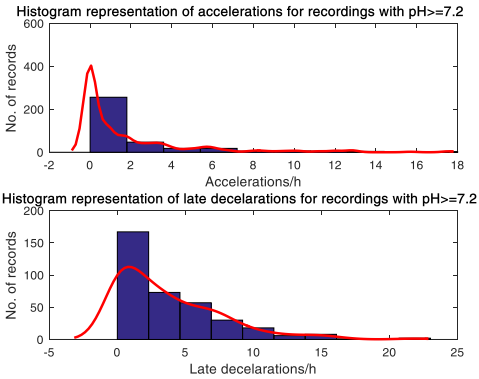

Fig. 10. Histogram representation of the recordings in the normal group for the parameters Acc/h and Ldcc/h

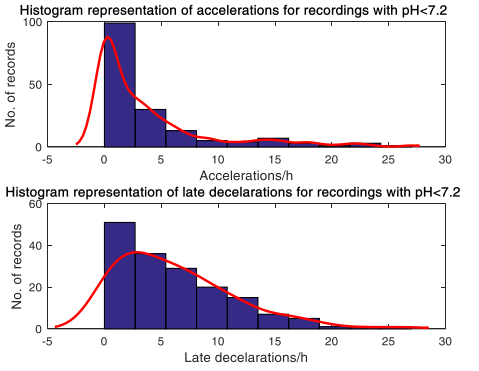

Fig. 11. Histogram representation of the recordings in the acidosis group for the parameters ACC/h and Ldcc/h

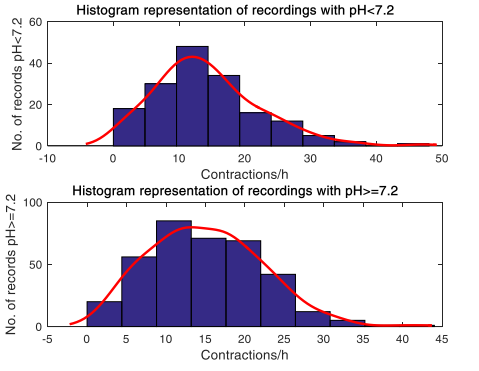

Fig. 12. Histogram representation of both groups for the parameter NC/h

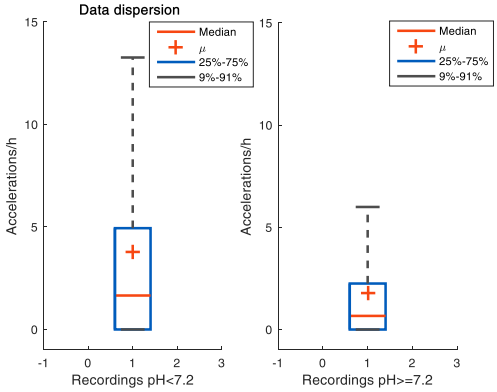

Fig. 13. Data dispersion for both groups when analyzing the Acc/h parameter

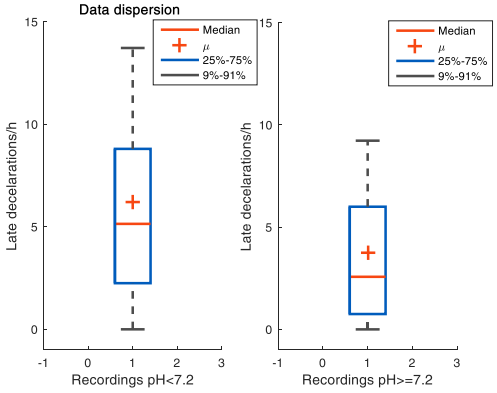

Fig. 14. Data dispersion for both groups when analyzing the Ldcc/h parameter

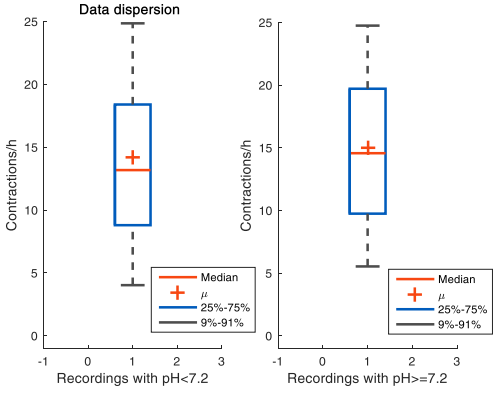

Fig. 15. Data dispersion for both groups when analyzing the NC/h parameter

The results presented in Table I are expressed as mean value ± standard deviation for each group. Table I, figures 14 and 15 show that the data groups are statistically different for the parameters Acc/h and Ldcc/h. The number of contractions per hour was not significant different between two groups. This is an indication that the NC/h parameter does not interfere in metabolic acidosis development.

TABLE I

Unpaired **Student's t-test** results expressed as mean ± standard deviation

Parameter	p	Metabolic acidosis group	Normal group	SD
Acc/h	0.016	3.77 ±5.42	1.8 ±3.05	3.95
Ldcc/h	0.022	6.22 ±5.2	3.73 ±3.91	4.36
NC/h	0.21	14.17 ±7.95	15.02 ±7.08	7.36

CONCLUSIONS

We developed and implemented a Matlab software used for CTG analysis, respectively identification of accelerations, decelerations and uterine contractions.

These parameters are used as indicators for the onset of acidosis.

The software was tested using a public database of intrapartum CTG recordings. We found a statistical difference for ACC/h, Ldcc/h and incidence of prolonged decelerations. However, the number of uterine contractions per hour of CTG trace were a constant parameter for the entire database and did not influence directly the onset of acidosis.

ACKNOWLEDGEMENTS

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NEWS

COFFEE INTAKE DECREASE THE MULTIPLE SCLEROSIS RISK

Health benefits and health risks of coffee drinking were evaluated by numerous studies that used human or animal models. Some studies have linked coffee consumption with neuroprotective effects by decreasing inflammation and demyelination.The influence of coffee consumption on the risk of multiple sclerosis (MS) has been investigated in two retrospective case–control studies conducted in Sweden and the US. Both trials included people aged over 15 years who were diagnosed with MS and have investigated consumption habits of caffeinated coffee in different periods of age. The two studies have involved a total of 2779 cases and 3960 controls. The risk of MS among participants which consumes coffee was compared with the risk of MS among participants who never drank coffee, by calculating ORs with 95% CIs.The results showed a reduced risk of MS among those who reported a consumption of coffee exceeding 6 cups daily (OR 0.70 in the Swedish study, and OR 0.69 in the US study) in comparison with participants who never drank coffee.Additional studies are needed to determine which of biologically active constituents of coffee determines these results, in order to use them in multiple sclerosis therapy (A K Hedström; E M Mowry; M A Gianfrancesco; X Shao; C A Schaefer; L Shen; T Olsson; L F Barcellos; L Alfredsson. High consumption of coffee is associated with decreased multiple sclerosis risk; results from two independent studies. *Neurol Neurosurg Psychiatry* 2016;87:454-460).

Cătălina Luncă

PATTERNS OF ROAD TRAFFIC ACCIDENT IN NORTH-EAST ROMANIA

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PATTERNS OF ROAD TRAFFIC ACCIDENT IN NORTH-EAST ROMANIA (Abstract): Romania is ranked first among mortality road traffic accidents in Europe. The **aim** of our study was to evaluate some statistical patterns of population group who unfortunately was admitted at an emergency hospital of Iasi, a large city in the North East of the country. **Material and Methods**: The study was performed on 1623 patients admitted in the Emergency Room ("Sf. Spiridon" Emergency Hospital of Iasi, Romania) suffering from road traffic accidents, during 2009 and 2013. **Results**: The prevalence of 19-45 age group cases represented more than 50% of all patients, with a high significance ($p<0.05$), and men were involved more frequently (69.69%) than women (30.31%); most of the patients were injured while driving an automobile; 6 PM was the "rush" hour (6.4%), Monday was the "black" week day (16.02% of total cases), September the "harvest" time (12.7%), and Summer the "hottest" season (48.49%). **Conclusions**: The "B plan" is imperative to be implemented in Romania, including **b**etter roads, **b**etter vehicles, **b**etter legislation, and **b**etter teens' education, in order to avoid road traffic accidents. **Keywords**: ROAD TRAFFIC ACCIDENT, INJURY, PEDESTRIAN, DRIVER.

Road traffic accidents are an important public health problem in all regions of the world, but especially in less and medium developed countries, which can be showed by a large and increasing number of deaths in these territories. In Romania, the road traffic accidents, resulting in injuries, are also a major health problem, representing: the first cause of premature death - PYLL (Potential Years of Life Lost) in males; the second cause of temporary disability (13.2% of a total); the fourth cause of death after cardiovascular diseases, tumours, and respiratory diseases; the fourth rank in adjusted life years' potential inability - DALY (Disability Adjusted Life Years); 6-7% of all hospitalized patients (1).

The risk of death from road traffic accidents in Romania is almost 14 times higher than the European countries average, Romania is ranked first among mortality road traffic accidents in the EU. The degree of civilization in a society is given also by the manner of the road traffic. Although human, material and social losses are huge, there are some interesting differences between countries in how they manage this problem. In some regions in Australia, Europe and North America, the number of deaths decreased from (25-30)/100,000 inhabitants in 1970 to (3-8)/100,000 inhabitants nowadays, although the number of cars has increased 5

times. These countries are not the richest, but they have created a safer traffic environment through traffic education, access to safe vehicles, and severe laws (2).

MATERIAL AND METHODS

The study was performed on 1623 patients admitted in the Emergency Room ("Sf. Spiridon" Emergency Hospital of Iasi, Romania) suffering from road traffic accidents, during 2009 and 2013. 902 patients (55.58%) were from rural areas, 721 (44.42%) from urban areas.

Scale types of variables presented in our research were nominal and ordinal, so they were analysed with *t* student-test. Statistical significant difference was at $p<0.05$, and confidence interval (CI) higher than 95%. The database was performed using MS EXCEL 2010 software and SPSS for Windows (version 16.0, SPSS Inc., Chicago, Illinois, USA) for statistical analysis of the collected data.

RESULTS

Annual incidence rates of road traffic accident showed ***increasing values in North-East Romania*** (fig. 1).

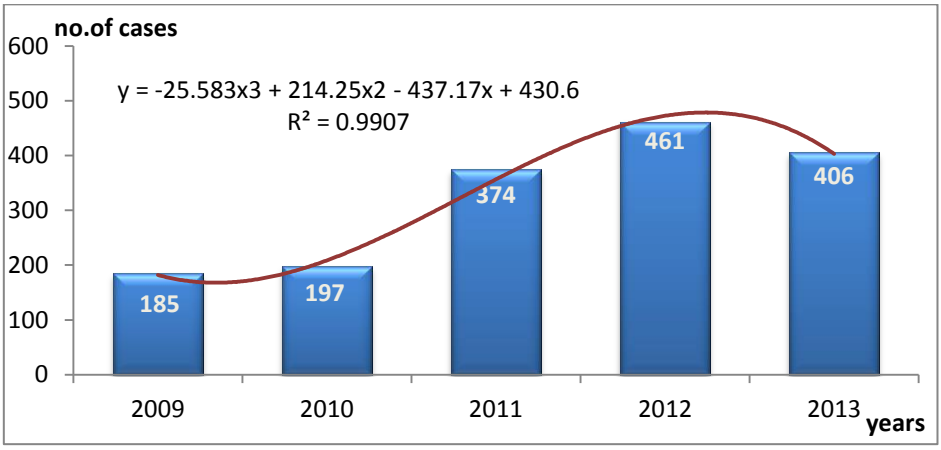

Fig. 1. Annual incidence rates of road traffic accidents

Comparing the five age groups recommended by the WHO, the annual prevalence values showed no significant differences, except for the age group under 18, because of their admission to "Sf. Mary" Emergency Hospital for Children, in Iasi. When assessing cumulative demographic range ***19-45 years old***, the prevalence of cases assisted in the ER represented more than 50% of all patients, the difference with other age groups was highly significant ($p<0.05$) (tab. I). The values series had a normal Gaussian distribution and Kolmogorov-Smirnov test was $p<0.01$ for each of the five years included in the study.

Statistical analysis showed a significantly higher percentage of ***male patients*** 1131 (69.69%) in relation to female patients 492 (30.31%) (t Stat = 2.33; $p<0.001$), from a total of 1623 persons.

The distribution by week day of road traffic accidents in Iasi showed that ***Monday*** was the "black" one (tab. II). Most of the patients were injured while driving an ***automobile*** (t Stat = 3.36; p=0.0199) (tab. III).

TABLE I

Age group distribution of road traffic accidents

Age group / *years*	*2009*		*2010*		*2011*		*2012*		*2013*	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
≤ 18	30	16.2	30	15.2	33	8.8	36	7.8	25	6.2
19-30	36	19.5	58	29.5	116	31.0	133	28.9	96	23.6
31-45	49	26.5	42	21.3	83	22.2	121	26.2	103	25.4
46-60	42	22.7	37	18.8	71	19.0	78	16.9	88	21.7
≥ 61	28	15.1	30	15.2	71	19.0	93	20.2	94	23.1
Total	**185**	**100**	**197**	**100**	**374**	**100**	**461**	**100**	**406**	**100**

TABLE II

Number of injured patients in motor vehicle accidents - by day of injury

Days of the week	Number of injured patients	
	No.	**%**
Monday	260	16.02
Tuesday	252	15.52
Wednesday	244	15.03
Thursday	187	11.52
Friday	222	13.67
Saturday	231	14.23
Sunday	227	13.98
Total	1623	100.0

t Stat = 23.86; $p<0.001$

TABLE III

Types of vehicles' occupants

Victims	No.	%
Pedestrian	301	22.68274
Motorcyclist	91	6.857573
Bicyclist	118	8.892238
Occupant of a car	473	35.64431
Driver	234	17.63376
Carter	110	8.289375
Total	1327	100.0

t Stat = 3.369146093; P(T<=t) two-tailed = 0.019908236

It is noted that a third of all victims recorded during the five years' analysis occurred in the ***summer*** season (48.49%) (fig. 2).

An evident assignation of months with the lowest frequency in road traffic accidents was not really possible. The incidence of occurrence of these accidents could not be linked to a specific cause so that the possibility of an appropriate interpretation is reduced. The "black" months in terms of an increased admission of injured persons were: ***September*** with 12.7% of cases, then August and July, with 11.6% and 10.7%, respectively (t Stat = 9.361431241; p<0.0001). This situation could be justified by the peak period of holiday and the beginning of school and university studies (tab. IV).

The daytime most involved in the occurrence of road traffic accidents was 18 hours (6.00 PM) (fig. 3).

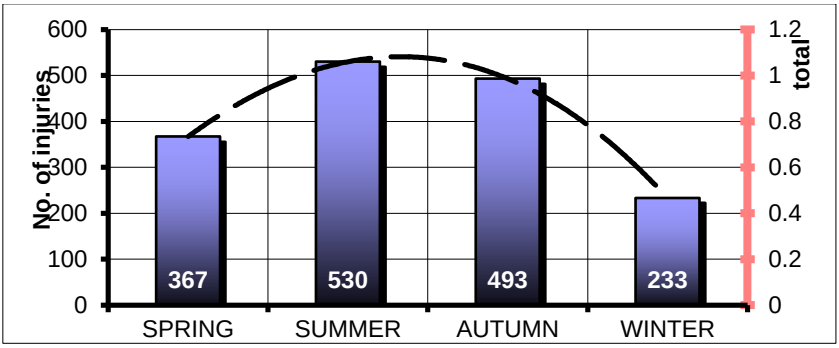

Fig. 2. Cases distribution in relation with seasons

TABLE IV

Number of injured persons in motor vehicle accidents - by month of accident

Month of the year	Injured persons	
	No.	%
January	67	4.2
February	51	3.2
March	107	6.6
April	119	7.3
May	141	8.7
June	167	10.3
July	174	10.7
August	189	11.6
September	207	12.7
October	154	9.5
November	132	8.1
December	115	7.1
Total	1623	100.0

t Stat = 9.361431241; p<0.0001

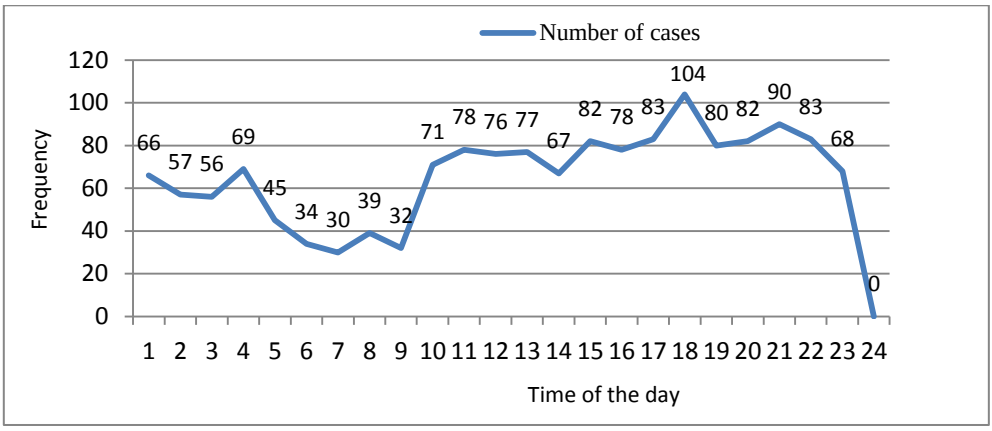

Fig. 3. Hours of road traffic accidents occurrence

The average ***length of stay*** for the entire study group (1,623 patients) registered low values during the first two years of the study, rising steadily in 2011 and 2012. If in 2009 the average length of stay was 5.79 days, it increased by almost 60% in 2012, until a value of 9.86 days (tab. V, fig. 4).

TABLE V

Length of stay between 2009 and 2013

Length of stay / *year*	*2009*	*2010*	*2011*	*2012*	*2013*	Total
Total	1071	1181	2931	4568	3763	**13514**
Average	5.79	5.99	8.03	9.86	9.26	**8.32**

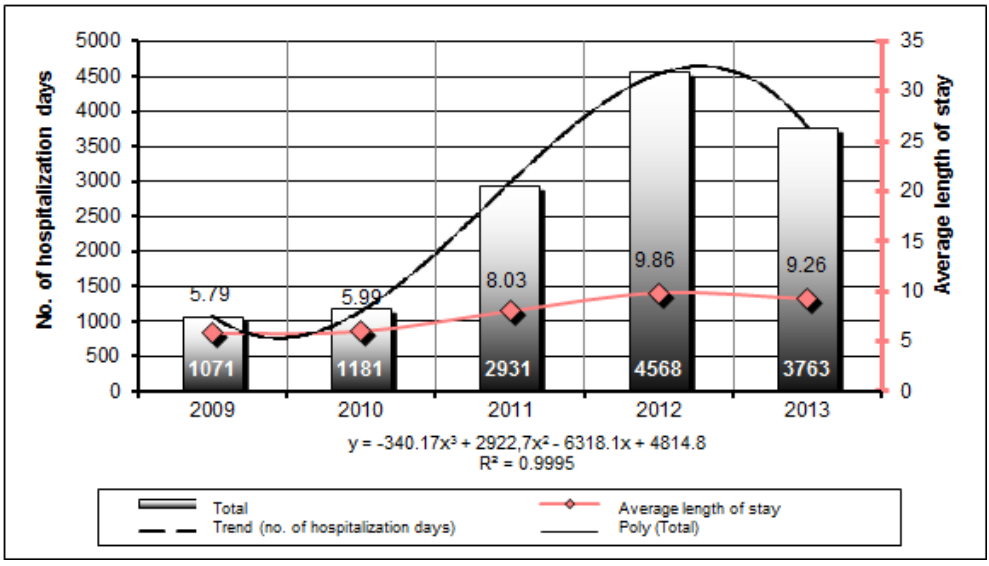

Fig. 4. Trend in the length of stay between 2009 and 2013

DISCUSSION

Regarding the data of those who were involved in road crashes, our analysis showed significant differences between females and males in injuries rates. Authors of some studies such as Holland et al. (3), Bíl et al. (4), Pino et al. (5), and Valent et al. (6) explained a higher mortality rate in males than in females.

In countries with distinct seasons, there are differences between the incidences of road traffic accidents at different times of the year. Dynamic for the distribution of road accident victims is characterized by almost constant and continuous increase in the number of this category of victims, between spring - summer – autumn. The general trend of the prevalence and mortality caused by road traffic accidents can be divided in two cycles: first, an ascendant uniform cycle, characteristic for spring-autumn period; second, a descendant abrupt cycle characterized for winter-spring period. Specific factors of variations in the incidence of accidents in relation to the seasons have certainly taken into account in any epidemiological study of traffic accidents.

Many factors come to explain increased values of the prevalence of road traffic victims among the 19-45-year age group, with a significant increase in males. Among the most frequently listed are: the number of male drivers is larger and therefore the risk is higher; women drive more cautiously, avoiding speeding and paying more attention to signs and restrictions on movement; young people are frequently involved in traffic, both as pedestrians and as drivers (7, 8, 9). Showmanship, drinking, ignoring the legislation, recklessness, excessive speeds, a.s.o., make the road pathology a disease of multifactorial etiology and a major problem of public health in many countries (10, 11).

CONCLUSIONS

To know the real impact of road traffic accidents would facilitate the adaptation of emergency rooms to the annual increasing rate of incidence and allow the medical staff to be more determined to wait for, to diagnose and to treat medical and surgical emergencies. More effective national programs for primary prevention of road crashes could be implemented starting from the performed data.

The “B plan” is imperative to be implemented in Romania and elsewhere, including **b**etter roads, **b**etter vehicles, **b**etter legislation, and **b**etter teens’ education, in order to avoid road traffic accidents.

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NEWS

HEPATECTOMY FOR GASTRIC CANCER METASTASES OFFERS SUPERIOR SURVIVAL

The liver is one of the most common sites of gastric cancer dissemination. There isn't any consensus regarding the benefits of the hepatectomy of gastric metastases. The authors review in a retrospective study 107 patients with hepatic metastases (34 synchronous, 73 metachronous) out of 5437 patients diagnosed and treated for gastric cancer in the Toranomon Hospital, Tokyo, Japan, between 1985-2014. Hepatectomies were performed in 9 synchronous and 4 metachronous metastases, that had ≤ 3 tumors and diameters < 3cm. The overall survival rates after hepatectomy were significantly improved in both synchronous and metachronous, compared with patients who did not undergo hepatectomy and had similar metastatic status. The multivariate analysis reveal that the hepatectomy is the most significant prognostic factor regarding the treatment of metastases, provided they are equal or less than 3 and smaller than 3 cm (Ohkura Y, Shinohara H, Haruta S et al.Hepatectomy Offers Superior Survival Compared with Non-surgical Treatment for ≤3 Metastatic Tumors with Diameters <3 cm from Gastric Cancer: A Retrospective Study., *World J Surg*. 2016;39(11):2757-63. doi: 10.1007/s00268-015-3151-0).

Filip Târcoveanu

CONSIDERATIONS ON ANATOMY AND PHYSIOLOGY OF LYMPH VESSELS OF UPPER AERO DIGESTIVE ORGANS AND CERVICAL SATELLITE LYMPH NODE GROUP

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CONSIDERATIONS ON ANATOMY AND PHYSIOLOGY OF LYMPH VESSELS OF UPPER AERODIGESTIVE ORGANS AND CERVICAL SATELLITE LYMPH NODE GROUP (Abstract): The almost constant local regional development of the cancers of upper aero digestive organs requires the same special attention to cervical lymph node metastases, as well as to the primary neoplastic burning point. The surgical therapy - alone or associated -has a mutilating, damaging character, resulting in loss of an organ and function, most of the times with social implications, involving physical distortions with aesthetic consequences, which make the reintegration of the individual into society questionable. The problem of cervical lymph node metastases is vast and complex, reason why we approached several anatomical and physiological aspects of lymph vessels of the aero digestive organs. Among the available elements during treatment, the headquarters of the tumour, its histologic degree, and its infiltrative nature, each of them significantly influences the possibility of developing metastases. **Keywords**: TOPOGRAPHIC ANATOMY, EMBRYOGENESIS, LYMPH NODES, HEADQUARTER OF TUMOR, HYSTOLOGIC DEGREE, INFILTRATIVE NATURE.

Taking into account the fact that the lymph from the entire body drains into the large vessels on the base of the neck, the research area of specialties extends in other medical fields too, in the event that the primary lesion is not detected in the head and neck.

Our knowledge on lymphatics of the neck is based on the works of Barteis, Iosipow, Kessel, and Octaviani, and especially on Rouviere's descriptions which are nowadays the starting point of study for every specialist (1).

On the basis of 600 various jugular carotid dissections, it results a description of position of pathologic lymph nodes, that is useful for completions on neck dissection (2).

General embryogenesis concepts

The lymphatic system is born phylogenetically and ontogenetically later than the blood system. Starting with the third embryonic month, the lymphatic meshwork communicates with right and left upper cardinal vessels through an orifice equipped with a valve. In the place where the lymph nodes will be formed, bundles of vessels appear, in whose loops mesenchymatous elements gather, lymph cells

arise, and the fibrous capsule is already shaped in the fifth month (3).

From marginal sinuses lymph vessels grow, which penetrate the parenchyma towards the hilum. At birth, the lymph nodes are formed organically, except for the ones that are born secondarily, postnatal. The weight of the lymph node system is 1% of the body weight.

This tissue is best developed around the age of 14-15 years old, coinciding with the complete growth of the adrenal gland (4).

From here on, it starts a slow atrophy process, stronger and stronger as years pass. There won't be a total regress, since they can react inflammatory or neoplastic any time, outreaching their initial volume (5).

General structural features of vessels and cervical lymph nodes

The cervical lymph system is formed of lymph vessels and capillaries, lymph nodes, and terminal collectors on the base of bilateral neck. As Rouviere (1) described, each lateral cervical area contains 23 - 48 lymph nodes (1).

Zechner believes that 1/3 of the total of approximately 400 lymph nodes in human body is located in the neck (6). Royster, dissecting the operatory parts of 39 radical neck dissections identifies, through histologic studies, lymph nodes of various sizes, ranging from 1 cm to 1.5 mm in diameter. Under these conditions, in a cervical dissection, approximately 146 ganglionic formations are collected. It is remarkable that the lymph nodes that were detected microscopically were found metastasized, therefore several authors concluded that the ganglionic dissemination is not directly proportional to their size (7).

In normal physiological conditions, lymph nodes are usually bean-shaped, smooth, soft and elastic. Structurally, they are surrounded by a fibrous capsule, and inside there are parenchyma areas: the outer cortical area and the inner medulla (3). The lymphoid tissue is organized in follicles in cortical area and follicular cords in medulla, being formed of a reticule in which there are lymph cells (lymphoblasts), which will generate lymphocytes. The capsule sends interfollicular septa through the cortical area, which are continued in medulla through intercordonal septa up to the hilum (6, 7).

The circulation pathways of the lymph into the lymph nodes are perifollicular sinuses in cortical area and cavernous pathways in medulla. Lymph capillaries are made of a single endothelium and form a network of various density in connective tissue. The lymphatic vessels have a thicker own wall and double valves, bond, open in the direction of the stream and separated by a distance of approximately 1-2. The lymph trunks have, like blood vessels, an intimate layer, the endothelium of the vessel, a medullar layer, formed of smooth muscle fibres, and a connective-elastic layer, called adventitia, more reduced. (7).

Lymphatic circulation and functions of lymphatic-ganglionic system.

There are several causes of the progress of the lymph into the lymph vessels, depending on their place.

According to Rouviere, in the area of head and neck, there are two important elements (1): the arterial element, which, during systole sends the arterial beat to the cerebrospinal fluid which sends it further to the lymphatic vessels of the meninges. The absorption power of the lymph vessel during diastole should not be neglected; the

respiratory element is also valuable for circulation within veins, where the thoracic suction is the main factor of blood progression, therefore of lymph progression (3).

Unlike the large descending cervical and thoracic veins, lymphatic vessels have valves that prevent the retrograde circulation, due to higher downstream pressure than the upstream one, the opposite of venous circulation. The pressure in the thoracic duct increases while expiring and decreases while inspiring. Research conducted by Rouviere and Valette on dogs reveal values of 6 cm^3 water, therefore a higher value than the venous pressure of large vessels on the base of the neck, to ease the passage of the lymph into blood (1).

Lymphatic collecting vessels reach the closest lymph nodes, according to Bartels' law, and, crossing the capsule, open in the marginal sinus. Other times they travel in remote ganglionic relays or the afferent and efferent vessel, or are closely and directly bond through a medullar sinus (3).

All these data may explain why a metastasis does not always undergo all dissemination stages of lymph nodes.

Lymphatic vessels exiting through hilum are afferent lymphatics, but the direction of the stream can change backwards towards lymph nodes, in certain pathological conditions, and then the metastasis might initially arise on hilum. The lymph does not bathe all the parts of the marginal sinus, therefore subcapsular parcelar metastases are possible. Direct links between lymph vessels and veins are rare; only exceptionally a lymphatic vessel can open in thoracic duct. A very important practical aspect, according to Pressman (quote 6), is the direct communication between lymph nodes and blood vessels. Inserting successively air and saline in the lymph nodes of a dog, Pressman observed a direct communication between the lymph nodes and are neighbouring blood vessels, due to the existence of a reach capillary plexus within the lymph node (1,3).

Many remarks revealed that the pressure needed for such substance to enter the blood is lower than the one needed to enter the efferent canals of the respective lymph node. In some cases, the presence of neoplastic cells in blood flow, especially in the area of the lesion, can be thus explained. Lymph is in a diffusion balance with interstitial fluids, respectively with blood plasma, in which the electrolytes, organic and inorganic substances differ little from the ones in blood plasma, except for the concentration of albumin. The walls of lymphatic capillaries are highly permeable and allow diffusion of large molecules and corpuscular tissues, so that lymph is able to transport plasma proteins from interstitial fluids into the blood, regulating the register of plasma albumin. In 24 hours, almost the entire quantity of plasma travels through blood capillaries into interstice, where they are sent back to the lymph. There is a so called lymphocrinia, when some of the secretions of endocrine glands, whose structure contains albumin, reach the general circuit through lymph vessels. Via the thoracic duct, in 24 hours, approximately 2-3 litres of plasma albumin travel, leading to an important loss of lymph during lesions of thoracic duct, with dehydrating effect, with a dangerous deficit of serum albumin; this is also possible during neck dissection on the base of the neck (3).

Lymph nodes have a several functions; the most important ones are:

- *Lymphopoiesis.* Through their structure, lymph nodes are the most important

and the most spread wearers of the lymphoreticular tissue. This tissue has a great capacity of cell genesis and formation of lymphocytes which float into the lymph in various mononuclear aspects and from here partially in blood, where they regulate the formula of blood. Formation and number of lymphocytes are regulated by the hormones of the adrenal gland. Hypofunction of this gland causes lymphopenia, while an increase in number of adrenocorticotropic hormones or the action of various stress factors causes lymphocytosis (3).

- *Formation of antibodies.* In 1898, Pfeiffer and Mark (quote 7) showed that inside lymph nodes there are antibodies against the choleric vibrio and that in general the titre of the antibodies is higher than the titre of afferent lymph vessels. Recent research has proved that only certain immunologic substances are formed in lymph nodes, together with some of the gamma globulins of plasma proteins, namely, in plasma cells (8).

- *Ant infectious and antitumor filter*. In 1860, Wirchow described the barrier function of the lymph nodes, afterwards extending this function for toxins, various particles, bacteria, and tumour cells (9).

This complex function materialises in three aspects: spasms of lymph vessels: Fuldi et al. showed that in the centre of the inflammation, the lymph vessels are clogged by a fibrin clot and distal from it there is a functional closure, a spasm (10). No doubt that the closure of the lymph pathways as a result of inflammatory oedema prevents the entrance of the toxins and bacteria in inflammatory burning point into the blood; the lymph that reached the marginal sinus and then in other intraganglionic directions decreases the circulation speed and changes the dynamics of circulation. The reticulum of sinus endothelium and the other free reticula enhance this stopping mechanism for foreign bodies, bacteria, and tumour cells. The reticular cells, fixed or free, start an active phagocytosis which becomes the main element of defence (3).

These defence mechanisms of lymph nodes can be overcome through the increase of the harmful action of foreign agents and, moreover, they themselves can become infectious or tumoral burning points of spreading.

It seems that not all researchers agree to the role of ganglionic filter. Thus, Pressman (11), conducting research on animals, stated that in reality, lymph nodes have the structure and function of a sponge, and that a mere pressure is enough to express the content, not only in efferent vessels, but also in the blood flow. As a result, during a clinic examination or a neck dissection, one should avoid the compression of suspect lymph nodes, which might contain malignant cells.

The problem of ganglionic filter was raised in order to decide whether to avoid or not the cervical neck dissection. An increase of frequency of metastases was noticed, and researchers concluded that the cervical lymph nodes are a barrier against dissemination of metastases; the lymph nodes can delay individually or as a group the cranial-caudal lymphogenic metastatic process.

Multiple research and clinical observations prove the existence of a defence function, but evidence of destroying the neoplastic cells in the lymph node, as well as a cancericidal function are missing. (11).

The lymph meshwork is extremely developed in the area of inferior and middle nasal fossae, olfactory area and around the

choana. The olfactory area communicates with the subarachnoid spaces through the lymph vessels which accompany the fillets of the olfactory nerve.

The lymph collecting vessels are directed towards the tubular area, receiving also the lymphatic vessels from the upper side of the veil, after which two pedicles are formed: an external pedicle, which criss-crosses the styloid process and discharges into the main digastric lymph node (Kuttner); a posterior pedicle, more important, which crossing the superior constrictor muscle, drains in Gillete's retropharyngeal lymph nodes, and after their involution – in the lateral retropharyngeal lymph node in posterior subparotid space. The lymphatics of the sinuses and ethmoidal cells are associated with the ones of the nasal fossae and follow the same destination.

The lingual lymph network is particularly rich and it is grouped in median and lateral collecting vessels. The median lymphatics travel down towards the median glossoepiglottic fold, where, changing direction towards the lower pole of the palatine tonsil on the same or opposite side, meet the lateral lymphatics (12). From here, together, they cross the lateral wall of the pharynx at various heights and in the end they become: some of collecting vessels in Kuttner's or Most's subdigastric lymph node; some in the middle jugular lymph node; some in the median lymph node of the spinal chain. Remarkable is the wide array of lateral and heterolateral spreading of lymph nodes of the lymphatics of the base of the tongue, as well as the connections with the lymphatics of the palatine tonsil on its lower pole (10).

Inside the pharynx, the mucous membrane is spread along the entire pharynx, very rich within the piriform sinuses and around Waldeyer's ring; establishes connections with the lymphatics of the nasal fossae, oral cavity and larynx in the anterior side, downwards connections with the lymphatics of the esophagus and outwards, with Eustachian tube network (13,14).

The collecting vessels are grouped depending on the three distinct regions (nasopharynx, oropharynx and laryngopharynx), with different directions and ganglionic destination.

The lymph on the walls of the nasopharynx is collected in two categories of collecting vessels, median and lateral. The median vessels originating on the floor, Luscka's tonsil and the posterior wall are the most numerous ones. They cross the two large anterior muscles of the head, tilt outwards and reach the lateral retropharyngeal lymph nodes. Some vessels avoid this stage and end in superior external jugular nodes or lower, in a spinal or subdigastric lymph node; the counter-lateral and bilateral drainage is possible. The lateral lymph vessels cross the superior constrict of the pharynx and discharge in the lateral retropharyngeal lymph node.

The collecting vessels of the oropharynx are grouped in the lymphatics of the palatine velum, palatine tonsils and the pillars of the palatine velum; very rich on the anterior side of the velum, especially on uvula. The collecting vessels have three directions: an anterior lymphatic path, which joins the lymphatics of the cheek and goes downwards to pre- and retrovascular submandibular lymph nodes; a median pathway, which criss-crosses the deep side of digastric muscle and ends in subdigastric lymph node and superior homolateral and counterlateral jugular lymph nodes; a posterior pathway, where they join

the lymphatics of the posterior pedicle of the nasal fossae and end in lateral retropharyngeal lymph node and sometimes in the counterlateral lymph node. There still are connexions between lymphatics of the velum and lymphatics of the palatine tonsil (15).

The lymphatics of laryngopharynx are numerous and large, and, especially on the piriform sinuses, they are grouped as: anterior collecting vessels, which, together with the lymphatics of the supraglottic area of the larynx follow the superior laryngeal pedicle and distribute to subdigastric lymph node or lower, to jugular lymph nodes, located between the thyrolinguofacial and omohyoid trunk; posterior collecting vessels cross the lateral pharyngeal wall and end in lateral retropharyngeal lymph node or superior exterior jugular lymph node (15).

Reference to cervical esophagus alone is arbitrary, due to the fact that the lymph network of the esophageal mucosa is continuous, so that it can drain both into a neighbouring lymph node, and into a lymph node located at a distance from the emission site of the lymph (16, 17).

The deep lymphatic network of the larynx is separate from the counterlateral one by a fibrous commissure that joins the two halves of the thyroid cartilage, distinct in embryonic terms.

The laryngeal lymphatic pathways are linked to the one of the barrier of the tongue and pharynx. As a general remark, the direction of the lymph stream goes upwards from the inferior margin of the cricoid.

Lymphatics of the trachea, although making connections with the subglottic lymphatics, drain especially towards the mediastinal lymph nodes; a subglottal cancer, extended to trachea, will need the therapy of the structures of the neck simultaneously with the therapy of the mediastinum.

Subglottic lymph vessels which receive a small portion of the lymphatics of the vocal cords are connected with the supraglottic lymph vessels through the posterior wall mucosa of the larynx and very few ventral connections (16).

Rejecting the idea according to which the lymph nodes are randomly spread in soft parts of the neck, they form a fixed, well-structured anatomical whole, enough and often complete for stopping any kind of aggression of the lesions of upper aerodigestive organs.

The anatomic study of these groups of lymph nodes is indispensable for understanding the pathogenic concept of cervical lymphadenopathy and for defining the physiologically normal direction of the lymphatic stream (4).

It is necessary to know them, each time a cervical lymph node dissection is performed, be it radical or traditionally functional (2,3).

For practical purposes, without ignoring the continuity of lymphatic system, the surgical anatomy describes several chains of lymph nodes, identified through certain limits, bones or muscles. Neck dissection contains certain chains which, in general, may fit certain geometrical shapes, so that the piece contains a coherent assembly whose sides are dissected (4).

Even if there still are some concerns related to cancerogenic factors, the lymphatic system looks like a unitary whole of performant pathways, which offers the tumour a way to evade, and the tumour will definitely follow it.

Histological research of the tumoral peripheral areas reveals a stroma, a pericarcinomatous reaction which does not limit or

cease the process. Thus, there are inflammatory (granuloma), constructive (connective tissue hyperplasia), and vascular phenomena (vascular neoformations), insufficient as barriers, which allow the effraction of tumoral cells from the local cohesion, which will borrow whatever dissemination pathway they find (17, 18). The lymphatic discontinuity causes embolism, usually in the lymph nodes of the first relay, the closest to tumour. They have a clinical and diagnostic importance because through their topography they are able to detect the headquarters of the primary lesion.

This rule is not absolute, since there are also abnormal pathways, or by blocking the existing pathways of previous inflammatory processes, the drainage takes place through collateral pathways. One needs to take into account the cross drainage, which can cause counter lateral metastases, solitary in 10% of the cases (19).

Hematogenous discontinuity pathway can usually provide the metastases of sarcomatous tumours and malpighian carcinoma in 45% of the cases.

Continuity pathway presented as neoplastic lymphangitis is observed along the preformed lymph vessels through the lymph sheathes of the nerves (10,15).

Morphological changes of the lymph nodes which are macroscopically visible for the clinician are reactive changes of the lymph nodes, tokens of defence of the reticular endothelial system, characterized by nonspecific reactions which are prone to produce a spontaneous closure of the lymphatic pathways during acclimation of the tumoral cells which reached the marginal sinus, and tumoral changes of the lymph nodes.

Related to reactive changes of the lymph nodes and the way the metastases approaches the lymph node, the most frequent way of dissemination is the orthograde way, and the tumoral graft is located in the marginal sinus of the convexities of the lymph nodes (16, 19).

If a lymph node is blocked by a tumour or inflammation and it is excluded from lymphatic circulation, the afferent vessels enlarge, the stream becomes reverse and it drains randomly, involving tumour cells as well, which explains the appearance of the lymphatic invasion.

CONCLUSIONS

In our evaluations, we can conclude that when the high percentage of responsibility of the lymph nodes was confirmed by statistics, compromising the results through local relapse or remote general metastases, therapists' main point of interest lays in solving this problem; a cancer whose first treatment failed is definitely lost.

The treatment approach on cervical lymph nodes with metastases evolved in the same pace with the development of general oncology principles and especially cervical-facial cancerology.

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HISTORY OF MEDICINE

A RENAISSANCE PROMOTER OF MODERN SURGERY

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A RENAISSANCE PROMOTER OF MODERN SURGERY (Abstract): The present paper aims, exploring the history of Renaissance medicine, to evoke the figure and work of the priest, surgeon and anatomist, Guido Guidi (Vidus Vidius) (1509-1569). The XVIth century is considered a period marked by artistic and scientific effervescence in the western part of Europe and Guido Guidi was a first order personality, grandson of Domenico Ghirlandaio and friend of Benvenuto Cellini. He was appointed by the King Francis I the first professor of anatomy and surgery at the newly founded Collège de France. On demand of the King, he wrote *Chirurgia è Graeco in Latinum conversa Vido Vidio Florentino interprete, cum nonnullis eiusdem Vidii comentariis* (1544), a beautifully illustrated original surgery book that became for the following two centuries the main source in teaching surgery. Our study realized a detailed assessment of the book and especially of its illustrations belonging to Francesco Salviati. Exploring the life of Guido Guidi, we were also able to point out other significant contributions in the field of anatomy and clinical medicine as *De anatome* the first book where are presented disarticulated, the bones of the skull base and also the discovery of the chickenpox. Some surgical personalities attributed to him both the elaboration of the term *appendix vermiformis* and the first description of an aneurysm, he treated with the help of Fallopio. Although forgotten today, Guido Guidi was a leading figure of the Renaissance medicine both in France and Italy. **Keywords:** GUIDI, SURGERY BOOKS, RENAISSANCE MEDICINE, SALVIATI, COLLEGE DE FRANCE.

At the beginning of the XVIth century Florence was the theater of major social and political convulsions occurring against the background of the economic prosperity and artistic effervescence at the peak of the Renaissance. The richness and patronage of the Medici family decisively contributed to the development of an unequaled Florentine artistic universe and to the apparition of brilliant masters and apprentices who scientifically studied the human body through dissection in order to achieve a realistic representation in works of art. This was the moment when the canons of anatomical drawing were established and the artistic anatomy began to be taught by cadaver dissection at the Arcispedale Santa Maria Nuova. Here Leonardo da Vinci (1452-1519) and Marcantonio della Torre (1481-1511) started to write a book with drawings

after dissection preparations but the plague of Florence (1511) interrupted this collaboration, due to the death of della Torre.

In 1540 was established, under the patronage of Grand Duke Cosimo I de Medici, the "Accademia degli Umidi", which later became "Accademia Fiorentina" (in 1541) (1). Here, anatomy was thought initialy by Cosimo Roselli (1439-1507) and later by his pupils, Piero di Cosimo (1462-1521), Andrea del Sarto (1486-1531), Domenico Ghirlandaio (1449-1494). This effervescent atmosphere marked by the enthusiastic desire for knowledge, developed the brilliant triumvirate Leonardo da Vinci, Michelangelo (1475-1564) and Benvenuto Cellini (1500-1571).

LIFE AND ACTIVITY

In this artistic Renaissance Florence was born, on February 10, 1509 (2, 3, 4). Guido Guidi, the son of the well-known Florentine physician Giuliano Guidi and Costanza Bigordi, daughter from a second marriage of the great painter Domenico Ghirlandaio.

The data of Guido Guidi's childhood and how he obtained his university degrees are missing (5), but it is believed that the young man, with artistic blood in his veins and raised by his father in the medical tradition required by the Hippocratic oath, received a humanist education of the highest quality. In the first phase of his life, until 1535, he studied in Florence, becoming a connoisseur of Greek and Latin. Given his high education and early erudition he was noticed by the Cardinal Ridolfi, who took him under his protection. In the Cardinal's entourage, the young Guidi had access to the famous Laurentian library that housed manuscripts of great value in all areas of science and art. Among them there was a codex of the Byzantine physician Nicetus from the X-th century, which contained, collected by Apollonius of Kitium, the Hippocratic works: *De ulceribus*, *De fistulis*, *De vulneribus capitis*, *De fracturis*, *De articulis* and *De officina medicinae*, the last three commented by Galen, in Galen's treatise *De fasciis* and Oribasus, *The laqueis and De machinamentes.*

Between 1535 and 1538, Guido Guidi went to Rome where he worked with famous surgeons of the time, Antonio Ponzanello and Giacomo Rastelli, the latter being the personal surgeon of Popes Clement VII and Paul III. Under the guidance of Roman masters he became familiar with the use of contention and continuous extension devices in the treatment of strains and fractures. It seems that in Rome Guido Guidi met for the first time Benvenuto Cellini and warm friendship developed between the two Great Men.

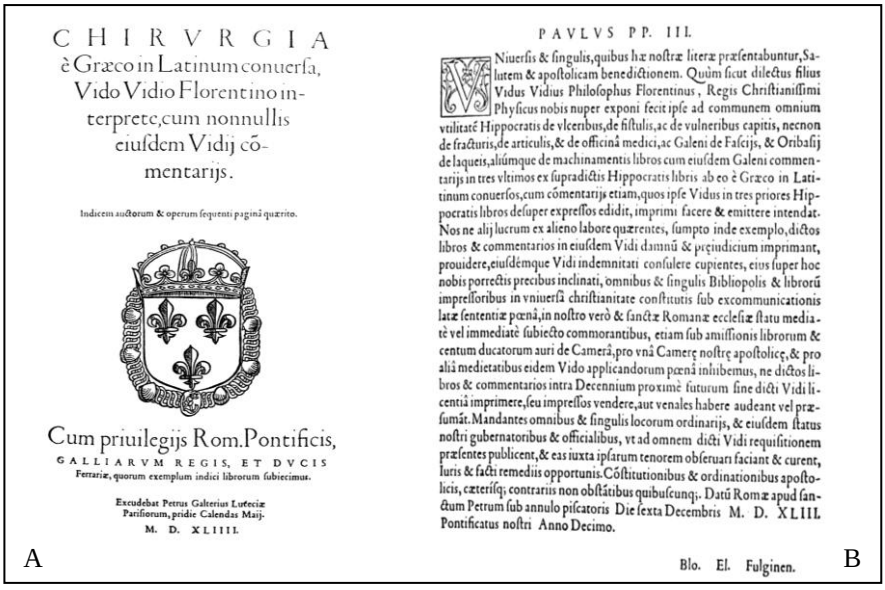
After returning to Florence, Cardinal Ridolfi encouraged Guido Guidi to translate *De ulceribus* and *De fistulis,* adding not only his own comments but also that of Aristotle (*causam nos docuit Aristoteles, quum tradit calorem in unu coactum augeri..)* and of Eudemus (370-300 BC), Cornelius Celsus (25 BC-50 AD), Paulus in Aegineta (625-690), and Soranus of Ephesus (ca. AD 98-138) (*Soranus, cuius scripta de vulneribus capitis adhuc extat...)* as is specified in *Chirurgia* pp. 67-72.

King Francis I of France informed by Primaticcio (1504 - 1570), by the diplomat Alamanni and by Cardinal Ridolfi himself about the audacious attempt of the young erudite, invited him to Paris in 1542. From that moment the life of Guido Guidi had sensationally changed. He presented to the King the original codex and his translation of it, making an incredible impression. He was appointed the first professor of medicine and surgery at the newly established

Collège de France, the personal physician of the King, and was requested to complete the translations in order to publish a book of surgery, according him important ecclesiastical benefits.

At Paris, where Guidi taught and practiced medicine between 1542-1547, for unknown reasons he changed his name, becoming Vidus Vidius as he will be cited in the classical anatomical literature, including *Nomina Anatomica*. We believe that the truth is more subtle; tempted by the advantages of belonging to the clerical elite, Guido Guidi, skillful user of Latin, Greek and Arabic, had chosen for himself this cognomen derived from "widower" or "alone", thus declaring his intention of becoming a priest. This idea is supported by at least two arguments: Tuscan contemporaries, including Cellini, continued to call him Messer Guido Guidi; in a pirate edition of *Chirurgia è Graeco in Latinum conversa* from 1553, the author was named Guy *i.e.* Guido the Widower (6).

In his private life, fate favored him again. The young Florentine became very good friend with Benvenuto Cellini who hosted him in his castle, Petit Nesle and, in his autobiography, made him an extremely flattering characterization. Here he met Pierre Galtier, the future editor of *Chirurgia è Graeco in Latinum conversa*, who worked in typography in the Paris castle of Benvenuto Cellini.



CHIRVRGIA
è Græco in Latinum conuerſa,
Vido Vidio Florentino interprete, cum nonnullis
eiuſdem Vidij cõmentarijs.

Indicem auctorum & operum ſequenti paginâ quærito.

Cum priuilegijs Rom. Pontificis,
GALLIARVM REGIS, ET DVCIS
Ferrariæ, quorum exemplum indici librorum ſubiecimus.

Excudebat Petrus Galterius Lutetiæ
Pariſiorum, pridie Calendas Maij.
M. D. XLIIII.

A

PAVLVS PP. III.

Nuerſis & ſingulis, quibus hæ noſtræ literæ præſentabuntur, Salutem & apoſtolicam benedictionem. Quùm ſicut dilectus filius Vidus Vidius Philoſophus Florentinus, Regis Chriſtianiſſimi Phyſicus nobis nuper exponi fecit ipſe ad communem omnium vtilitatẽ Hippocratis de vlceribus, de fiſtulis, ac de vulneribus capitis, necnon de fracturis, de articulis, & de officinâ medici, ac Galeni de Faſcijs, & Oribaſij de laqueis, aliúmque de machinamentis libros cum eiuſdem Galeni commentarijs in tres vltimos ex ſupradictis Hippocratis libris ab eo è Græco in Latinum conuerſos, cum cõmentarijs etiam, quos ipſe Vidus in tres priores Hippocratis libros deſuper expreſſos edidit, imprimi facere & emittere intendat. Nos ne alij lucrum ex alieno labore quærentes, ſumpto inde exemplo, dictos libros & commentarios in eiuſdem Vidi damnũ & prejudicium imprimant, prouidere, eiuſdémque Vidi indemnitati conſulere cupientes, eius ſuper hoc nobis porrectis precibus inclinati, omnibus & ſingulis Bibliopolis & librorũ impreſſoribus in vniuerſa chriſtianitate conſtitutis ſub excommunicationis latæ ſententiæ pœnâ, in noſtro verò & ſanctæ Romanæ eccleſiæ ſtatu mediatè vel immediatè ſubiecto commorantibus, etiam ſub amiſſionis librorum & centum ducatorum auri de Camerâ, pro vnâ Camerę noſtrę apoſtolicę, & pro aliâ medietatibus eidem Vido applicandorum pœnâ inhibemus, ne dictos libros & commentarios intra Decennium proximè futurum ſine dicti Vidi licentiâ imprimere, ſeu impreſſos vendere, aut venales habere audeant vel præſumãt. Mandantes omnibus & ſingulis locorum ordinarijs, & eiuſdem ſtatus noſtri gubernatoribus & officialibus, vt ad omnem dicti Vidi requiſitionem præſentes publicent, & eas iuxta ipſarum tenorem obſeruari faciant & curent, Iuris & facti remediis opportunis. Cõſtitutionibus & ordinationibus apoſtolicis, cæteriſq; contrariis non obſtãtibus quibuſcunq;. Datũ Romæ apud ſanctum Petrum ſub annulo piſcatoris Die ſexta Decembris M. D. XLIII. Pontificatus noſtri Anno Decimo.

Blo. El. Fulginen.

B

Fig. 1. A. Main opening page of *Chirurgia è Graeco in Latinum conversa*, decorated with the royal crown with the lilies of France. B. The papal prohibition against copying, reproducing or republishing the book (copyright) (7).

Guido Guidi became the first professor of medicine at the Collège de France, his clear and attractive public lectures on medicine and surgery, as well as his elegant style (also reflected in his writings) brought new students and a large audience and at their request he began to carry out public demonstrations of anatomy. He worked

intensively untill 1543 completing (fig.1) the book entitled *Chirurgia e Graeco in Latinum conversa, Vido Vidio Florentino, cum nonnulus eiusdem Vidii comentariis* (7).

It seems that the book was printed under the guidance of Benvenuto Cellini himself, as demonstrated by the analysis of the capital letters at the beginning of the chapters, in which can be seen the floral ornaments used by Cellini for the armor of Cosimo I de' Medici (8) (fig. 2).

Fig. 2. Capital letter from *Chirurgia è Graeco in Latinum conversa*, decorated with floral motifs that are found in Benvenuto Cellini, on the bronze bust of Cosimo de' Medici, *Museo Nazionale del Bargello* in Florence (7, 20).

Guidi presented the book to the King Francis I, requiring a protection against plagiarism and republication by others. The King wrote him an eulogistic foreword, calling him *"notre cher & bien ayme maistre Vidus Vidius de Florence, docteur en medecine, notre medecin ordinaire & lecteur en Paris"* and then he added " *nous a faict dire & remostrer, quil estoit apres avoir laisser a imprimer a ses grandz fiz, mises & depenz, & au gand proffit & promotion des estudians en medecine & principalement en la chirurgie ung liure intitule....pour le teps & terme de six ans prochainement venant a commencer du jour & date de ces presentes, autres imprimeurs ne puissent imprimer ne vendre ledict liure ensemble, ou par ses parties, que ceulx a qui ledict permectra"* establishing penalties and significant punishments for infringement of that prohibition. The royal document dated January 2, 1543 (7, p. 22 iij) is the first known written copyright. But the author was not satisfied with that. He presented the manuscript to Pope Paul III and received, after 10 months of thorough review of the book in the papal chancery, a brief preface that proclaimed the originality and usefulness of the book and prohibited the partial or total reproduction under the penalty of excommunication (7, p. 22 ij).

Chirurgia è Graeco in Latinum conversa that became a reference book for the study of surgery in the XVI to XVIII centuries, begins as a result of author's erudition, with a bilingual alphabetical index in Greek and Latin, where, across 14 pages, the correspondence of the surgery terminology is specified in both languages. Moreover, Guidi presents an index of authors and works, outlining that the first six chapters, *De ulceribus, De fistulis, De vulneris capitis, De fracturis, De articulis, De officina doctors,* belong to Hippocrates and are commented by Vidius (the first three chapters) and the following three chapters to Galen.

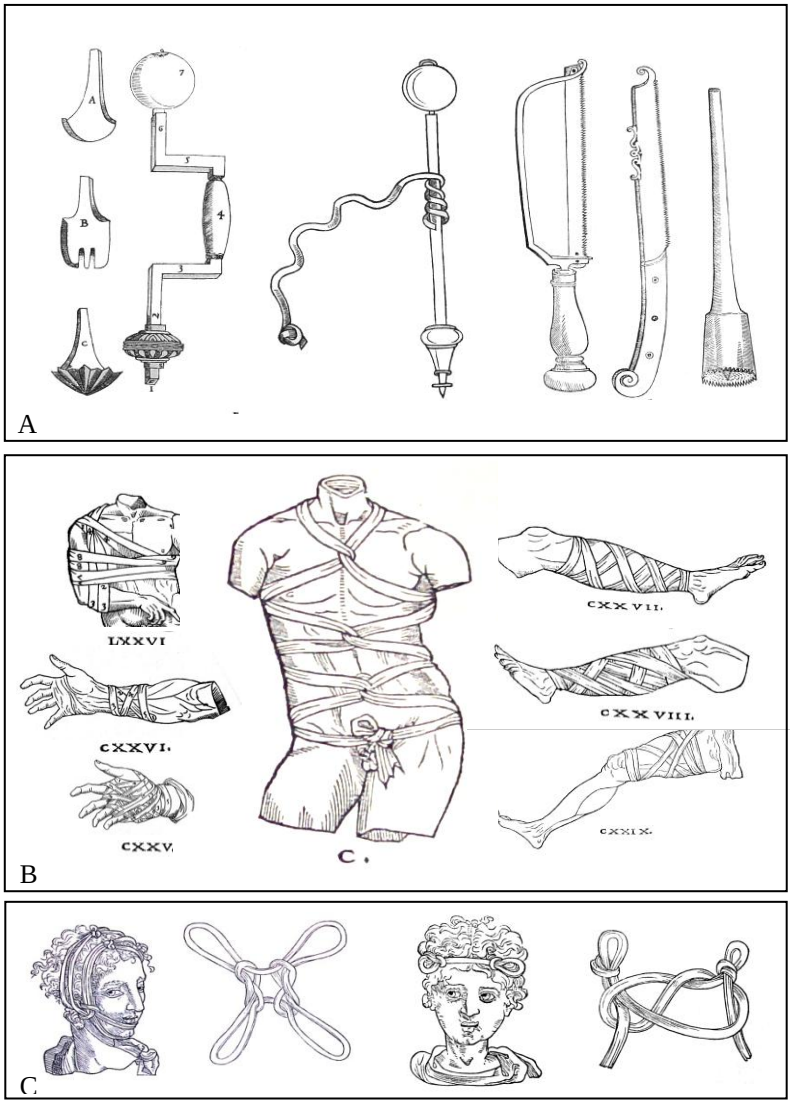


Fig. 3. A. The instruments imaged for different interventions on the bones (commentary illustrating *De fracturis*); B. Types of bandages for limbs and trunk; C. Models of surgical knots (7).

Chapter VII contains Galen´s *De fasciis,* and Oribasius´ *De laqueis* and *De machinamentes,* commented and illustrated by Vidus in collaboration with the Florentine painter Francesco Salviati (9) (fig. 4).

If, in the study of the six Hippocratic works, Vidus had only a theoretical contribution, the work of Galen *De fasciis* was richly illustrated with original designs of head bandages such as a complete capeline, single or combined with a gonion/chin double support sling, other types of bandages for craniocervical injuries, upper limb and thorax bandages, seen in

the Tabula LXXII, showing a shoulder immobilization technique used until today, such as the Desault bandage. Of the thoracoabdominal bandages, we mention the contention system used in the diastasis recti, where the bandages are arranged alternately, in the direction of the fibers of the oblique external and internal muscles of the abdomen and united in the midline as a raphe, which doubles the white line (fig. 3).

Of the periarticular bandages, the intercrossed pattern slings of the thumb, groin and talocrural bandages conclude the series of 138 drawings of the *De fasciis* chapter.

From the work of Oribasius (AD 320-403), Vidus selected, commented and supplemented the chapters on knots and orthopedic devices to reduce dislocations and fractures.

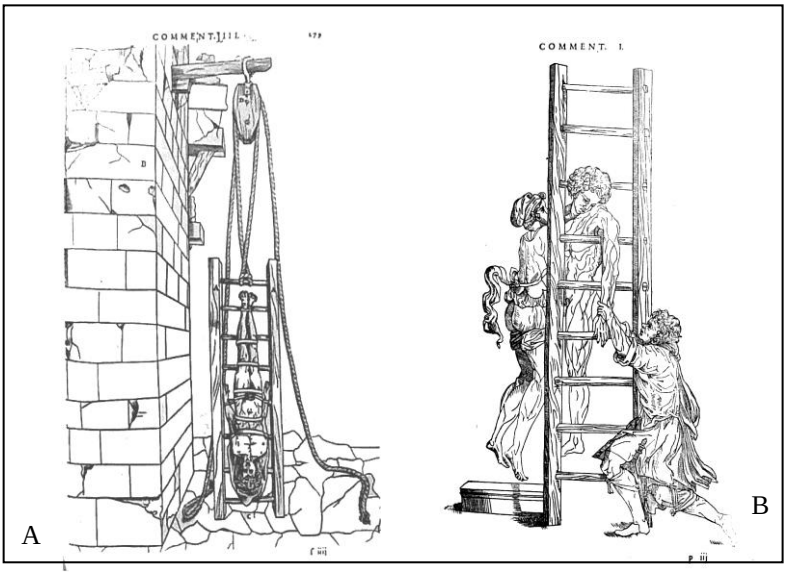


Fig. 4. Devices (machinamentes) for continuous extension (A) and luxation reduction (B) reproduced from original engravings of Francesco Salviati, preserved in Paris, at the Ecole Nationale Supérieure des Beaux-Arts, Inventario Masson, 210 (A) and Bibliothèque Nationale de France (B) Départements des Manuscrits (9).

In the work of Oribasius, the contribution of Vidus consisted in the diversification and improvement of the knot systems and especially of the outstanding graphical presentation that facilitated learning. Finally, the systems for the reduction of dislocations and fractures are initially presented segmentally, and then gradually assembled, indicating where to put the sprockets and pulleys thus making possible the replication of the device for any physician. The images of this last chapter of Guidi`s *Chirurgia* were also made by Francesco Saviati by engravings on copper plates (fig. 4).

After the death of King Francis I (1547), Guido Guidi returned to Italy and settled in Pisa, where he practiced surgery and held public lectures of surgical anatomy through demonstrative dissections of human cadavers (10, 11, 12).

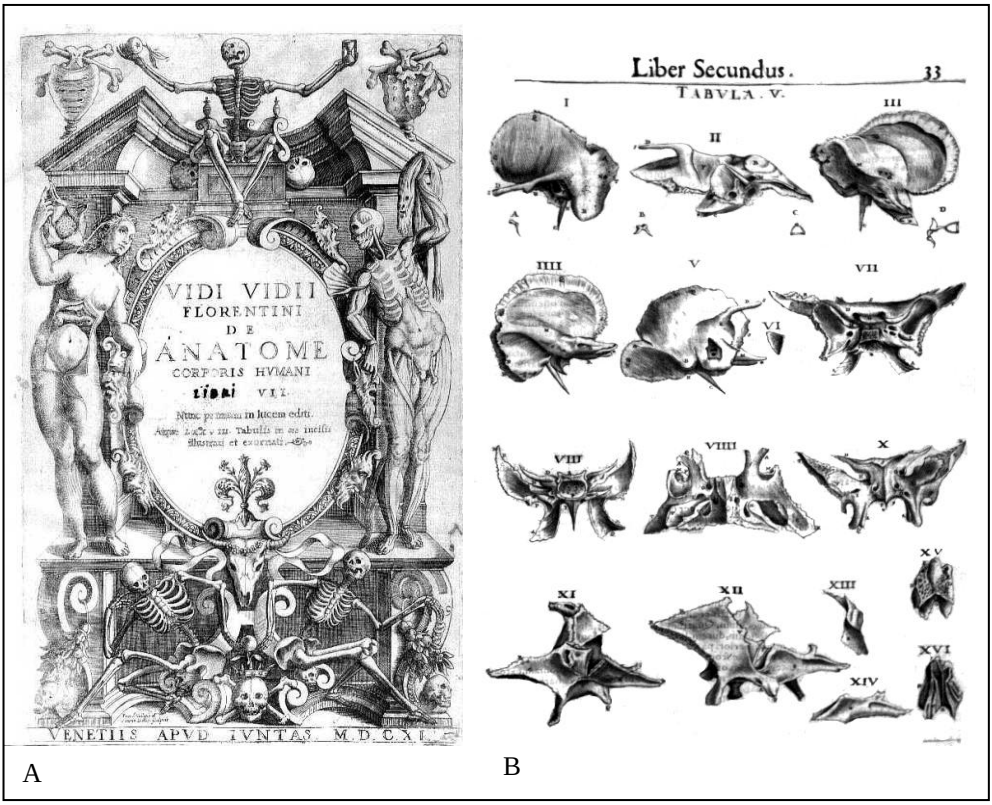


Fig. 5. A. The opening page of the Treaty *De Anatome*; B. Tabula V of Chapter II (Liber Secundus), which presents for the first time, the disarticulated bones of the skull base (7).

This allowed him to accumulate a notable experience in surgery and anatomy that enabled him to add new discoveries to the scientific heritage of the time. In the surgical field he was the first to describe the arterial aneurysms, an original technique of tracheotomy, a procedure of lengthening the foreskin of the penis incurvatus or other sequels of circumcision, cutaneous plasty of trophic limb ulcers, nose plasty, plaster or wax dental implants to improve phonation and he established the axillary lines as landmarks for the thoraco-brachial approach (13, 14).

He named in Italian language *vermiforme*, the ileocecal appendix, discovered by Berengario da Carpi (1460-1530) and described the *ostia* of the hepatic veins openings in the inferior vena cava. In the accompanying drawing it can be observed a rare anatomical variation of five such *ostia*, demonstrating that the study was done by dissection on human corpses.

In the medical field, he was the discoverer of chickenpox. By studying diabetes, he concluded that it was a consequence of a single glandular insufficiency (without specifying the gland). He successfully applied the physiotherapy, using the therapeutic qualities of the baths of Pisa (15).

In the anatomy field, he wrote a remarkable treatise – *De anatome* (fig. 5) – in which he established the conditions of

building a *Theatrum Anatomicum* (5), presented, for the first time, the disarticulated bones of the skull base, described the orbital process of the palatine bone and the pterygoid canal that bears his name – *the vidian canal* (16).

CONCLUSIONS

Although forgotten today, Guido Guidi was a leading figure of the Renaissance medicine, both Italian and French (17, 18, 19). He was the first professor of anatomy and surgery at the Collège de France, and his *Chirurgia*, remained the reference book for teaching surgery in the following two centuries. It is considered the first original surgery book, beautifully illustrated by a famous contemporary Florentine painter. In the field of anatomy Guido Guidi was a prominent personality with important contributions that lasted until today as eponyms in *Nomina Anatomica*.

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IN VITRO TESTING OF XANTHAN/LIGNIN HYDROGELS AS CARRIERS FOR CONTROLLED DELIVERY OF BISOPROLOL FUMARATE

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IN VITRO TESTING OF XANTHAN/LIGNIN HYDROGELS AS CARRIERS FOR CONTROLLED DELIVERY OF BISOPROLOL FUMARATE (Abstract): **Aim**: To develop sustained release matrix tablets based on xanthan as highly water-soluble, cost-effective, nontoxic, easily available, and suitable hydrophilic systems. **Material and methods:** Xanthan and lignin epoxy-modified resin (LER) mixture were crosslinked using epichlorohydrin as crosslinking agent leading to superabsorbent hydrogels with high swelling rate in aqueous mediums. **Results and conclusions**: These hydrogels were tested as carries by the loading/delivery behaviour of bisoprolol fumarate in physiological conditions and based on the obtained results these hydrogels may show interest for application in medical and pharmaceutical areas. The amount of drug loaded in polymer networks was found to be ranging between 14.4 % and 19.2 %. Drug release was retarded and the release mechanism of the active principle was found to depend on matrix composition. **Keywords**: HYDROGELS, XANTHAN, CONTROLLED DELIVERY, BISOPROLOL FUMARATE.

The exopolysaccharide xanthan is produced by the bacterium *Xanthomonas campestris*. Xanthan is a high molecular mass, double stranded anionic polysaccharide which forms solutions that are both highly viscous and very stable under conditions of extreme temperature and salinity. Given these properties and its ability to be manufactured cheaply in large amounts xanthan has gained widespread use as a viscosity controller in a number of different commercial applications ranging from a gelling agent and thickener in food industry (1) and as a rheology controller of the oil-well drilling and for enhancing well-bore recovery in oil industry (2) to a hydrophilic matrix for controlled release of drugs in pharmaceutical industry (3, 4).

Efforts devoted to incorporate lignin preparations into useful polymeric materials have started in 1944, but intensive work has been done in this field, especially in the last twenty years. There are numerous studies on the application of lignin – synthetic polymer blends (5) and very few studies on the application of lignin – synthetic polymer blends in order to obtain new materials (6). Lignin incorporation is expected to

lead to increased thermal stability, hydrophilicity, biocompatibility, biodegradability, antioxidant activity, etc., of different materials (7, 8, 9) and their use in the medical field.

Bisoprolol is a drug belonging to the group of beta blockers, a class of medicines used primarily in cardiovascular diseases. The U.S. Food and Drug Administration approved an application by Duramed Pharmaceutical for Zebeta Oral Tablets (Bisoprolol Fumarate) as a new molecular entity since1992 (10). It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic health system (11). Chemically, bisoprolol fumarate is 1-[4-[[2-(1-methylethoxy) ethoxy]-methyl]phenoxy]-3-[(1-methylethyl) amino]-2-propanol(E)-2butenedioate (12).

In this study the crosslinking of a xanthan and lignin mixture in the presence of the epichlorhydrine, leading to superabsorbent hydrogels with high swelling rate in aqueous mediums is discussed. Bisoprolol fumarate was loaded in these matrices followed by drug release in order to obtain information regarding their application as.

MATERIAL AND METHODS

Materials. The food grade xanthan gum (X) used in this study was purchased from CP Kelco – France, and used without any further purification. Epoxy modified ammonium lignosulfonate (LER) used for the study was obtained by reacting ammonium lignosulfonate with epichlorohydrine in 1/10 ratio, in the presence of a 35-40 wt% NaOH solution at 75 ^{o}C, for 5 hours (13). Bisoprolol fumarate (BF) – 100.07 % pure reference substance was purchased from Unichem Laboratories LTD, India.

Hydrogels preparation. Xanthan/ modified lignin hydrogels were prepared in various mixing ratios of the two polymers in the presence of NaOH and epichlorohydrine (EPCl) at 80°C. The hydrogels have been washed repeatedly with twice distilled water until neither lignin nor epichlorohydrine were detected in the washing waters by UV spectroscopy.

Swelling degree of xanthan/LER hydrogels. The swelling behavior of hydrogels was gravimetrically determined. The swelling degree was calculated using the following equation:

$$\%SD = [(m_t - m_0) / m_0] \cdot 100 \qquad (1)$$

where m_t is the mass of the swollen hydrogel at time t and

m_0 is the mass of dry hydrogel at time 0. The experiment was carried out in twice distilled water at room temperature.

Drug loading. The applicability of xanthan/lignin (X/LER) hydrogels as carriers for controlled delivery of active principles in pharmaceutical and medical fields was tested by using bisoprolol fumarate, a synthetic beta1-selective (cardioselective) adrenoceptor blocking agent. The incorporation of bisoprolol fumarate in the polymeric matrix was performed by immersing the sample in 50 mL twice distilled water solution with a drug concentration of 0.05 % at room temperature for three days. The bioactive compounds were then dried in the vacuum oven at a temperature of 60 ^{o}C for 24 h. Drug loading degree was estimated using an UV-Vis spectrometer (Cary 60 Spectrophotometer). The amount of active drug loaded in hydrogel matrices was determined according to the standard curve of bisoprolol fumarate absorbance in twice distilled water. Thus, the estimation of drug content was performed based on calibration curve of maximum absorption of bisoprolol fumarate, depending on drug concentration,

on UV-Vis range.

In vitro drug release. The *in vitro* dissolution profiles of the designed formulations were carried out using an Agilent 708–DS Dissolution Apparatus connected at UV-Vis Cary 60 Spectrophotometer. Each hydrogel specimen, with a mass of 0.018 g, was placed in basket support and suspended in 100 mL phosphate buffer pH. 7.4 (14, 15). Drug release tests were performed at 37°C and the stirring speed was 100 rpm.

RESULTS AND DISCUSSIONS

Swelling study

The water sorption kinetic curves of X/LER hydrogels (fig. 1) showed that water uptake is very fast in the first 5-6 minutes, after that all samples reaching equilibrium. The equilibrium swelling degree (the plateau of the curves) is considered the maximum swelling degree.

The slopes of the curves, which represent the swelling rate, were determined for the first 10 minutes by using the following equation:

$$dq/dt=Kq^n \qquad (2)$$

Both, the maximum swelling degree and the swelling rate are decreasing with the increase in lignin content (fig. 2, 3).

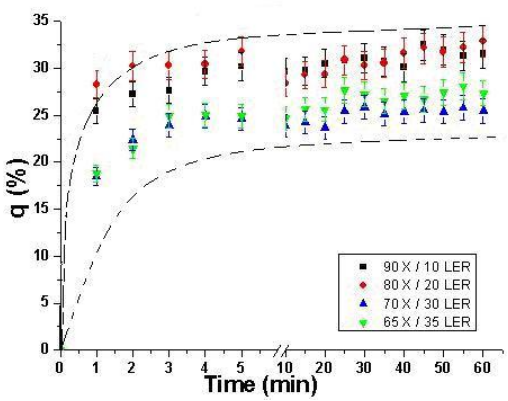

Fig. 1. Time variation of swelling degree for X/LER hydrogels

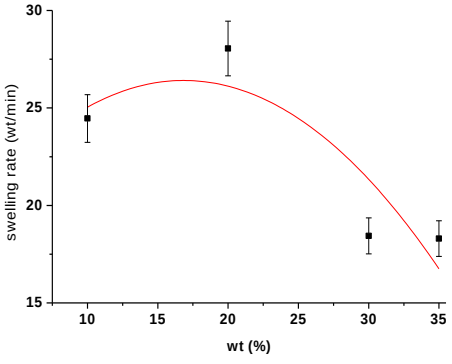

Fig. 2. The variation of the maximum swelling degree with lignin content

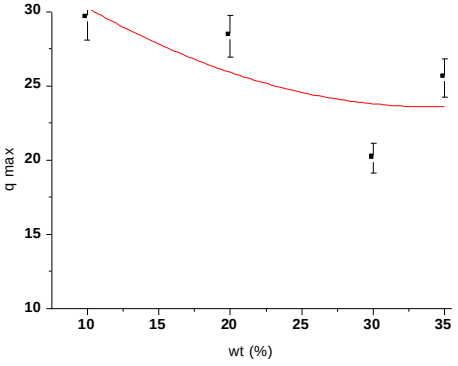

Fig. 3. The variation of the swelling rate with lignin content

In vitro drug load / release studies

The drug loading process of xanthan/lignin polymeric networks were found to be influenced by the composition of hydrogels (tab. I). An increase in the networks loading capacity with the decrease in xanthan content was found, with a maximum load capacity of 19.2 % recorded for 65 X/35 LER hydrogel.

Drug delivery profiles showed a fast release of approximately 60% (fig. 4) of the total drug content loaded in the polymeric network within the first 10 minutes followed by controlled drug release for 72 hrs. In the selected interval for the experiment there was no plateau, the release continuing after this time range. The experimental data related to the release of bisoprolol fumarate were processed to determine the kinetic parameters by the Korsmeyer - Peppas model (3), according to the equation:

$$Mt/M_0 = K \times t^n$$

where, M_t is the mass of the released drug at predetermined time (t), M_0 is the mass of the released drug at the end of the process, K is the rate constant and n is the release exponent used for the estimation of release mechanism.

TABLE I
Drug loading degree of hydrogels

Sample code	Drug loading degree determined by UV-Vis (%)
90X/10LER	14.4
80X/20LER	14.8
70X/30LER	16.4
65X/35LER	19.2

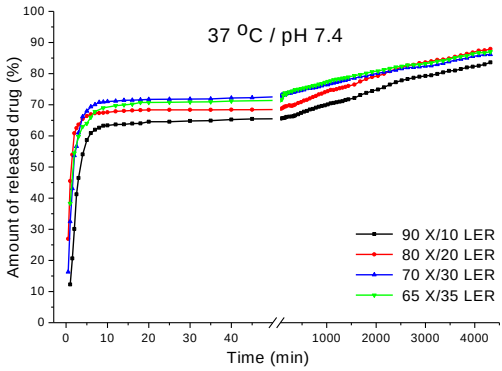

Fig. 4. Drug release profiles of BF from X/LER hydrogels with different composition

TABLE II
Drug release kinetic characteristics

Sample code	N	R^2_n	K, min $^{-n}$	R^2_k
90X/10LER	0.80	0.993	0.13	0.998
80X/20LER	0.42	0.999	0.45	0.999
70X/30LER	0.72	0.999	0.32	0.999
65X/35LER	0.41	0.97	0.39	0.97

n = the release exponent, R^2 = correlation factor, K = the rate constant

Table II shows that drug release kinetics of polymeric networks 90X/10LER and 70X/30LER respects an order of 0.5 - 1, indicating a non-Fickian diffusion behavior meaning that the diffusion rate is higher than the rate of relaxation in the polymer chains. Samples 80X/20LER and 65X/35LER present a drug release kinetics of the order

of 0.5 which indicates a Fickian diffusion behaviour where the drug release takes place by diffusion at a rate equal to the rate of relaxation of the polymer chains.

CONCLUSION

In this study the ability of xanthan with epoxy modified lignins in the presence of epichlorhydrine hydrogels to release *in vitro* bisoprolol fumarate in a controlled retarded manner. The ability of hydrogels to swell in water depends of their composition. The high swelling degree indicates their superabsorbent properties. The amount of drug loaded in polymer networks ranged from 14.4 % to 19.2 %, the maximum value being obtained for the hydrogels with the highest amount of lignin (LER). The release of bisoprolol fumarate from hydrogels was influenced by hydrogel composition. The X/LER-based hydrogels loaded with bisoprolol fumarate may be of interest for application in medical and pharmaceutical areas.

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NEWS

THE EVALUATION OF THE ANTIMICROBIAL SUSCEPTIBILITY AND MICROBIAL PROFILE OF SUBGINGIVAL BIOFILMS IN INDIVIDUALS WITH AGGRESSIVE PERIODONTITIS

The study realized by a group of Brazilian researchers aimed to evaluate the antimicrobial susceptibility and composition of subgingival biofilms in case of generalized aggressive periodontitis patients treated using mechanical/antimicrobial therapies (chlorhexidine, amoxicillin and metronidazole). Generalized aggressive periodontitis patients allocated to the placebo (C, n = 15) or test group (T, n = 16) received full-mouth disinfection with chlorhexidine, scaling and root planning, and systemic amoxicillin (500 mg)/ metronidazole (250 mg) or placebos. Subgingival plaque samples were obtained at baseline, 3, 6, 9 and 12 months post-therapy from 3-4 periodontal pockets, and the samples were pooled and cultivated under anaerobic conditions. The minimum inhibitory concentrations of amoxicillin, metronidazole and chlorhexidine were assessed using the microdilution method. Bacterial species present in the cultivated biofilm were identified by checkerboard DNA-DNA hybridization. At baseline, no differences in the minimum inhibitory concentrations between groups were observed for the 3 antimicrobials. In the T group, significant increases in the minimum inhibitory concentrations of chlorhexidine ($p < 0.05$) and amoxicillin ($p < 0.01$) were detected during the first 3 months; however, the minimum inhibitory concentrations of metronidazole decreased at 12 months ($p < 0.05$). For several species, the minimum inhibitory concentrations significantly changed over time in both groups, i.e., Streptococci the minimum inhibitory concentrations tended to increase, while for several periodontal pathogens, the minimum inhibitory concentrations diminished. A transitory increase in the minimum inhibitory concentrations of the subgingival biofilm to amoxicillin and chlorhexidine was observed in generalized aggressive periodontitis patients treated using enhanced mechanical therapy with topical chlorhexidine and systemic amoxicillin / metronidazole. Both protocols presented limited effects on the cultivable subgingival microbiota (Lourenço TG, Heller D, do Souto RM, Silva-Senem MX, Varela VM, Torres MC, Feres-Filho EJ, Colombo AP. Long-term evaluation of the antimicrobial susceptibility and microbial profile of subgingival biofilms in individuals with aggressive periodontitis. *Braz J Microbiol.* 2015 1;46 (2) :493-500).

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CANCER RESEARCH INVESTMENT: "TOP-DOWN" *VERSUS* BIBLIOMETRIC APPROACH

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CANCER RESEARCH INVESTMENT: "TOP-DOWN" VERSUS BIBLIOMETRIC APPROACH (Abstract): Cancer is a complex chronic disease with a real burden that goes beyond the individuals and their families to healthcare budgets and society. At the global level there are still many gaps in the knowledge on cancer research funding and activity. **The aim** of this paper is to present two approaches that can be used for the collection of data on cancer research investment. **Material and methods**: A scientific literature search was performed within the three databases (PubMed, Science Direct and OVID MEDICAL), using different combinations of some key words**. Results:** We identified 7 papers that used the "top-down" method and 4 that used the bibliometric approach for data collection on cancer research investment. Using the first method, usually the cancer research funding from public domain were cached, while using the second the overall expenditure (including indirect funding) could be estimated. **Conclusions**: The best approach for the collection of cancer research funding information is a combination of bibliometric with "top-down"/ "bottom-up" methods, which allows the assessment of oncological research activity and impact, but also the evaluation of the amount of money invested on cancer research from different perspectives. **Keywords**: CANCER, RESEARCH, FUNDING, BIBLIOMETRIC APPROACH, TOP-DOWN APPROACH.

Cancer is a complex chronic disease, which change forever the life of an individual affected by it. Also, cancer is one of the leading causes of death worldwide (1). The real burden of this disease goes beyond the individuals and their families to healthcare budgets and society.

With more than 14 billion euro global expenditure on oncological research (2) and an upward trend in the burden of this disease worldwide (3), cancer is one of the major global policy issues. Many financial resources were invested to study cancer initiation, etiology, diagnosis, prognosis, prevention, and treatment (4) and different methods were used to assess these expenses. But these financial resources are limited and all these methods have advantages and disadvantages. So, the correct allocation of the funds is very important. In order to help this

allocation of funds according to the burden of cancer in a specific population/ society, there is a need to obtain information on oncological research investment in different countries, regions, continents or worldwide (Europe, United States of America – USA, Canada, Australia and Asia). There are some examples of studies in the literature which provide such an analysis (4, 5, 7, 19, 20), but at the global level there are still many gaps in the knowledge on cancer research funding and activity.

To map the funding priorities in cancer research in order to correspond to the actual population/society needs is a major policy issue, but the collection of this data is usually hard (27).

The aim of this paper is to present two approaches that can be used for the collection of data on cancer research investment.

MATERIAL AND METHODS

We performed a scientific literature search within the three databases (PubMed, Science Direct and OVID MEDICAL), using different combinations of some key words: "cancer (or neoplasm or malignancy) AND research AND funding (or expenses or expenditure or costs)". Also, another search was performed using the following combinations: "cancer research funding in Europe"; "cancer research funding in Asia"; "cancer research funding in Australia"; "cancer research funding in Canada" and "cancer research funding in USA".

The total number of paper (including duplicates) obtained in PubMed was 20,957, in Science Direct was 87,560, and in OVID MEDICAL [Ovid MEDLINE (R) 1946 to February Week 4, 2015] was 5,617. After duplicate removal, the titles and abstracts of the remaining articles were evaluated against the following inclusion criteria: (1) published between 2005 and 2014; (2) published in English; (3) full text; (4) humans as subjects; (5) worldwide. A secondary literature search was performed using the references cited in the selected papers. A total of 22 articles were identified, for which the full-text version were obtained.

From these papers 7 used the "top-down" method and 4 used the bibliometric approach for data collection.

RESULTS

The scientific papers reporting on oncological research funding were conducted in the following geographical regions: Europe (2, 5, 6, 7, 8, 9, 10, 11, 12, 13), USA (4, 14, 15, 16), Australia (17, 18, 19), Canada (20, 21, 22) and Asia (23, 24).

The methods used to collect data on cancer research expenditure for the studies identified in the literature were: letters sent to the relevant Institutions/ Organizations (2, 5, 6, 7, 11, 16, 17, 18, 19) or to leading cancer researchers (2, 18, 19), interrogation of the most relevant databases regarding the data on cancer research papers (the outputs of cancer activity) (2, 5, 16, 24), information from institutional websites (where available) (2, 5, 6, 7, 11, 17, 18, 19) or interrogation of common databases regarding cancer research funding (2, 4, 11) (tab. I).

From all these methods the most used was the "top-down" approach (25, 26). This method involves a direct survey that collects data from organizations that fund cancer-related research or from organizations that administer cancer-related projects and programs (universities and hospitals). First step is the identification of these organizations. Then letters is sent to each institution and specific data related to can-

cer research expenditure are required (such as the name of funding organization, the name of the project, the name of the chief investigator, type of funding organizations, collaborators, source of funding, the amount of money spent by Common Scientific Outline – CSO – categories, and by cancer site) (7, 19). Besides, the researchers involved in the assessment of oncological research investments evaluate the amount of money spent by political group, by countries/territories, and by Gross Domestic Product (GDP)/ per capita. Where the information is incomplete or it is not provided, the chief investigators are contacted and asked for this data. Also, the websites are checked were available. Follow up letters are sent to the organizations which do not respond to the first request. Many attempts (letters, emails, and phone calls) are used to contact these institutions. The information has to be cross checked. If there are data provided in other currencies than the official currency used in the survey, they are converted within maximum two days of receipt of the information. Other type of currency conversion could be the usage of an average conversion rate over the calendar year. This approach avoids the involvement of variations in currency conversion rates as a confounder (4). A final verification letter could be sent to all organizations providing them with the figures for their institution as well as for the other institutions in the country and ask them for updates (7, 19). The response rate for "top-down" method is high (62-96%) compared with "bottom-up" approach, which means to directly contact the leading cancer researchers (27-42%) (2, 17, 19).

TABLE I

Methods used in the literature to collect data on cancer research investment

No.	Methods used in different studies to collect data on cancer research investment [authors / year of publication (reference)]				
	"Top-down" approach	**Bibliometric approach**	**"Bottom-up" approach**	**Web sites interrogation**	**Common databases interrogation**
1	Cancer Australia / 2014 (17)	Eckhouse S et al. / 2008 (2)	Eckhouse S et al. / 2008 (2)	Cancer Australia / 2014 (17)	Eckhouse S et al. / 2008 (2)
2	Eckhouse S et al. / 2008 (2)	Eckhouse S et al. / 2007 (5)	Shiraze N et al. / 2011 (18)	Eckhouse S et al. / 2008 (2)	International Cancer Research Partnership / 2012 (4)
3	Eckhouse S et al. / 2007 (5)	Gillum LA et al. / 2011 (16)	Shiraze N et al. / 2011 (19)	Eckhouse S et al. / 2007 (5)	Kanavos P / 2010 (11)
4	Eckhouse S, Sullivan R / 2006 (6)	Hong W, Dong E / 2014 (24)		Eckhouse S, Sullivan R / 2006 (6)	
5	Eckhouse S et al. / 2005 (7)			Eckhouse S et al. / 2005 (7)	
6	Gillum LA et al. / 2011 (16)			Shiraze N et al. / 2011 (18)	
7	Kanavos P / 2010 (11)			Shiraze N et al. / 2011 (19)	
8	Shiraze N et al. / 2011 (18)				
9	Shiraze N et al. / 2011 (19)				

Using the "top-down" approach, Eckhouse et al. showed that in 2004 the non-commercial funding organizations spent €1.97 billion (on direct funding of cancer research) in Europe and €5.15 billion in USA. Besides, there were €1.36 billion in Europe and €0.10 billion in USA from national healthcare systems and universities ("hidden" expenses or indirect funding). Also, there were €3.09 billion from Pharmaceutical Industry (direct spending) worldwide contributing to public domain (not all companies). So, in 2004 a total of €11.67 billion were invested from public domain on cancer research (2, 5).

The bibliometric method for the assessment of cancer research investment was taken into account for the evaluation of direct (2, 5, 16, 24) or indirect costs (2, 5).

There are many ways of using the bibliometric approach. First, it could be evaluated the total number of scientific articles on cancer, over a specific period. The relevant databases are searched (ISI Thomson Web of Science, PubMed, Google scholar etc.) using specific filters / queries (2, 5, 24, 27). There is the possibility to select the topic, the language, the type of documents, the timespan and other settings (*eg*. Citation Indexes in the case of ISI Thomson Web of Science). There are Boolean operators, parentheses, field tags, and query sets available to help the researcher with the creation of his own query. When the final results were obtained, an Excel database could be used to store the bibliometrical details of each scientific article. The number of papers related to a country / region / organization could be evaluated using two methods of counting: the integer counting (which means that a paper is counted as unity for each country / institution appeared among the addresses) or fractional counting (which means that the researcher have to divided the number of addresses for a particular country / institution by the total number of addresses identified in the article) (28). The analysis could provide data on the type of research (basic/ clinical), the type of funding organization, the type of collaboration (national/ international) and regarding the values by CSO or by cancer site. Second, the quality of cancer research outputs could be evaluated using the impact factor of the journals which published the scientific paper (24) (the scientific value of the journal in which were published the outputs) or the actual citation impact, defined as the number of citations received by a specific article in the next 5 years after its publication (28) (the scientific value of the papers itself). Also, the potential citation impact could be used in this respect. The last indicator is calculated as the number of citations expected to be received by an individual paper, considering that is cited with the average frequency for papers of that journal in that year (28).

Some authors evaluated the number of research papers on cancer over a specific period (usually a 10-year period) in a specific country / region (2, 5, 24), others provided a deeper analysis and calculated the impact of the outputs on cancer research or estimated the amount of money spent on oncological research (using the number of research outputs identified for a specific country / region / institution and the mean cost per paper (2, 5, 27).

Using the bibliometric approach was shown that the Global Public Sector cancer research expenditure was around €11.03 billion, from which: USA = €5.27 billion, Europe = €3.33 billion, Japan = €1.00 billion, Canada = €0.27 billion, Australia =

€0.16 billion and the Rest of the World = €0.98 billion. The major funder for oncological research were the Government (€4,712 million), followed by Charity (€456 million) and healthcare systems and universities (€109 million) in the USA, whilst the universities and healthcare systems (€1,364 million), followed by Government (€992 million), and, Charity (€872 million) were the relevant funders in EU (5). Although, the USA invested a higher amount of money in cancer research compared to Europe (5), these regions were comparable when analyzed their overall oncological research productivity as a volume of publications (2).

In China, the scientific papers resulting from breast cancer research followed an upward trend over the period of 2004-2013. Although, the number of articles from China rose from 267 in 2004 to 3,414 in 2013, was still far behind the USA (with more than 7,000 papers), but well in advance compared with other countries such as Japan (1,030), England (1,157) or Germany (1,196) (24).

DISCUSSION

The bibliometric method is a relatively new tool used for the assessment of cancer research funding priorities (2, 27, 28).

The expenditure evaluated using the bibliometric approach was 41% greater in Europe than the amount of money found using the top-down method (directly reported funds) combined with other methods, except the bibliometric one, and 2% greater in the USA (5). Over a 10-year period, from 1994 to 2003, when the assessment was done in absolute terms (% of total number of publications), European cancer research activity was dominated by Germany (9.6%), UK (9.1%), Italy (7.3%), and France (6.79%), followed by Netherlands (3.6%) and Sweden (2.5%), but when the analysis taken into consideration the number of papers / billion euro GDP, Sweden (2.2), Greece and Netherlands (2.1 each) had the greatest outputs (2, 5).

The difference between data collected using the bibliometric approach (national estimation) and "top-down" methods (directly reported investments) in Europe resulted from the funds added by the universities and healthcare services (money spent for infrastructure), so called indirect costs (5).

The "top-down" approach is a widely used method for the data collection on cancer research funding, with the advantage of providing directly reported information, usually with high accuracy. Generally, this method catches data on public domain. But, there are some aspects that could be considered as disadvantages. First, in some cases there is a need for many follow-up letters, phones, and emails to get the expected percentage of answers. So, sometimes the rate response could be too low. Second, the rate response from private organizations could be even lower.

The number of articles published is considered as a good measure of oncological research efforts. Vanderelst D et al. showed that the volume of publications represents a valid estimate of the magnitude of research funding (27). So, the bibliometric approach is a reliable way to collect information on cancer research investment. Even more, some authors found that the number of published articles was correlated with the burden of a disease on the population / society (Swingler G et al., 2003, cited by Vanderelst D et al.) (27). Cancer research scientific papers are one of the major outputs of investment and an

objective surrogate of the global cancer research activity and impact (2, 28). Besides, the scientific productivity in specific areas of research (including cancer) could stimulate further interest from researchers and the funding organizations and could support some the targeted investments (16). This method is useful when there is a short period of time available for the estimation of oncological research expenditure. However, the analysis of cancer research investment in Far East (especially China and India), but also in Latin America and Russia, is difficult because the names of many authors are the same and an important percent of these papers are published in other languages and national journals that do not appear in the most relevant databases such as ISI Thomson Web of Science, PubMed, Google Scholar and so on, which are English-databases (2). Also, the bibliometric approach provides estimated values.

CONCLUSIONS

1. There are many different methods that could be taken into account for data collection on oncological research investment, with advantages, but also with disadvantages.

2. The "top-down" approach is a widely used method for the assessment of cancer research expenses, especially from public domain, which provides directly reported data, but sometimes at a lower response rate.

3. The bibliometric approach is a relatively new tool for the evaluation of cancer research funding, but also on the impact of this activity. This method helps the setting of cancer research funding priorities and the appropriate financial resources allocation, according to the actual population/society needs.

4. The best approach for the collection of cancer research funding information is a combination of bibliometric with "top-down" / "bottom-up" methods, which allows the assessment of oncological research productivity and scientific impact, but also the evaluation of the amount of money invested on cancer research from different perspectives.

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STUDY ON HEALTH PROFESSIONALS' PERCEPTION OF QUALITY OF HEALTHCARE PROVIDED TO PATIENTS

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STUDY ON HEALTH PROFESSIONALS' PERCEPTION OF QUALITY OF HEALTHCARE PROVIDED TO PATIENTS (Abstract). The quality of the health care system is an essential condition for in improving the quality of health services and satisfying both the patients and healthcare professionals. **Research objectives**: To identify the characteristics of medical profession, the factors of professional satisfaction or dissatisfaction and health care team-related factors that influence the quality of care. **Material and methods**: Qualitative study using a SurveyMonkey online questionnaire consisting of 14 open-ended questions. The respondents were 1013 health professionals in university and non-university hospitals with various unit profiles. **Results**: According to healthcare professionals, medical profession is defined by: humanism and personal sacrifice (33.37%) and also high professional competence (33.07%). Satisfaction factors are: patient health (40.57%), high social status (36.33) and saved lives (33.07%). Dissatisfaction may be due to: low salary (39.98%), disorganization (38.10%) and lack of procedures (33.96%). Performance may decrease as a result of: stressful working conditions, lack of adequate medical supplies, lack of recognition at work, routine, and strained relations with superiors. **Conclusions**: The study shows the need for correlating the medical education system and health systems, and for an integrated analysis of both systems by determining the required skills and modeling the medical team behavior in accordance with the performance variables which take into account satisfaction among both patients and health care professionals. **Keywords:** PROFESSIONAL, PERCEPTION, QUALITY, HEALTHCARE.

Every healthcare professional knows that the needs of patients vary from individual to individual. Also, patient safety and quality of care make a difference for patients and their families (1). Thus, on the one hand, healthcare professionals use their knowledge and skills, and on the other hand their experience, intuition and creativity to meet these needs. Health professionals must know that the quality of care is assessed by outcome measurement (2). Implementation of a monitoring system is essential for a successful quality improvement program. Even with limited resources systematic methods can be used to constantly monitor the quality of care. Quantifying the effect that nurses and nursing interventions have on the quality of care processes, and on patient outcomes, has become increasingly important to support

evidence-based staffing plans, understand the impact of nursing shortages and optimize care outcomes" (3, 4).

Monitoring the quality of care is the regular collection and analysis of a set of quality indicators, and is frequently directed toward the measurement and analysis of processes. This process allows health care providers to assess whether the program or its performance targets are met (5, 6).

Aim of the study: To improve health system performance in accordance with the elements identified by assessing the degree of satisfaction of both patients and healthcare professionals.

Research objectives: To identify the significant characteristics of medical profession, factors of job satisfaction or dissatisfaction, and health care team-related factors that influence the quality of care.

MATERIAL AND METHODS

The qualitative survey research was conducted by using SurveyMonkey online questionnaire consisting of 14 open-ended questions. It has been applied to a number of 1013 health professionals in hospitals from 6 university centers in Romania: Iasi, Bucharest, Cluj-Napoca, Târgu Mures, Timiş oara, Craiova and non-university hospitals in 9 cities. The profile of these hospitals was varied: obstetrics and gynecology, neurology and psychiatry, lung disease, pediatrics; medical rehabilitation; infectious diseases or general hospitals (emergency hospitals). Their inclusion in the study was possible after obtaining the agreement of hospital managers, doctors or other healthcare professionals.

The questionnaires included open-ended questions aimed at identifying the degree of satisfaction of responders. Thus, 10 important issues were addressed: delivered health care, humanism reflected in treatment, absence of pain, prompt response to all requests and needs. Respondents' opinions on dissatisfaction factors that have bothered them most were expressed about the 30 possible factors that contribute to a disturbing degree of dissatisfaction during hospitalization. The non-response rate/refusal rate was 0.1%, not influencing significantly the quality of results. Data were processed according to the studied qualitative variables for a statistical significance of the results with 95% confidence interval.

RESULTS

Description of study population

The analysis of study group composition showed that respondents were people with higher education. According to the variable "age" the group showed a relatively homogeneous distribution, consisting of 149 persons under 30 years (14.7%), 207 persons aged 30-35 years (20.4%), 218 aged 35-45 years (21.5%), 287 people aged 45-50 years (28.3%) and 152 people aged over 50 years (15%). According to "length of service" 37.8% had more than 20 years professional experience; 25% of 10-20 years; 22.2% between 5-10 years, and a relatively small percentage (14.7%) had worked in the healthcare system less than 5 years.

The results obtained after processing the responses to the questions reveal the following:

1. "What are the three main characteristic features defining the medical profession?" Responses revealed that an equal number of physicians, 338 (33.4%) believe that sacrifice and humanity, respectively are defining characteristics of their profession. Other responses were: competence (335/33.1%), dedication and thorough theoretical knowledge (0.1% each).

Dedication and knowledge were for 170 physicians each (16.8%) the basic characteristic features of medical profession, while 16.7% each opted for talent and perfection. Similar percentages were obtained for responsibility and self-control. Respect and rigor were preferred by 170 respondents each (16.8%) and experience and intelligence by 169 respondents each (16.7%). Work speed was selected by 168 respondents (16.6%) as an essential condition that must be met in order to practice medicine. In conclusion, according to our respondents he 3 main characteristic features of medical profession are: **humanism and sacrifice** (33.37% each), and **competence** (33.07%).

2. "What are the three factors of satisfaction with medical profession?" The results show that: the highest percentage of responders (40.57%) considered patient health as the most important factor impacting professional satisfaction; other responses were: saving lives (33.1%); social status (265 respondents (26.2%); freedom in decision making (1respondent/0.1%). Patient gratitude is considered by 332 physicians (32.8%), as the main factor contributing to professional fulfillment and satisfaction, while the same is true for respect in the community according to 289 physicians (28.5%). 19.9% of respondents find professional satisfaction in their status, and 9.9% believe that nothing can please them more professionally than one recovered patient. Synthesizing the answers it can be concluded that there are 3 primordial factors of professional satisfaction: **patient health** (40.57%), high social status (36.33) and **saved lives** (33.07%).

3. "What are the three main factors of dissatisfaction in the medical profession?" The results showed that: for the highest percentage (40.0%) of respondents the main reason for professional dissatisfaction is related to low salary, while 34.6% attribute it to physical wear and 25.2% to lack of respect. Another factor responsible for the lack of satisfaction is the absence of procedures, according to a number of 344 physicians (34.0%). A percentage of 26.7 believe that lack of discipline leads to professional dissatisfaction, and 21.4% attribute it to lack of culture. "Insufficient funds to equip the hospital" is the reason for professional dissatisfaction for a number of 98 physicians (9.7%). One physician (0.1%) considers denigration as the key factor of professional failure to which the lack of professional recognition and community appreciation is added. The lack of organization in the health system is a factor that causes professional dissatisfaction in the opinion of a number of 386 physicians (38.1%). Interpretable legislation is another plausible cause of achieving the level of professional dissatisfaction for 252 physicians (24.9%). After analyzing all responses it can be concluded that the main factors of professional dissatisfaction are: **low salary** (39.98%), **lack of organization** (38.10%) and the **absence of procedures** (33.96%).

4. "Sorting by relevance the factors related to patient care". The analysis of a first group of factors (group 1) aimed to determine the importance of: equipment, medical resources, prompt response to patient requests or needs, patience, and kindness. The variable "equipment" was ranked second among the factors related to patient care by 71.08% of responders, third by 6.52%, fourth by 6.91%, sixth by 0.10%, seventh by 8.39, and eighth by 7.01%. "Medical resources" (medical supplies) were ranked second by 0.10% of the respondents and fourth by 8.49%, while the

highest percentage of responders (61.8%) ranked it ninth. The variable "patience" was ranked: first by 6.42%, second by 6.92%, third by 8.39%, while most respondents (66.54%) ranked it ninth. According to 0.10% of the respondents "kindness" is the third most important factor related to patient care, being ranked sixth by 8.39%, seventh by 7.01%, eighth by 6.91% and tenth by 77.59% of the respondents. In the second group of factors related to patient care (group 2) the following variables were analyzed: competence, hospital environment, empathy, counseling time allocated to each patient, nurse dedication, responsiveness and patient attitude. The synthesis of results showed the following ranking in order of importance: "competence" (71.27% of respondents), "equipment" (71.08%), "counseling time allocated to each patient (51.53%), "responsiveness and patient attitude" (55.97%), "nurse dedication" (44.82%), "hospital environment" (51.04%), "empathy" (51.04%), "prompt response to the patient requests and needs" (51.04%), "patience" (66.54%), "kindness"(77.59%), "medical resources" (61.8%). According to the cumulative summary of respondents' opinion on issues that can diminish work performance at work, these were in order of importance the stressful working conditions, lack of adequate medical resources, lack of recognition, routine and strained relations with superiors (tab. I).

DISCUSSION

The responses of the study subjects related to ranking the factors influencing the quality of patient care revealed that a significant percentage of respondents (70%) ranked the variables "competence" and "equipment" first and second, and over half of respondents considered the variables "counseling time allocated to each patient" and "responsiveness and patient attitude. A significant proportion of respondents (60%) believe that the following variables influence the quality of care to a lesser extent: "patience", "kindness" and "medical resources (medical supplies)". As to respondents' opinion on issues that may negatively influence job performance, over 50% of respondents ranked first the variable "stressful working conditions" and about 40% also considered important the variable "lack of appropriate medical resources ", ranking it second (out of nine). In a performance health system the most important variables are "competence" and "equipment", variables depending on the education system and the level of funding (7). A highly trained staff cannot compensate for lack of funding and modern equipment, but the latter cannot replace professional competence (8).

CONCLUSIONS

1. Our research demonstrates the need for correlating the education and health systems in view of establishing the knowledge and skills required for modeling the medical team behavior in accordance with patient satisfaction.

2. An organizational culture that creates an environment based on respect, kindness, courtesy, recognition for a job well done has to be developed.

3. Based on the obtained results the introduction into educational curricula in Romania of research directions aimed at developing behavioral and ethical practices specific to health professional-patient relationship, knowledge and respect of patient rights, and increased resistance to demanding work is recommended.

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NEWS

EU RECOMMENDS APPROVAL OF *GARDASIL 9* VACCINE

The European Medicines Agency (EMA) has recommended approval for the new vaccine *Gardasil 9*, which provide protection against the infection caused by nine serotypes of *Human Papillomavirus* (HPV) – 6, 11, 16 and 18 (from the earlier version of the vaccine), and other five - 31, 33, 45, 52, 58, which are new. The latter HPV types are responsible for around 1 in 5 cases of cervical cancer. EMA highlights on the implications of the infections with HPV: about 100% of cervical cancers, 90% of anal cancers, 70% of vaginal cancers, and 15% of vulvar cancers; also, certain types of HPV are linked to mouth and throat malignancies. The indications of this new vaccine include: 1. girls and young women, between 9 and 26 years (it offer protection for cervical, vulvar, vaginal and anal cancers caused by HPV types 16, 18, 31, 33, 45, 52, 58; genital warts produced by HPV types 6 and 11; and other dysplastic lesions of the cervix, vulva, vagina and anus, caused by these all HPV serotypes). 2. boys, between 9 and 15 years (to prevent anal cancer caused by oncogenic HPV types 16, 18, 31, 33, 45, 52, 58; genital warts caused by the benign HPV types 6 and 11; and anal intraepithelial neoplasia). The EMA recommendation is based primarily on four studies that analysed the efficacy of *Gardasil 9* for the protection against the infections caused by HPV types 31, 33, 45, 52 and 58, but also assessed the protection against the HPV types 6, 11, 16, 18, compared with the first variant of the vaccine, *Gardasil* (which was authorized for use in the European Union since 2006). One of these studies was conducted on 14,000 young women, between 16 and 26 years, who were negative for vaccine HPV types at the beginning of the study. The results showed that *Gardasil 9* was 97% effective in preventing cervical, vulvar and vaginal cancers caused by the additional HPV types (31, 33, 45, 52 and 58) and had similar efficacy as *Gardasil* against 6, 11, 16 and 18 (on the basis of similar antibody responses). The EMA notes that the safety of *Gardasil 9* was evaluated in more than 23,000 people included in seven clinical trials. The most frequent side effects were injection site pain, swelling, redness and headaches. *Gardasil 9* is already approved by US Food and Drug Administration for marketing in the USA (since the end of the last year) (Nelson R. EU Recommends Approval of Gardasil 9 Vaccine. *Medscape*. March 27, 2015).

Mioara Matei

QUALITY OF LIFE FOR AMBLYOPIC CHILDREN AND THEIR PARENTS

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QUALITY OF LIFE OF AMBLYOPIC CHILDREN AND THEIR PARENTS (Abstract). **Aim:** To highlight the changes in the quality of life in patients with anisometric, refractive, strabic and mixed amblyopia and to assess the psychosocial implication for their family. **Material and methods:** Prospective observational transversal study of 44 ambulatory patients (88 eyes) treated in the Ophthalmology Clinic of Iaşi "Sf. Spiridon" Hospital and Iaşi Stereopsis Ophthalmology Clinic. Clinical parameters: visual acuity, objective refraction (autorefractometry), optical correction type, stage of binocular vision, ocular motility, strabic deviation. The patients or their attendants accepted to fill out a questionnaire comprising 46 items pertaining to 9 domains to assess patient (or parents) adherence to treatment and issues related to quality of life. **Results:** Mean age of study patients was 8.19±2.92 years (range 4-16 years) and age at first diagnosis was: 3.90±2.15 years. Moderate and severe amblyopia in the right eye was present in 47.72% of cases and in the left eye in 43.18%. Right eye uncorrected visual acuity was 0.45±0.31 and corrected 0.63±0.31 ($p<0.0001$). Left eye uncorrected visual acuity was 0.49±0.33 and corrected was 0.69±0.32 ($p<0.0001$). No statistically significant difference between the right and left eye were found. A 46-item questionnaire focused on child health, visual acuity, binocular vision, time to occlusion or penalization, child emotions/activity, family and society integration was used. **Conclusions:** The first presentation to the ophthalmologist was at age 3.9091±2.1597 years (range 3 months-7 years). Health promotion and education is important to determine parents to seek help from an ophthalmologist not later than age 3. The main symptoms that led the parents to seek eye care were: strabic deviation – 45.45%, difficulty seeing the blackboard – 11.36%, and difficulty in seeing closer – 20.45%. Quality life questionnaires detect life or school problems of children with amblyopia and educate parents. In 15,91% of parents they did not knew that before treatment the child had poor school results. This study represents the first step in Romanian ophthalmologists concern for socio-professional integration of children with amblyopia, because as we know some jobs require healthy eyes and for those who do not receive appropriate treatment their dream will be denied. **Keywords:** AMBLYOPIA, QUESTIONNAIRE, QUALITY OF LIFE.

The word amblyopia is derived from the Greek amblyos which means "blunt vision" (1) and the first description was made by Hippocrates in 400 BC. Amblyopia is an important condition that can affect up to 5% of the general population (2,3). Many people with amblyopia, especially those who only have a mild form, are not even aware they have the condition until tested at older ages, since the vision in their

stronger eye is normal. However, people who have severe amblyopia may experience related visual disorders, most notably poor depth. Amblyopic patients may suffer from poor spatial acuity or low sensitivity to contrast (4).

The health-related quality of life (HRQoL) implications of amblyopia are specifically related to amblyopia treatment, impact on family life, social interactions, difficulties in undertaking daily activities, as well as feelings and behavior (5,6). The impact of amblyopia on HRQoL has not been adequately explored (7). Existing patient reported outcome (PRO) measures for amblyopia do not meet current recommendations for the methods adopted during their development, or the way in which the instruments are administered. One study was to produce a self-complete PRO instrument for amblyopia for children aged 4–7 years that uses children's responses in the development phase (8).

Currently in Romania there is high interest in preventing amblyopia but there are no studies on the quality of life of amblyopic children. A study on the quality of file in glaucoma patients has been published (9).The aim of this study was to produce a pediatric disease-specific HRQoL instrument for amblyopic children to be used in research or routine clinical practice.

MATERIAL AND METHOS

This is a prospective observational transversal study of 44 patients examined and followed-up in the outpatient Ophthalmology Clinic of the Iasi l "Sf. Spiridon" Hospital and Iaşi Stereopsis Ophthamology Clinic over a 12-month period (January – December 2013). The mean age of children with amblyopia was 8.1932±2.9278 years (range 4-16 years). It must be noted that most of the patients were aged 5 to 10 years at the time of the survey, which corresponds to peak detection of amblyopia cases. The mean age of children at the first presentation to the ophthalmologist (which in most cases coincided with the date of the questionnaire) was 3.9091±2.1597 years (range 3 months-7 years). This age was lower in children with highly educated parents. The mean age at the time of diagnosis was 3.9091±2.1597 years (range 1- 7 years).

Parents/tutors of selected children diagnosed with amblyopia were asked to fill out a 46 item questionnaire aimed at measuring the quality of life of parents and especially of children with amblyopia. The clinical parameters recorded during the first examination were: visual acuity (uncorrected and corrected), objective refraction, type of optical correction, binocular vision, ocular motility. Data were statistically processed using Student t test, correlation coefficient – r and p – value.

RESULTS

The following refractive and ocular changes were found: ocular deviation (mainly esotropia) in 45.45% of children, refractive amblyopia in 37% and amblyopia in 14% of cases. Anisometropia was the causes in 13.63% of cases (most commonly hyperopic or myopic in two cases), percentage higher than recent estimates in the literature.

In 93.18% of patients a treatment had been previously recommended by an ophthalmologist. It consisted of optical correction (spectacles or contact lenses) in 88.64%, surgical treatment was performed in 11.36% of cases, and occlusion (covering of the dominant eye) was used in 63.64% of cases. More than half of the

cases associated two or three types of treatment. As to treatment by optical correction (fig.1a and b), in the right eyes (RE) we found: hyperopia in 14.77% of cases (7 cases with mild hyperopia and 5 cases with moderate hyperopia), only 2.27% presented moderate myopia and 25% had mixed astigmatism, mean spherical equivalent = 2.9286±1.4772 diopters (range 0.75-6.25).

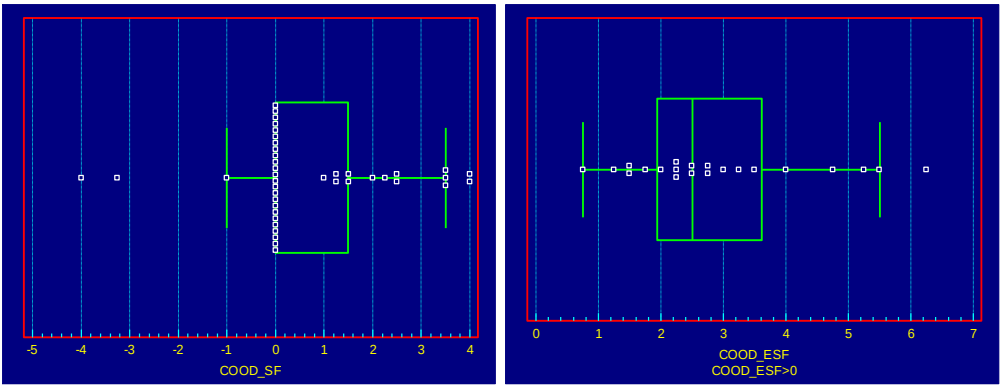

Fig. 1. a and b. Dioptric range of optical correction - RE

In the left eye (LE) we observed (fig. 2a and b) hyperopia in 17.04% of cases: 7.95% mild hyperopia and 9.09% moderate hyperopia (range 3.5 – 6 diopters). In 2.27% of cases we found mild myopia (-1.25) and moderate myopia (-5.25) and 21.59% had mixed astigmatism, mean spherical equivalent 2.9079±1.5097 (range 0.75- 6.5 diopters).

Refractive amblyopia caused by the presence of anisometropia was present in 6.81% of the eyes (tab. I).

There were cases that had more than 2 diopters of cylinder. Mean right eye (RE) uncorrected visual acuity (VA) was 0.4516±0.3104 (range 0.02-1), and the corrected one (with spectacles or contact lenses) 0.6334±0.3161, range 0.08-1 (correlation coefficient r=0.9195 and p <0.0001- statistically significant) (fig. 3).

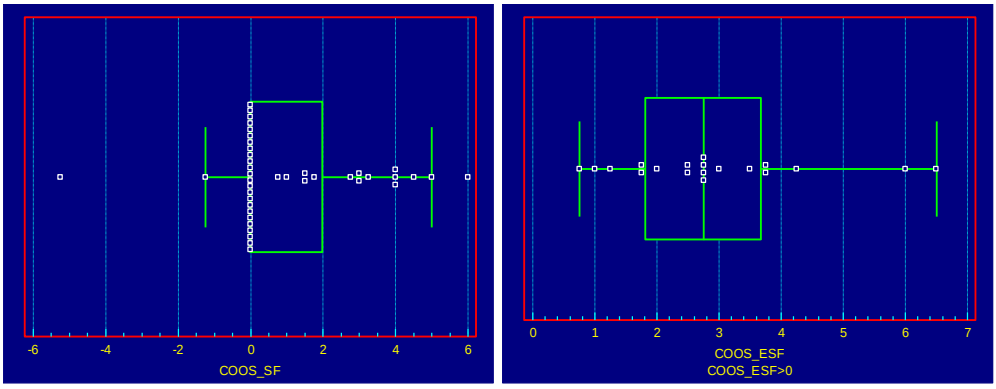

Fig. 2. a and b. Dioptric range of optical correction - LE

TABLE I

Distribution of objective refraction

OCRE/COLE	2,25 Eqsf	5,25 qEsf	-3,25 sf	-4 sf	+1,25 sf	+2,5 sf
2,75 Esf	0	1	0	0	0	0
3,5 Esf	1	0	0	0	0	0
- 5,25 sf	0	0	1	0	0	0
0	0	0	0	1	0	0
+3,25 sf	0	0	0	0	1	0
+4 sf	0	0	0	0	0	1

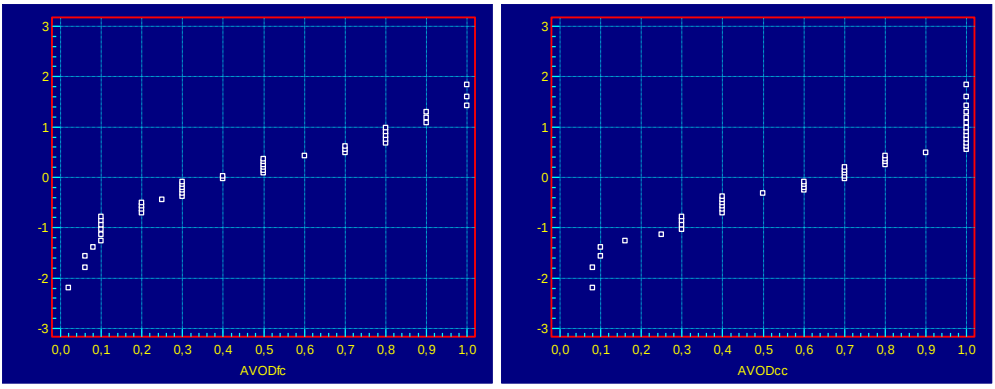

Fig. 3. RE visual acuity without and with correction

In the left eye (LE) mean uncorrected and corrected visual acuity were (fig. 4): 0.4909±0.3378 (range 0.00041-1) and 0.6977±0.3225 (range 0.0004-1), respectively (correlation coefficient r=0.8701, p <0.0001- statistically significant).

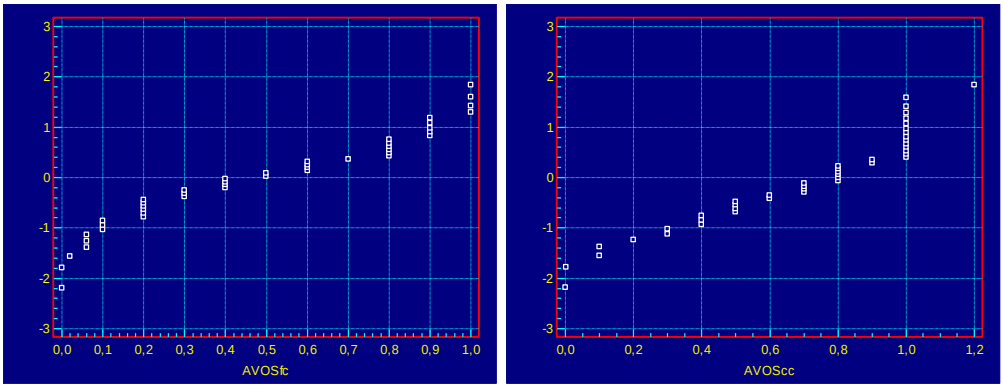

Fig. 4. LE Visual acuity without and with correction

According to the information obtained following the processing of data collected from the questionnaire administered to parents, the main symptoms for which parents sought eye care were: strabic deviation – 45.45%, difficulty seeing the blackboard – 11.36%, and difficulty in seeing closer - 20,45%. Of the parents who filled

out the quality of life questionnaire of their child with amblyopia, most were females (56%), age range 21-50 years (70.45% aged 31 to 50 years). Parental level of education: the majority completed high school (34.09%) or academic education (31.82%). The questions were answered by parents if they spent most of their time with their child (50% of parents) or a family member (grandparents, aunts).

Responses to the questionnaire revealed the following: most parents believed that their child health was very good, binocular VA was good (even though VA was very low in one eye), visual acuity of the good eye was reasonable. Most caregivers said they were concerned with child's vision and eye health spending long time with him/her most weekdays at the expense of time spent with their own family, in other words a form of sacrifice for the greater good. In relation to social life, the children were generally happy and enjoyed the attention received from the caring parents. They tend to bond easily and prefer to play with friends. However in some cases children are excessively shy or nervous, becoming more sociable and calmer after simple optical correction.

Regarding basic daily activities, most parents believe that visual impairment does not affect the ability of their child to recognize faces from a distance, can dress themselves, wash themselves, can ride a bicycle and read their watch or cell phone, can dial a number and manage drinking with a cup. When asked whether the child was in an isolated community, some said that the children remained indoors using computer and watching TV.

As observed from the responses to the questionnaire, children loved to read but it was difficult for them to find certain object in dark places, and commitment to treatment was not so good due to the child disliking it or forgetful or busy caregivers. Before treatment 84.09% of parents were aware that the child had poor school results.

Family support is important as it is related to treatment compliance. Amblyopia therapy is influenced by both the child and the parent/guardian. Whilst the child may object to the wearing of glasses or a patch on a personal level, a parent's perspectives can influence the success of such treatment.

DISCUSSION

Some Quality of Life instruments used in the identified studies were derived from consultations with ophthalmic professionals and/or parents of children with amblyopia. Therefore, the items included in the instrument design, are deemed to be of importance from an adult perspective (10). The included items may be of importance to adults but not necessarily to the child. For example, a parent may feel that educational attainment and the ability to see well at school is of great importance; however, this view may not be shared by the child. In some of the identified studies, the reported findings are taken from a parental perspective (5).

It is not possible to state that the impact of amblyopia treatment felt by the child is the same as that perceived by adults on how, or what the child should feel or experience. Some of the questions asked included how well the child could see whilst undertaking treatment. The parent/guardian cannot directly assess this; they can only make a judgment on how they perceive the child is able to see whilst on treatment. Their judgment could be influenced by how important they judge the activity to be (such as school work or interacting with friends).

Treatment compliance in amblyopia therapy is influenced by both the child and the parent/guardian. Whilst the child may object to the wearing of glasses or a patch on a personal level, a parent's perspectives can influence the success of such treatment. This may incorporate their own experiences or impressions of patching/glasses-wear, or their understanding of the condition and the importance of treatment. Parental understanding of the condition and belief in the prescribed treatment are key components for good treatment compliance (11). However, parents can be well-informed and positive, yet compliance may still be poor. Another argument against using treatment compliance as a measure of quality of life is that a child may consent to wearing the patch but their daily activities and social interactions may still be affected.

Amblyopia it is commonly treated with patching of the nonamblyopic eye, preferably before the age of 6. Approximately 1/3 of the affected children do not reach visual acuity of 6/12 in the amblyopic eye, thereby increasing the risk of bilateral visual impairment due to loss of vision in the nonamblyopic eye and decreasing quality of life in adulthood. A lot of studies have suggested that one of the factors influencing outcome with treatment is the level of compliance (12-22). Low compliance was found to be associated with social deprivation (23) and lower attendance rates were reported among amblyopic patients from more deprived areas (24).

CONCLUSIONS

1. The first presentation to the ophthalmologist was at age 3.9091±2.1597 years (range 3 months -7 years). The sooner the amblyopia is diagnosed and treated, the better the results are regardless of amblyopia type. Health education for seeking attention from an ophthalmologist not later than age 3 is needed.

2. The main symptoms that determined the parents to seek eye care were: strabic deviation (45.45%), difficulty seeing the blackboard (11.36%), and difficulty in seeing closer (20.45%). It is difficult for a child to integrate into school and community but sometimes only after optical correction, the situation improves being reflected in his results.

3. Most parents believe that child health is very good, binocular VA is good (despite a very low VA in one eye), and VA of the good eye is reasonable. Quality life questionnaires detect life or school problems of children with amblyopia and educate parents. In 15.91% of parents they did not knew that before treatment the child had poor school results.

4. This study work represents a first step in Romanian ophthalmologists concern for socio-professional integration of children with amblyopia, because as we know some jobs require healthy eyes and for those who do not receive an appropriate treatment at a young age their dream will be denied.

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A POSSIBILITY TO IDENTIFY THE VEHICLE DRIVER THROUGH COMPLEX FORENSIC AND CRIMINALISTIC EXPERTISE – CASE REPORT

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A POSSIBILITY TO IDENTIFY THE VEHICLE DRIVER THROUGH COMPLEX FORENSIC AND CRIMINALISTIC EXPERTISE – CASE REPORT (Abstract): Traffic accidents can have more or less dramatic consequences that involve penal and civil responsibility with amplitude extending over long periods of time. In many cases, substitution of the driver with the passenger in order to avoid criminal responsibility is often remarked. The substitution takes place with the passenger's agreement or, in cases with dramatic consequences (coma or death), without his/her consent. These situations are encountered in civil cases regarding insurance fraud. In addition to forensic medical expertise, to aid the experts, mathematical modeling and computer simulation of the dynamics of vehicle passengers is a tool that completes the criminal expertise of traffic accidents. This paper presents the method of identification of the person driving the vehicle based on the computer simulation of vehicle occupants' dynamics. **Keywords**: EXPERTISE, TRAFFIC ACCIDENTS, COMPUTER SIMULATIONS

Identification of the vehicle driver is usually accomplished by correlating the injury panel with the vehicle marks (1). These marks can be shape marks, trace substances, biological traces, imprints, etc.

In some cases, individuals assume the fault for an accident without considering long-term consequences. After the case shapes towards a solution, they realize they must face long-term consequences and restate their initial positions. Since a significant amount of time lapses between the date of the traffic accident and the date of the new investigation, during which the vehicle was repaired, totaled or changed ownership, and also due to the fact that the victims were buried or the wounds healed without marks, an answer is difficult to obtain based solely on forensic medical certificates. There could be cases where one of the occupants of the vehicle was projected from the vehicle, and if deceased, the driver may substitute to the passenger in order to be exonerated. In these circumstances, computer simulation of the traffic accident may offer additional information to strengthen the outcome of forensic expertise, and it may even provide answers in cases where forensic expertise is incon-

clusive.

Computer simulations of traffic accidents

Computer simulation of traffic accidents involves correct input of dynamic and physical parameters into a mathematical model. Some parameters are already known while others are found through iterative trials. Mathematical modeling involves a large number of calculations, impossible to accomplish without a computer. If correctly chosen, input parameters, correlated with the shape of the vehicle and the road geometry must yield results coinciding with the real life situation via mathematical modeling. For example, tire marks, final vehicle positions and their trajectories must coincide with those documented by on site investigation.

Here we present a modeling approach using V. Crash 2.2 software. For mathematical modeling of occupant's dynamics as well as in the case of simulations of pedestrian accidents, the V. Crash software takes into consideration higher rigidity coefficients of the vehicle parts as compared to those of the human body and therefore considers as negligible the vehicle deformations.

The model used is composed of a group of ellipsoid bodies which make up the humanoid somatic structure.

Nagoya University researchers (2) developed a mannequin build up of ellipsoid parts (fig. 1) and a mathematical model based on statistics of Japanese population. Based on these data, a model was developed based on the 50est percentile of an adult man (Polar II (3)) whose height was determined at 175 cm and body weight of 75 kg. The sitting height of Polar II mannequin is 88.4 cm. The mannequin used by V. Crash simulations has 16 joints which interconnect 15 hyper-ellipsoids, corresponding to human body parts and their associated degrees of freedom.

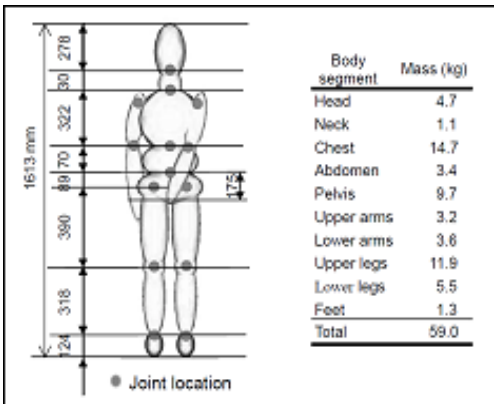

Fig. 1. Japanese model of test mannequin

CASE STUDY

A group of young individuals were inside a vehicle (make Renault, model Megane) moving on an asphalt county road with shoulders and ditches. The motorway was covered with snow and spots of ice. Suddenly the driver lost control of the vehicle and entered the ditch on the right side of the road where it rolled over on the right side and hit the end of a concrete bridge. Following the accident all four occupants suffered injuries (fig. 2, 3).

Several people proceeded to the site and helped pulling the occupants out of the vehicle. Eye witness declarations coincided only in part with the occupants statements. The identity of the occupants on the back seat and their respective positions were confirmed unequivocally, however, regarding the driver and the front passenger, the declarations of the witnesses were contrary to the statements of the vehicle occupants. A forensic medical expertise was ordered in this matter but the investigation did not establish which of the occupants were,

respectively, the driver and the passenger. Therefore, judicial organs ordered a criminalistic expertise whose sole objective was to establish the identity of the driver.

Fig. 2. The vehicle seen from behind

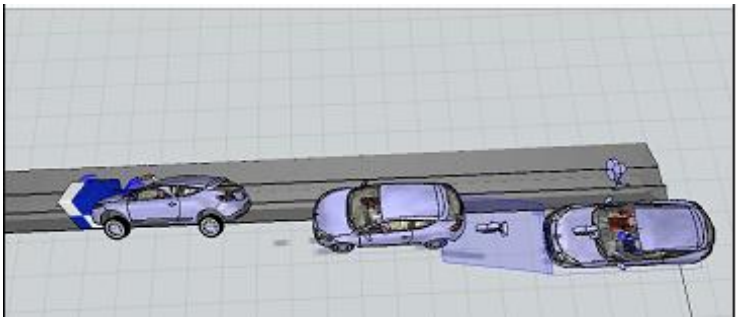

Fig.3. Overview of accident dynamic

The injury profile of the back seat passenger on the right side showed remarkable similarities with that of one of the front seat occupants. Both had lesions of the right fist and right forearm as excoriations and cut wounds. The passenger in the right back seat had a foreign body at the level of the right distal epiphysis (glass fragment) while the front passenger had a fracture of the distal epiphysis (fig. 4).

The front seat occupant suffered an elongation of the right brachial plexus with right acromial – clavicle disjunction (fig. 5), paresthesia of the right arm and shoulder, jaw wound, multiple excoriated and cut wounds at the level of the face, which required 30 – 35 days of medical care.

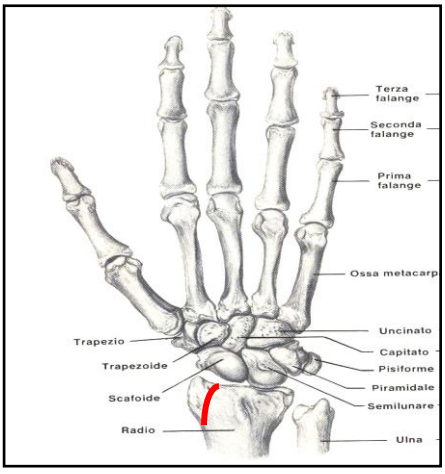

Fig. 4. Right distal epiphysis fracture (marked with red line – according to attached Rx)

The elongation of the brachial plexus is engendered by anterior-posterior hyperextension of the arm that is through front-to-back movement of the arm over the normal limit. This kind of trauma produces the swelling of the shoulder joint with injury of the vascular and nervous bundles with shoulder paresthesia of the shoulder.

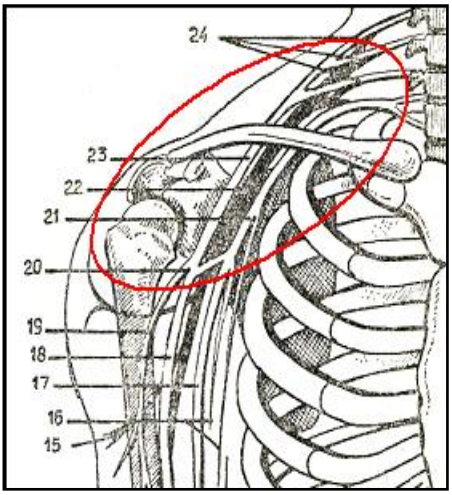

Fig. 5. Diagram of the brachial plexus and its innervation (positions 22-24).

The Rx administered as evidence in the case, obviating the elongation of the brachial plexus and acromial-clavicular disjunction

The injuries suffered by the other occupant were minor and consisted of cut wounds at occipital and upper lip levels. Spots of reddish-brown liquid were found on the vehicles which were later identified as human blood (fig. 6, 7). The blood in the back belonged to the O group while the one in front to group A.

The occupant of the back seat was the brother of one of the front seat occupants and it was assumed that both brothers may have the same blood group. For unknown reasons, until the date of ordering the expertise, the blood group of the brother of the individual occupying the right back seat was not administered as evidence in the case.

Fig. 6. The front seat where samples of reddish brown liquid were taken

Fig. 7. The right back seat where blood traces were sampled

Starting from the data yielded by the minutes of the site investigation regarding the geometry of the road, computer simulations of the accident was performed using V.Crash software. The speed at the impact with the concrete bridge was relatively low, under 10 km/h, as demonstrated by the integrity of the front bumper (fig. 8)

Fig. 8. Aspect of the front side of the vehicle

The injuries suffered by the vehicle occupants were due for the most part to rolling over and not to the impact with the concrete bridge. Following the simulation, starting from a speed of 55 km/h an impact speed of approximately 3.4 km/h was obtained and it was found that the dynamics of the front seats occupants coincides with a dynamic that could produce the injuries suffered by the two occupants, the driver being affected to a minor degree.

Starting from the initial position (fig. 9), front seat occupants were placed and the safety belts were deactivated.

During the first phase of the vehicle rolling over on the right side, it was found that the driver suffered head injuries, due to contact with elements inside the vehicle (fig. 10).

Immediately following the rollover, the passenger on the right suffered injuries at the level of the external aspect of the right hand at the level of the joint (fig. 11). Similarly, these injuries inflicted to the back seat passenger on the right.

Finally it is found that the driver falls over the passenger on the right provoking the brachial plexus dislocation at the level of the right shoulder (fig. 12).

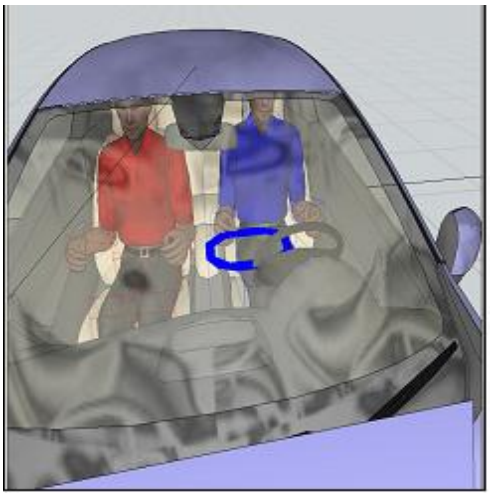

Fig. 9. Starting position of the computer simulation

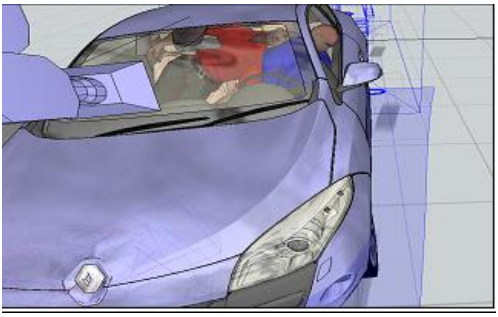

Fig. 10. Impact of the driver with elements inside the vehicle

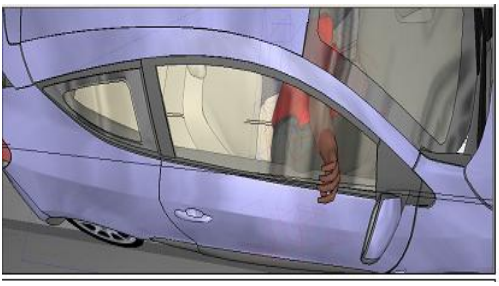

Fig. 11. Hyperextension of the right arm of the passenger of the right and impact of the right arm at the level of the radial-cubital-carpian joint with the right window and later with the dashboard

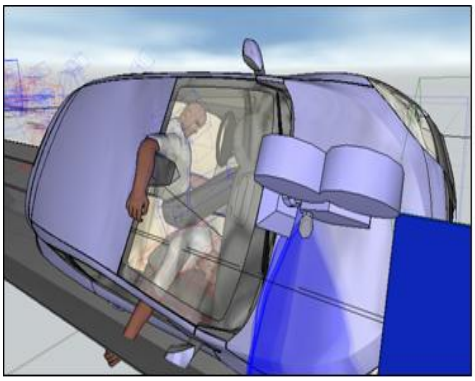

Fig. 12. The mechanism of right brachial plexus dislocation of the passenger

Although during testimonial investigation the passenger and the driver state that they inversely occupied the places in the vehicle, witnesses declarations proved to be true, with computer simulation through mathematical modeling being a sufficiently solid argument towards this conclusion.

CONCLUSIONS

In cases where the driver of the vehicle is unknown, in addition to the injury panel, computer simulation of the traffic accident becomes a very useful and often essential tool.

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EPIDEMIOLOGICAL ASSESSMENTS ON INCIDENCE OF ACUTE GASTROENTERITIS IN 0-14 AGED CHILDREN, IN THE IASI COUNTY, ROMANIA, BETWEEN 2009-2012

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EPIDEMIOLOGICAL ASSESSMENTS ON INCIDENCE OF ACUTE GASTROENTERITIS IN 0-14 AGED CHILDREN, IN THE IASI COUNTY, ROMANIA, BETWEEN 2009-2012 (Abstract): The study **aim** was to assess the epidemiological potential of acute gastroenteritis (AGE) in children between 2009 and 2012. **Material and methods**: We studied two groups; first, a lot of 7237 cases (*group A*) recorded in the Iasi County through passive surveillance system, reported monthly by the network of primary health care, the assisted outpatients, respectively, those reported by hospitals. The second group (*group B*) was composed of 4191 hospitalized cases reported also in the passive surveillance system m by telephone, weekly, during only June / end of May to October / beginning of November. Structural peculiarities of the two groups are due to different reporting methodology, so that they were analyzed separately. **Results**: AGE cases come from urban or rural areas in relatively stable proportions and slightly increased in rural areas compared to urban. The distribution by sex of cases from the group B is maintained at the same shape from one year to another, with a slight predominance in males. The best represented age group in every studied was 0-3 (54.3% of all cases), followed by children between 5 and 10 years with 17.5%). Laboratory investigations have enabled the etiologic diagnosis for hospitalized cases, which ranged from 15.3 to 17.5%, with the exception of 2009 when the diagnosis was noted for almost half of the discharged patients (47.5%). **Conclusions**: AGE remains a public health problem in the Iasi County and Romania also, by affecting all age groups, but especially children under 3, an insufficient knowledge of the etiology, and economic losses insufficiently evaluated. **Keywords:** ACUTE GASTROENTERITIS, CHILD, SURVEILLANCE

Acute gastroenteritis (acute diarrheal disease, AGE) is a widespread disease worldwide in all age groups, responsible (after common acute infections of respiratory tract) of a large number of consultations in primary care network, but high levels of school and employment absenteeism (which include sick child care leave) (1, 2, 3). AGE in developing countries remains an important cause of death among

children, especially aged 0 to 5 years. Annually, worldwide, approximately two million deaths in children in the age group 0-5 years are reported. AGE mortality in developed countries is no longer a health emergency to the population, although the admission rate is high, causing significant economic losses (4, 5).

Many of episodes are not found in AGE reports, even in industrialized countries, due to the inaccuracy of reporting and under diagnosed. In the United States, where it is estimated that annually produces about 5 million cases of AGE, in fact are reported only 4 million (6, 7, 8, 9, 10, 13). In Romania, AGE cases are reported monthly, cumulating data provided by family physicians and hospitals and during the seasonal epidemiological surveillance, the reporting is made by telephone, weekly. In 2008, AGE surveillance methodology in Romania has changed so that only hospitalized cases were reported between 2009 and 2012.

MATERIAL AND METHODS

The study aim was to assess the epidemiological potential of AGE in children between 2009 and 2012. Thus, we studied two groups; first, a lot of 7237 cases (*group A*) recorded in the Iasi County through passive surveillance system, reported monthly by the network of primary health care (family physicians), the assisted outpatients, respectively, those reported by hospitals. The second group (*group B*) was composed of 4191 hospitalized cases reported also in the passive surveillance system m by telephone, weekly, during only June / end of May to October / beginning of November. Structural peculiarities of the two groups are due to different reporting methodology, so that they were analyzed separately.

The database was performed in Excel software. Statistical analysis was performed with *SPSS 16.0*. For comparison, we used the $\chi 2$ (chi-square). The value of p (significance threshold), which reflects the probability that the difference between analyzed variables is randomly, was considered greater than 0.05.

RESULTS AND DISCUSSION

AGE cases come from urban or rural areas in relatively stable proportions and slightly increased in rural areas compared to urban (values between 51.5 and 54.8%) (tab. I).

TABLE I

Distribution of AGE cases from group A in relation to residence area (Iasi County, 2009-2012)

Year	Total	urban		rural		p
		No.	*%*	*No.*	*%*	
2009	1306	830	**63.5**	476	**36.5**	<0.01
2010	1571	717	**45.6**	854	**54.4**	>0.05
2011	2103	951	**45.2**	1152	**54.8**	>0.05
2012	2257	1008	**44.7**	1249	**55.3**	>0.05
Total	**7237**	**3506**	**48.5**	**3731**	**51.5**	>0.05

Changing in AGE reporting methodology in 2008 to 2009 was followed by recording higher proportion of cases from urban areas (63.5%) in 2009, but in coming years,

the situation is comparable to previous years before those modifications. It notes, however, that the absolute number of reported cases increased in both urban and rural areas (from 978 and even 640 reported cases in 2007-2008, 1306 subjects in 2009 to 2,257 cases reported in 2012) (fig. 1).

The distribution by sex of AGE cases from the group B (phone reporting between June and October) is maintained at the same shape from one year to another, with a slight predominance in males (fig 2).

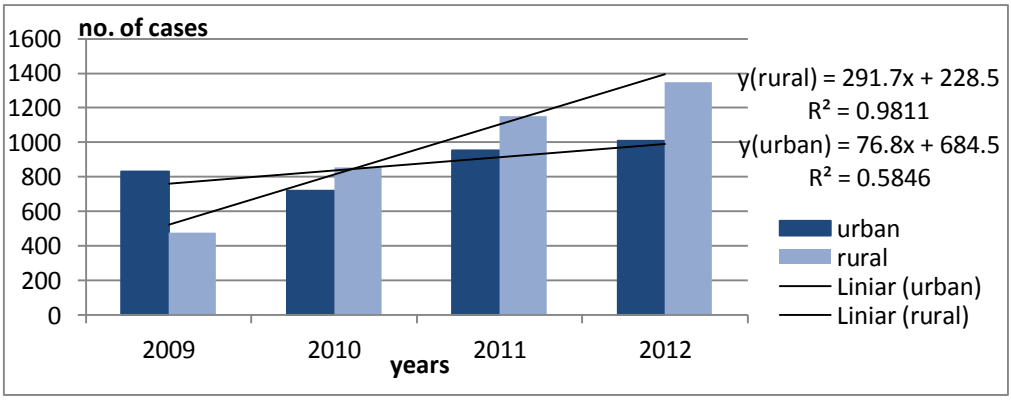

Fig. 1. Distribution of AGE cases from group A in relation to residence area (Iasi County, 2009-2012)

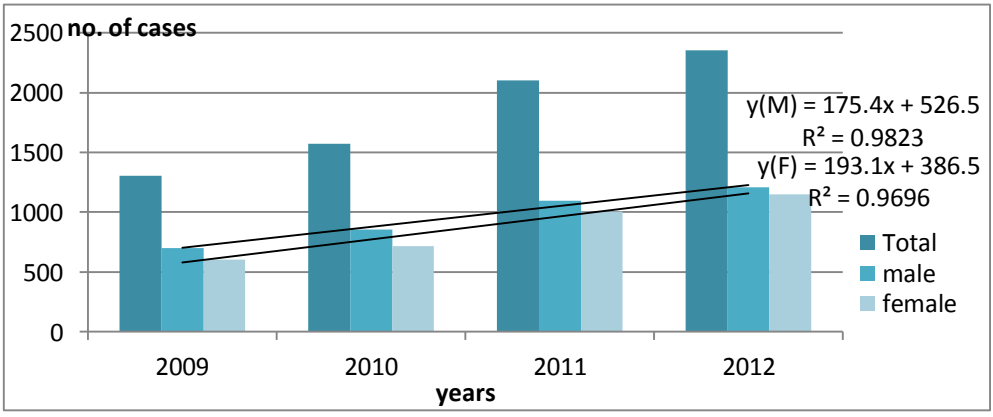

Fig. 2. Distribution by sex of AGE cases from group B (Iasi County, surveillance period June – October, 2009-2012)

The best represented age group in every studied was 0-3 (54.3% of all cases), followed by children between 5 and 10 years with 17.5%), according to data from the literature (7, 8, 9, 10, 11) (tab. II, fig. 3).

Risk factors such as the hygiene status and intra-familial transmission, or gastric hypoacidity and antibiotic treatments could be add an increased vulnerability to any type of infection, including the digestive system and the immune system immaturity specific in children at 3 or younger (15, 16).

TABLE II

Distribution by age of AGE cases from group B (Iasi County, surveillance period June – October, 2009-2012)

Year	Age groups (years)								Total
	0-3		3-5		5-10		10-14		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*
2009	774	59.2	183	14.0	200	15.3	149	11.5	**1306**
2010	872	55.4	268	17.0	255	16.4	176	11.2	**1571**
2011	1070	50.8	341	16.2	403	19.2	289	13.8	**2103**
2012	1213	53.7	358	15.9	410	18.2	276	12.2	**2257**
Total	**3929**	**54.3**	**1150**	**15.9**	**1268**	**17.5**	**890**	**12.3**	**7237**

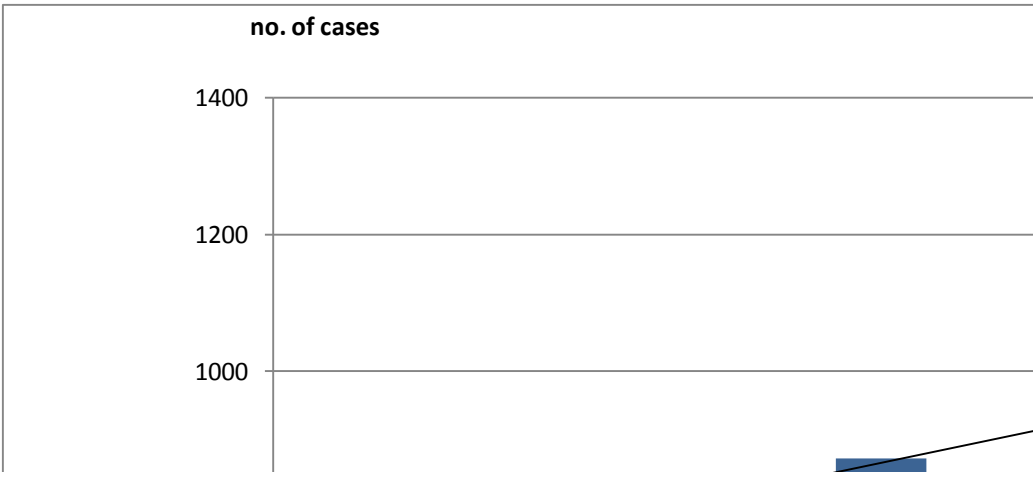

Fig. 3. Distribution by age of AGE cases from group B (Iasi County, surveillance period June – October, 2009-2012)

Risk factors such as the hygiene status and intra-familial transmission, or gastric hypoacidity and antibiotic treatments could be add an increased vulnerability to any type of infection, including the digestive system and the immune system immaturity specific in children at 3 or younger (15, 16).

Distribution of AGE cases in children hospitalized during 2009-2012, highlighted significant differences in the total number of cases (from the group B), during seasonal surveillance ($p<0.05$). The age group most affected was 0-4 years (73.8%, 80.5%, 78.5%, and 78.2%) followed by 5-9 years (14.2% of total cases) and 10-14 years age group with only 8.2% (tab. III, fig. 4).

TABLE III

Distribution by age of AGE cases from group B in relation to the month of discharge (Iasi County, surveillance period June – October, 2009-2012)

Age group (years)		**0-4**		**5-9**		**10-14**		**Total**
Year	**Month**	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*
2009	June	198	71.6	55	20.0	23	8.4	**276**
	July	201	68.3	66	22.4	27	9.3	**294**
	August	139	65.9	33	15.7	39	18.4	**211**
	September	148	84.0	16	9.0	12	7.0	**176**
	October	128	88.2	14	9.6	5	3.2	**147**
Total		**814**	**73.8**	**184**	**16.7**	**106**	**9.5**	**1104**
2010	June	153	81.0	27	14.2	9	4.8	**189**
	July	173	76.7	26	11.6	25	11.7	**224**
	August	162	80.2	28	13.8	12	6.0	**202**
	September	154	79.5	22	11.3	18	9.2	**194**
	October	133	87.0	10	6.5	10	6.5	**153**
Total		**775**	**80.5**	**113**	**11.7**	**74**	**7.8**	**962**
2011	June	152	82.1	22	11.9	11	6.0	**185**
	July	123	74.9	25	15.3	16	9.8	**164**
	August	186	78.4	29	12.3	22	9.3	**237**
	September	179	77.5	36	15.5	16	7.0	**231**
	October	140	79.0	22	12.5	15	8.5	**177**
Total		**780**	**78.5**	**134**	**13.5**	**80**	**8.0**	**994**
2012	June	148	77.4	34	17.8	9	4.8	**191**
	July	168	82.3	24	11.7	12	6.0	**204**
	August	273	81.4	41	12.2	22	6.4	**336**
	September	162	80.5	28	14.0	11	5.5	**201**
	October	133	66.9	38	19.1	28	14.0	**199**
Total		**884**	**78.2**	**165**	**14.6**	**82**	**7.2**	**1131**
Total		**3253**	**77.6**	**596**	**14.2**	**342**	**8.2**	**4191**

Regarding the distribution of the months included in the report, most cases were reported each year in June, July and August (tab. III, fig. 4). The difference of the reported cases by age group in the summer months is statistically significant ($p < 0.05$).

In the group B (season reporting), a large cluster of AGE cases is noticed in the summer months (70.5% of cases in 2009,

64.0% and 64.7% in 2010 and 2012); only 59% of cases were recorded in the summer months of 2011, most cases are concentrated in August and September, contrary to previous years. In the same group B, a number of 1131 of AGE cases with a peak in August is reported in 2012. A higher number of cases reported in 2009 and 2012 in the Iasi County did not follow the trend of the situation at the country level; in contrast, a smaller number of cases reported in the Iasi County in 2010 did not reflect the situation at national level, with greater number of diseases to other years (tab. IV).

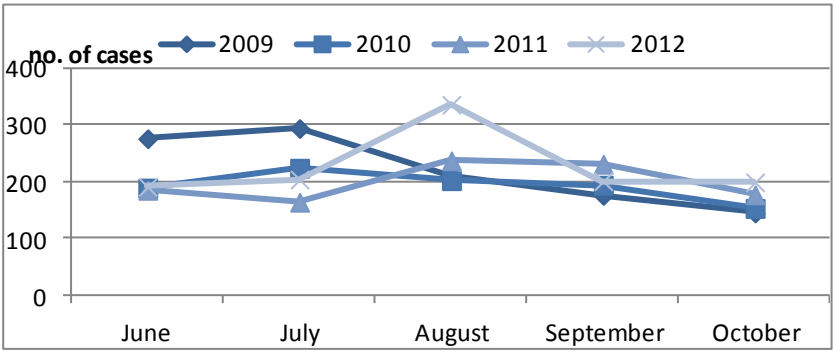

Fig. 4. Distribution of AGE cases from group B in relation to the month of discharge (Iasi County, surveillance period June – October, 2009-2012)

TABLE IV

Number of AGE cases registered in Romania and Iasi County, phone reported during the seasonal surveillance, June – August, 2009 – 2012

Year	Romania	Iasi County	
		No.	%
2009	39,649	1104	2.8
2010	41,651	962	2.3
2011	35,172	994	2.8
2012	36,962	1131	3.0
Total	**153,434**	**4191**	**2.7**

Etiologic diagnosis of AGE cases is a challenge for the epidemiologist, clinician, and laboratory physician (15, 17, 18). Laboratory investigations have enabled the etiologic diagnosis for hospitalized cases, which ranged from 15.3 to 17.5%, with the exception of 2009 when the diagnosis was noted for almost half of the discharged patients (47.5%). Greater attention and better clinical guidance referred to samples from children 0-1 children in which the percentage of positivity is somewhat higher than other age groups (tab. V).

TABLE V

Number of AGE hospitalized cases reported by seasonal surveillance system in relation to age, with identified (IdE) or non-identified etiology (nonIdE) (group B, June-October, 2009-2012)

Year / age group		0-1		1-4		5-9		10-14		Total	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
2009	Cases with IdE	149	45.3	234	48.3	90	49.0	46	43.3	529	47.5
	Cases with NonIdE	180	54.7	251	51.7	94	51.0	60	56.7	585	52.5
	Total	**329**	**100.0**	**485**	**100.0**	**184**	**100.0**	**106**	**100.0**	**1104**	**100.0**
2010	Cases with IdE	55	16.7	71	16.0	18	16.0	12	16.2	156	16.2
	Cases with NonIdE	274	83.3	375	84.0	95	84.0	62	83.8	806	83.8
	Total	**329**	**100.0**	**446**	**100.0**	**113**	**100.0**	**74**	**100.0**	**962**	**100.0**
2011	Cases with IdE	55	22.0	66	14.0	22	16.4	9	11.2	152	15.3
	Cases with NonIdE	251	78.0	408	86.0	112	83.6	71	88.8	842	84.7
	Total	**306**	**100.0**	**474**	**100.0**	**134**	**100.0**	**80**	**100.0**	**994**	**100.0**
2012	Cases with IdE	74	20.0	75	14.8	33	20.0	14	17.0	196	17.5
	Cases with NonIdE	295	80.0	430	85.2	132	80.0	68	83.0	925	82.5
	Total	**369**	**100.0**	**505**	**100.0**	**165**	**100.0**	**82**	**100.0**	**1121**	**100.0**
Total		**1333**	**100.0**	**1910**	**100.0**	**596**	**100.0**	**342**	**100.0**	**4181**	**100.0**

CONCLUSIONS

In the Iasi County, acute gastroenteritis recorded averages values, comparable to other counties from Romania, representing between 2.3 and 3% of all cases reported nationwide.

Seasonal surveillance highlighted a cluster of AGE cases in the summer months (June, July, and August).

The etiologic diagnosis was not possible for most laboratory tests, but the 0-1 age group positivity rate is higher.

Acute gastroenteritis remains a public health problem in the Iasi County and Romania also, by affecting all age groups, but especially children under 3, an insufficient knowledge of the etiology, and economic losses insufficiently evaluated.

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INFLUENCE OF ANTIBIOTIC THERAPY PRIOR TO ADMISSION ON THE EFFICACY OF CLASSICAL METHODS FOR THE DIAGNOSIS OF MENINGOCOCCAL DISEASE

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INFLUENCE OF ANTIBIOTIC THERAPY PRIOR TO ADMISSION ON THE EFFICACY OF CLASSICAL METHODS FOR THE DIAGNOSIS OF MENINGOCOCCAL DISEASE (Abstract): **Aim:** To assess the influence of preadmission antibiotic therapy on the results of the classical methods for bacteriological confirmation of meningococcal disease (MD). **Material and methods:** Retrospective study of the MD cases diagnosed in the "St. Parascheva" Universitary Clinical Infectious Diseases Iași between 1994 and 2011. **Results:** The etiological diagnosis was made by identifying the meningococcus in the CSF (cerebrospinal fluid) in 71.9% of the 323 patients and by blood culture in 8%. Preadmission antibiotic therapy received 39% of the patients, thus the sensitivity of test was significantly reduced: direct examination from 64.6% to 43.2% ($p < 0.001$), cultures from 55.9% to 27.2% ($p<0.001$), and latex-agglutination from 84.6% to 58.8% ($p=0.003$). The rate of positive CSF decreased from 82.1% to 56% ($p<0.001$). Preadmission antibiotic therapy significantly increased the ratio of cases in which meningococcus was not detected in CSF by any of the classical methods (44% compared to 17.9% in the cases without prior treatment). The proportion of cases in which meningococcal isolation was done by two methods decreased from 38.5% to 19.2%, and of those by all three methods from 16.9% to 5.6% ($p<0.001$). Preadmission antibiotic therapy also decreased the rate of positive blood cultures from 14.7% to 3.5% (Fisher's exact test, $p=0.009$). **Conclusions:** Antibiotic treatment prior to admission significantly decreases the percentage of patients with MD in which meningococcal isolation can be done; this requires the use of a more sensitive diagnosis method (ex. qPCR). **Keywords**: MENINGOCOCCAL DISEASE, PREADMISSION ANTIBIOTIC THERAPY, DIRECT CSF EXAMINATION, CSF CULTURE, BLOOD CULTURE

Meningococcal infection is a leading cause of morbidity and mortality worldwide. It is a disease with lethal potential, which begins suddenly and death can occur within hours. The disease may be present in various forms, from the simple rhinopharyngitis to severe purpura fulminans. All levels of severity are possible within these extremes. Meningococcal meningitis and meningococcal septicemia (meningococcemia) are more frequently drawing the attention of clinicians. The disease has a

higher mortality rate in case of delayed diagnosis and treatment.

Early diagnosis is essential for therapeutic success. Meningococcal disease is normally suspected on clinical grounds and confirmed by laboratory tests. Still, the clinical diagnosis of meningococcal disease can be difficult because of symptom variability. Furthermore, in order to improve the prognostic, in cases suspected of meningococcal infection, the current guidelines recommend the administration of an antibiotic as quickly as possible, sometimes even before arrival at the hospital (1).

MATERIAL AND METHODS

The present study aimed at assessing the influence of antibiotic therapy administered prior to admission on the results of classical methods for bacteriological confirmation of meningococcal disease in the cerebrospinal fluid (CSF) (direct examination, culture and latex agglutination), blood (blood culture) and pharynx (throat swab). This is a retrospective analysis which included the cases of meningococcal disease diagnosed in the „St. Parascheva" Universitary Clinical Infectious Disease Iaşi between 1994 and 2011. We analyzed the etiologic diagnosis [identification of *N. meningitidis* in CSF (direct examination, culture and latex agglutination (LA), blood (blood culture) and pharynx (throat swab)] in relation with the absence or presence of preadmission antibiotic therapy.

The database was created in Microsoft Access. The statistical analysis was performed using SPSS 17.0. The differences between groups were calculated with the help of χ^2 test, Fisher exact, with a significance threshold of $p < 0.05$.

RESULTS

During the study period (1994–2011) 323 cases of meningococcal disease were recorded, 80% (257 cases) being cases of meningococcal meningitis and the remaining 20% (66 cases) meningococcemia cases. The etiologic diagnosis was made in the Laboratory of the "St. Parascheva" Universitary Clinical Infectious Diseases Iaşi. 320 CSF specimens, 248 blood cultures and 288 throat swabs were obtained from the 323 patients with meningococcal disease. All 320 CSF specimens were tested by direct examination and culture, but CSF latex agglutination (LA) was performed in only 122 cases. The macroscopic appearance of CSF was purulent in 58% of the meningococcal disease cases and clear in 18%. Classically, CSF was purulent in most cases of meningococcal meningitis (67.8%) and clear in most cases of meningococcemia (69.2%). The etiologic diagnosis was made by the identification of *N. meningitidis* in 71.9% of the cases. The meningococcus was identified by direct CSF examination in 56.3% of the cases, and CSF culture in 44.7%. The use of CSF LA reaction made the etiologic diagnosis especially in the patients receiving antibiotic therapy prior to the test, in 76.8% of the cases. *N. meningitidis* was identified in CSF by one of the classical methods of diagnosis in 28.4% of the cases. In 30.9% of the patients, the meningococcus was identified by two methods and in 12.5% by three methods. CSF was positive in approximately 72% of the cases. Direct examination was positive in 56.3% of the cases, and the culture was positive on average in 45%; all these percentages remained relatively constant over the study period. The percentage of positive LA tests was high and varied significantly during the study period. Meningococcus was also identified by blood culture in 8% of the cases and in the throat swab in 15%. Thirty-nine percent of the patients received antibiotic therapy prior to admission. The annual distribution of the patients on antibiotic therapy prior to admis-

sion showed an average of 38.7%, relatively constant over the study period. When studying the influence of antibiotic therapy prior to admission on CSF, no statistically significant effects on the macroscopic aspect of CSF were noticed, either in meningococcal meningitis or meningococcemia. On the other hand, the effects on the etiologic diagnosis methods were statistically significant. Thus, the percentage of positive direct examinations decreased from 64.6% to 43.2% by using antibiotic therapy prior to admission (χ^2 = 14.195, p < 0.001) (fig. 1). The percentage of positive CSF cultures decreased significantly from 55.9% to 27.2% (χ2=25.379, p<0.001) in case of prior antibiotic use (fig. 2). The percentage of positive CSF LA test decreased from 84.6% in the absence of prior antibiotic use to 58.8% in the cases of antibiotic therapy prior to admission (χ2=8.84, p=0.003). The rate of positive CSF decreased significantly from 82.1% to 56% when antibiotic therapy was administered prior to admission (χ2=25.57, p<0.001) (fig. 3**).** Antibiotic therapy prior to admission determined a significant increase in the proportion of cases in which meningococcus could not be identified in CSF by any of the classical methods: 44 % compared to 17.9% in the cases without antibiotic use prior to admission. On the other hand, no significant changes were noticed in the cases in which meningococcus was identified by a single method. The percentage of cases in which the meningococcus was identified by two methods decreased as a consequence of prior antibiotic use, from 38.5% to 19.2%. The percentage of cases in which meningococcus was identified by all three methods decreased significantly from 16.9% without prior antibiotic use to 5.6% with antibiotic use prior to admission (χ2= 35.88, df=3, p<0.001) (fig. 4). A decrease in the proportion of positive blood culture from 14.7% to 3.5% due to antibiotic therapy prior to admission was also found (Fisher exact test, p=0.009) (fig. 5). Antibiotic therapy prior to admission had no statistically significant effect on throat swab tests (18.9% *vs.* 15%, χ2=0.7, df=1, p=0.4). The main antibiotics administered prior to admission were penicillin G (17.2% of cases) and third-generation cephalosporins (C3G) (7.5%). No statistically significant differences were found between the effects of penicillin G and C3G administered prior to admission on the etiologic diagnostic methods of meningococcal disease.

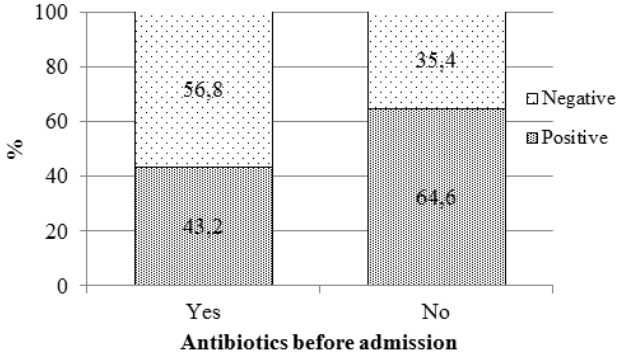

Fig 1. Meningococcal disease – influence of antibiotic therapy prior to admission on the direct examination of CSF.

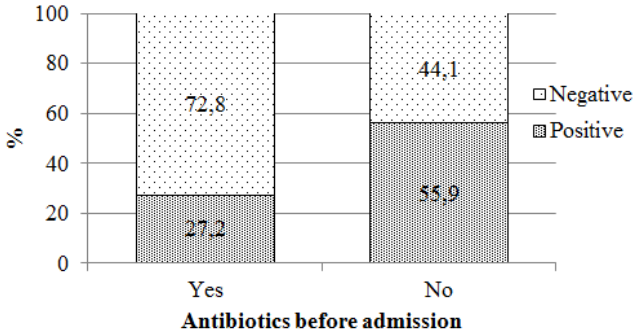

Fig 2. Meningococcal disease – influence of antibiotic therapy prior to admission on the CSF culture

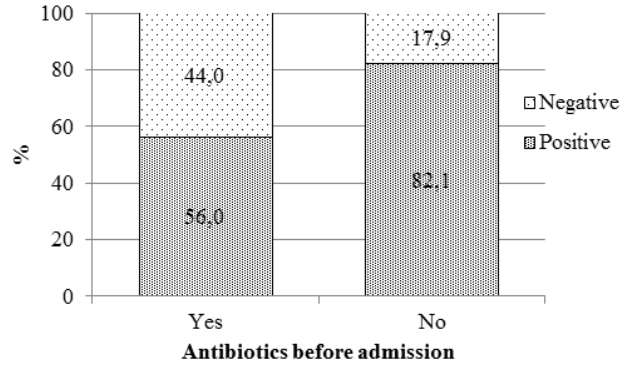

Fig 3. Meningococcal disease – influence of antibiotic therapy prior to admission on the CSF results, all methods of analysis considered together.

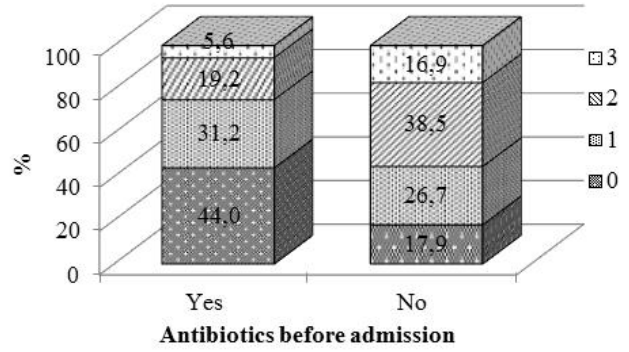

Fig 4. Meningococcal disease – influence of antibiotic therapy prior to admission on the number of positive CSF analysis methods: direct examination, culture and latex agglutination (counted from 0 to 3).

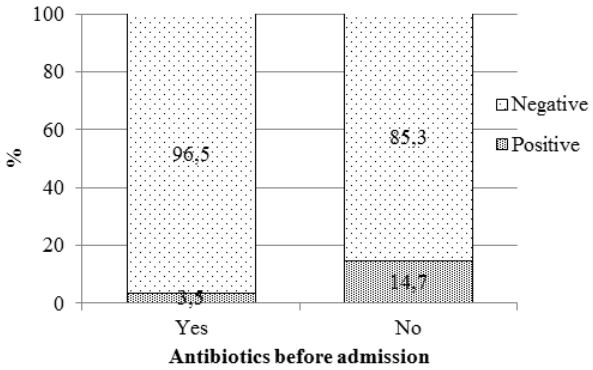

Fig 5. Meningococcal disease – influence of antibiotic therapy prior to admission on the blood culture

DISCUSSION

Neisseria meningitidis (meningococcus) causes a spectrum of disease that varies from the asymptomatic carriage to meningitis and even septicemia (meningococcemia), sometimes with fulminating evolution. Due to the difficulties of early diagnosis and fast progression, the invasive meningococcal disease (sepsis and/or meningitis) is a potentially fatal condition that can also cause long-term sequelae (2, 3). Therefore, fast and accurate diagnosis is essential for optimal management of patients and prompt implementation of preventive measures (4). In meningococcal disease the clinical diagnosis can be difficult due to the variability of symptoms**.** CSF examination (biochemical data and polymorphonuclear cells count) can be useful for differentiating acute viral meningitis from bacterial ones. But the gold standard in the microbiological diagnosis remains *Neisseria meningitidis* culture from sterile body fluid, like blood or CSF. Nowadays, confirming the diagnosis of meningococcal infection by conventional microscopy and culture techniques is becoming increasingly difficult. This is due to the early initiation of antibiotic treatment, which is recommended by current guidelines to improve the management and prognosis (5, 6). Thus, the positive blood culture rate ranges from 25 to 50 % and decreases to about 5 % when antibiotics are administered prior to admission. In our study a decrease in the positive blood culture rate from 14.7% to 3.5% in case of prior antibiotic use. Direct CSF microscopic examination and culture are positive in 80-90% of untreated cases of meningococcal meningitis, but this rate also decreases in case of antibiotic therapy prior to admission, fact also noticed in our study where the effects of antibiotic therapy prior to admission on the etiologic diagnosis were significant (7-10). Thus, antibiotic therapy prior to admission caused a decrease in the rate of positive direct CSF examinations from 64.6% to 43.2% and of the rate of positive CSF cultures from 55.9% to 27.2%. In our study, the rate of positive CSF latex agglutination also decreased from 84.6% in the cases without prior antibiotic use to 58.8% in the cases receiving antibiotic therapy prior to admission. The rate of positive CSF tests in relation with the absence or presence of prior antibiotic therapy decreased significantly from 82.1% to 56%. Furthermore, in many patients a lumbar puncture cannot be performed in early stages of the disease because of the fear of clinical deteriorations

(11). Other limitations of conventional diagnostic methods include the time to culture positivity and the low sensitivity and specificity of the rapid Ag-Ac tests (latex agglutination) (12, 13).

CONCLUSIONS

This study shows that the antibiotic treatment administered prior to admission significantly reduces the percentage of patients with meningococcal disease in which *N. meningitidis* can be identified by standard microbiological techniques. The use of CSF latex agglutination reaction allowed the etiologic diagnosis especially in patients with preadmission antibiotic use. A limitation of conventional diagnostic methods is the relatively long time to culture positivity and low sensitivity and specificity of latex agglutination test. Consequently, the use of a fast and more sensitive diagnostic method is mandatory (qPCR).

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THE INFLUENCE OF FUNCTIONAL THERAPY ON FACIAL AND DENTAL STRUCTURES. CASE PRESENTATION

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THE INFLUENCE OF FUNCTIONAL THERAPY ON FACIAL AND DENTAL STRUCTURES, IN CLASS II DIVISION 1 CASES. CASE PRESENTATION (Abstract): In the era of fixed appliances, some orthodontic practitioners seem to forget about functional therapy. Functional appliances are the only capable of orthopedically changes during the growth spurt. Activators of all types, classic or opened, are elected appliances in growing subjects with class II/1 anomalies. The appropriate case selection, along with patient compliance, lead to improved facial aesthetic and dental occlusion, at affordable prices. With this case presentation, we want to show our protocol in the management of these cases. **Keywords:** ACTIVATOR APPLIANCE, FACIAL AESTHETIC, DENTAL OCCLUSION.

Class II division 1 malocclusions through mandibular deficiency are common in Eurocentric societies. Many studies have shown that orthodontic-orthopedic treatment with functional appliances during growth is capable to stimulate the anterior repositioning of the mandible and to correct the occlusion and the facial appearance as well (1-6). The classic activator is one of the most frequent used appliances in class II/1 treatment but one of its disadvantages is the large volume that makes it uncomfortable for the patient. One alternative could be the open activator that allows more lack of restrictions for tongue movements because the acrylic mass is reduced, that′s why it is more comfortable and can be worn almost permanently, not only during sleeping. (7)

CASE REPORT: A 12 years old male with class II/1 malocclusion. Extraoral examination revealed an ovoid facial pattern with short lower face, protruded lower lip and deep labial-mental fold, retrognathic chin and convex profile (fig.1).

Fig.1. Pretreatment extraoral photographs

Intraorally, the canine relationship was half a cusp class II on both sides, the right first molar was in class I and the left first molar was in half a cusp class II relationship. The upper incisors were extremely protruded. The upper right canine was expected to erupt. In the lower arch, there was a little crowding in the front teeth and the curve of Spee was very deep (fig.2, 3). The over jet was of 9.1 mm and the overbite was of 5 mm. (fig. 4).

Fig. 2. Pretreatment photographs of the dental arches

Fig. 3. Pretreatment lateral occlusion

Fig. 4. Pretreatment frontal occlusion

The lateral cephalogram showed a stage 3 in maturity of the cervical vertebrae, what means pubertal growth, the best time for functional treatment; 4 mm skeletal class II, deep bite (FMA angle=20°), upper incisors protrusion (I -Frankfurt line angle=123°) and normal position of the lower incisors (IMPA angle=90°). Gonion angle was decreased (120°),the angle of Y axis with Frankfurt plane was 57° that means an anterior direction of growth.The skeletal and soft tissue profile was convex (NAPog angle= -8.2°, GlSnPog angle= -22°) (fig. 5.)

Fig. 5. Pretreatment lateral cephalogram

The case was treated with an open activator (fig. 6).

Fig. 6. Open activator used

After one year of treatment, the over jet was completely reduced through upper incisor retrusion and mandibular advancement. The overbite was 1/3 with a leveled curve of Spee, because of lower lateral extrusion, that was obtained progressively trimming the lateral acrylic mass. The same appliance was used for retention until the completion of growth (fig. 7).

The skeletal and soft tissue facial profile was substantially corrected into a straight one (fig. 8, 9).

Fig. 7. Post-treatment frontal occlusion

Fig. 8. Tracings of pretreatment (blue) and post-treatment (red) cephalograms

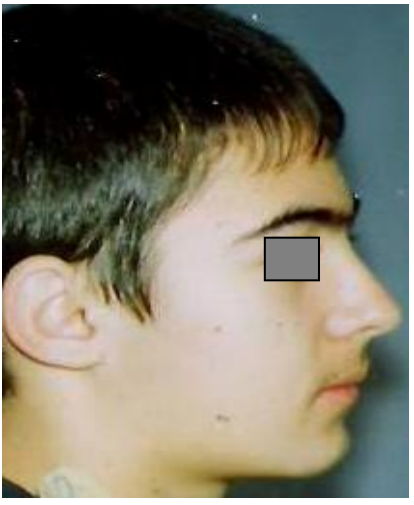

Fig. 9. Post-treatment extra-oral photograph

DISCUSSION

Regarding our case and the existing theories in the literature about functional therapy, properly case selection is a guarantee for successful treatment and good results. The criteria that must be fulfilled are: the skeletal maturation at the peak (pubertal age), distal occlusion in canines and molars, moderate to severe overjet (up to 12 mm), skeletal class II because of mandibular posterior position sometimes associated with mandibular deficiency, deep-bite or normo-bite, mandibular counter clockwise rotation and good patient compliance. (8,9)

The reason for we have chosen an open activator was to allow the tongue to move anterior during the treatment , in the same direction with the mandible determined by the construction bite and to apply in the anterior third of the palatal vault. Also, it is more comfortable for the patient compared with a full, classic one.

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SPECTROMETRIC DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY IN CHLORPROMAZINE RADICAL CATION – ASCORBIC ACID SYSTEM

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SPECTROMETRIC DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY IN CHLORPROMAZINE RADICAL CATION – ASCORBIC ACID SYSTEM ***(***Abstract). Through univalent oxidation chlorpromazine forms a colored and relatively stable radical cation with maximum absorbance at 525 nm, considered a redox mediator in a number of biochemical reactions. **Aim**: To develop a spectrometric method for the determination of total antioxidant activity based on the reaction of chlorpromazine radical capture by ascorbic acid (standard antioxidant). **Material and methods**: The calibration curve was drawn by monitoring the decrease in the absorbance of the preformed radical solution (obtained by oxidation of chlorpromazine by potassium persulfate in an acidic environment) depending on ascorbic acid concentration**. Results**: The method was validated. In the ascorbic acid concentration range 10-100 μM/L linearity was good (r^2 = 0.9991). Limit of detection (LOD) was 3.13 μM/L and limit of quantification (LOQ) was 9.49 μM/L. System precision (RSD = 0.75%), method precision (RSD = 4.50%) and intermediate precision (RSD = 4.63%) were determined. The average recovery of 101.7% for the concentration range 91.1 -105.9% confirmed the accuracy of the method. **Conclusions**: The method has a good linearity, precision, accuracy, and is easy to use for evaluation of antioxidant action of different products. Total antioxidant activity is expressed as ascorbic acid molar equivalents (AAE). The method has the advantage of using a radical involved in redox processes in the body**.** ***Keywords***: CHLORPROMAZINE RADICAL CATION**,** ANTIOXIDANT ACTIVITY.

In order to assess the antioxidant activity of some products (plants, medicines, food, nutritional supplements, cosmetics, etc.) various methods have been developed, most of them based on the quenching of preformed colored free radicals by the antioxidants in the analyzed samples. The most commonly used free radicals are DPPH and ABTS, but they have the disadvantage of not existing as such in biological environments (1-5).

Chlorpromazine ([2-chloro-N-(3-dimethyl aminopropyl phenothiazine]) forms by univalent oxidation a colored and relatively stable radical cation; it is considered that these radicals are important intermediates in the biochemical activity and metabolism of phenothiazines in general and of chlorpromazine, in particular.

Chlorpromazine (CP) is an efficient peroxidase substrate acting as redox mediator (mediator of electron transfer reactions)

through the cation radicals it forms ($CP^{\bullet +}$). Due to the high reduction potential of CP-$CP^{\bullet +}$ system (860 mV), these radicals exert a pro-oxidant action in the body (6).

It has been demonstrated by UV-VIS and ESR spectroscopic studies that chlorpromazine radical cation is able to oxidize aminopyrine (aminophenazone) to its radical cation (7).

Similarly, chlorpromazine radicals can stimulate the production of toxic intermediate free radicals from various drugs and other xenobiotics.

In this context, chlorpromazine cation radicals, with major implications in biological systems, can be used for the assessment of antioxidant action of various products.

The aim of this paper was to use chlorpromazine radical cation in the development of a simple, rapid and inexpensive spectrometric method for the assessment of antioxidant activity applicable for different products.

MATERIAL AND METHODS

Reagents:

- chlorpromazine maleate (Sigma) 0.01 M aqueous solution;
- potassium persulfate (Merck) 0.001M aqueous solution;
- hydrochloric acid 37% (Farmitalia) 1M solution;
- L(+) ascorbic acid (Merck) standard solution 0.01M. Etalon solution 2.10^{-4} M was prepared by dilution of standard solution at the time of use.

Apparatus:

- UV-VIS Spectrophotometer (Hewlett Packard 8453);
- Analytical balance (Scaltec).

Principle of the method: Chlorpromazine maleate reacts with potassium persulfate in an acidic medium, resulting in radical cation $CP^{\bullet +}$ with absorption maximum at 525 nm. Ascorbic acid captures the chlorpromazine free radicals and determines a decrease in radical solution absorbance; solution discoloration is proportional to ascorbic acid concentration.

Obtaining a calibration curve. In order to prepare a radical solution, for a volume of 1 mL chlorpromazine maleate solution 0.01 M, 8.5 mL 1M HCl and 0.5 mL 0.001M $K_2S_2O_8$ solution were added.

For larger amounts these quantities are multiplied.

20 minutes after adding 0.001M $K_2S_2O_8$ solution, at 2mL radical solution 1 mL ascorbic acid etalon solution was added having in concentrations between 10- 100μM /L. Absorbance of solutions was measured at 525 nm in a 1 cm cell against water.

Method validation. To validate the method the following parameters have been determined: response function linearity, limit of detection (LOD), limit of quantification (LOQ), precision (system precision, method precision and intermediary precision), and accuracy (recovery method) (8).

RESULTS AND DISCUSSION

The analytical domain was studied by determination of optimum ascorbic acid concentration. It was established that good results had been obtained in the concentration range $1.5.10^{-4} – 2.10^{-4}$ M.

The optimum ascorbic acid concentration was 2.10^{-4} M because the slope was greater and the difference between absorbance values was enough in the concentration range considered (fig. 1).

Linear response function was studied in triplicate in the range of 10-100 μM /L ascorbic acid concentration (tab. I).

From the calibration curve, obtained by using the average absorbance values, it could be seen that the developed method was linear

in the analyzed concentration range.

The equation of calibration curve was calculated using the least squares method.

The linearity of results was assessed by plotting the variation in concentration levels calculated by the equation of calibration curve according to theoretical ascorbic acid concentration (fig. 3).

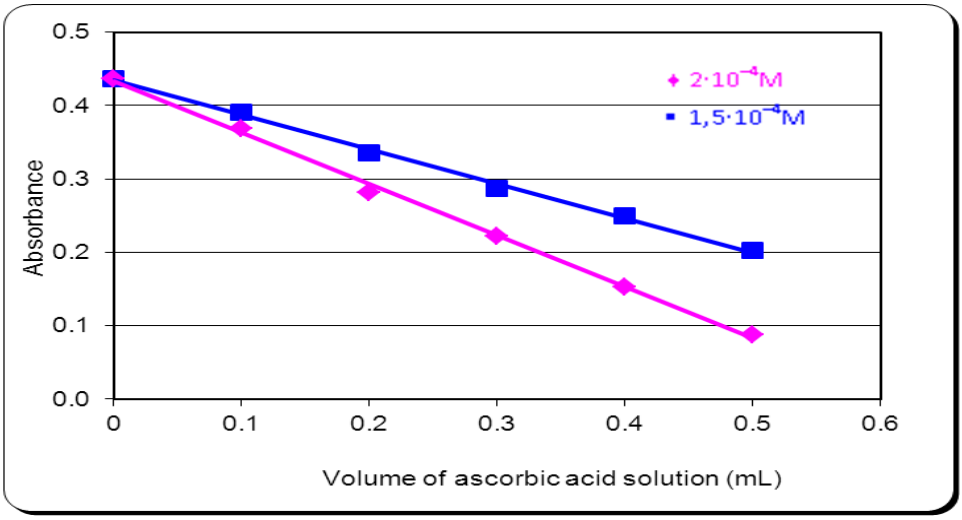

Fig. 1. Analytical domain

TABLE I

Linearity of the method

No.	Ascorbic acid concentration (μM/L)	Absorbance			
		I	II	III	Average
1	10	0.50143	0.49367	0.49991	0.498337
2	20	0.47917	0.48485	0.48152	0.481847
3	30	0.44793	0.48137	0.45829	0.462530
4	40	0.42057	0.45785	0.45164	0.443353
5	50	0.42158	0.41835	0.42021	0.420047
6	60	0.38454	0.42068	0.39405	0.399757
7	70	0.36134	0.40562	0.38487	0.383943
8	80	0.33152	0.39640	0.35993	0.362617
9	90	0.31616	0.38851	0.32778	0.344150
10	100	0.29738	0.38376	0.29846	0.326533

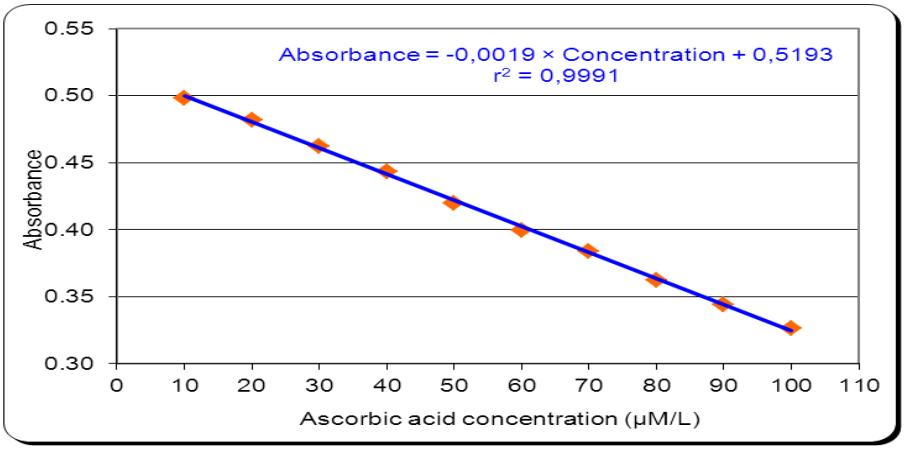

Fig. 2. Calibration curve

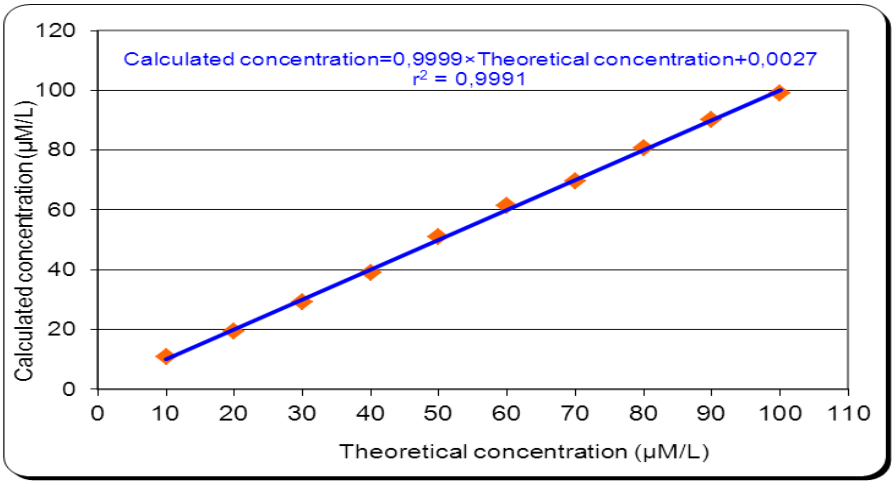

Fig. 3. Linearity of results

It was found that between the calculated and theoretical concentration values there was a linear dependence, the curve slope being 0.9999 and intercept 0.0027.

Limit of detection (LOD= 3.13 µM/L) and *limit of quantization* (LOQ= 9.49 µM/L) were calculated based on standard deviation and slope of regression straight line.

In order to estimate *precision* the following were determined:

- the repeatability of determination (*system precision*) for a number of 10 determinations, the relative standard deviation (RSD) value being 0.7535%;

- the repeatability of analysis (*method precision*) and *intermediate precision* for three assays at three different concentrations values.

Samples concentration was calculated using the calibration curve equation and the percent of calculated concentration from theoretic concentration was established. The results were statistically processed. For intermediate precision determination, this

experiment was repeated on the following day in the same way but with different reagents (tab. II).

From the experimental data we found that in the range ±20% of the value of interest the proposed method was precise, the relative standard deviation (RSD) being less than 5%.

To assess *the accuracy* of the method, a known ascorbic acid concentration was added to a determined amount of ascorbic acid solution. The samples were spectrometrically analyzed and accuracy was calculated based on the concentration of the recovered analyte and that of the added analyte; the recovery for a number of three samples at three different levels of concentration is presented in table II.

TABLE II

Method precision, intermediate precision and accuracy of the method

Nr.	Theoretical concentration (μM/L)	The method precision		Intermediary precision		Accuracy of The method	
		Calculated concentration (μM/L)	%	Calculated concentration (μM/L)	%	Calculated concentration (μM/L)	Recovery %
1	40	36.23812	90.6	42.87770	107.2	40.35250	100.9
2		40.43479	101.1	39.45248	98.6	36.42841	91.1
3		41.86968	104.7	37.28729	93.2	40.57879	101.4
4	50	52.15048	104,3	51.47675	103.0	48.84355	97.7
5		51.70819	103.4	50.60759	101.2	52.26363	104.5
6		51.51790	103.0	48.94641	97.9	52.96307	105.9
7	60	63.81989	106.4	63,33645	105.6	63.25931	105.4
8		61.64441	102.7	63.26959	105.4	62.53415	104.2
9		62.42100	104.0	63.92275	106.5	62.41586	104.0
Statistics		**Average**	**102.2%**	**Average**	**102.1%**	**Mean recovery**	**101.7%**
		SD	**4.6046**	**SD**	**4.7331**	**Minim**	**91.1%**
		RSD	**4.5036%**	**RSD**	**4.6373%**	**Maxim**	**105.9%**

The data obtained shows that the proposed method is accurate, average recovery being 101.7% in the 91.1% to 105.9% range.

In order to determine the antioxidant activity of some biological products, a certain amount of the sample, obtained by the appropriate processing of the product, is added to 2 mL of preformed radical solution, absorbance is determined and from the calibration curve the antioxidant activity expressed as molar equivalents (micromolars) of ascorbic acid (AAE) per unit of measure chosen for the sample can be estimated.

CONCLUSIONS

The capture of chlorpromazine chromophore radical cation ($CP^{\bullet+}$) by ascorbic acid (standard antioxidant) causes a decrease in the absorbance at 525 nm of the preformed radical solution proportional to ascorbic acid concentration.

Based on this reaction a spectrometric method for the determination of antioxidant activity in the $CP^{\bullet+}$ - ascorbic acid system was developed and validated. The proposed method was linear over the concentration range 10-100 μM/L ascorbic acid. Precision (system precision, method precision and intermediate precision), limit of detection (LOD), limit of quantification (LOQ) and accuracy have been assessed. The obtained experimental data show that the proposed method is sensitive, precise and accurate. The method is simple, inexpensive and easy to use requiring usual reagents and equipment; it can be used to determine the antioxidant activity of medicinal plants (lyophilisate, aqueous extracts, and tea) or pharmaceutical products containing antioxidant components, the result being expressed as ascorbic acid molar equivalent (AAE) per unit of measure chosen for the test sample.

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VALIDATION OF A SPECTROPHOTOMETRIC ASSAY METHOD FOR BISOPROLOL USING PICRIC ACID

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VALIDATION OF A SPECTROPHOTOMETRIC ASSAY METHOD FOR BISOPROLOL USING PICRIC ACID (Abstract): Bisoprolol is a drug belonging to beta blockers drugs used primarily for the treatment of cardiovascular diseases. **Aim:** A spectrophotometric method for quantitative determination of bisoprolol was developed based on the formation of a complex combination between bisoprolol and picric acid. **Material and methods**: The complex combination of bisoprolol and picric acid has a maximum absorbance peak at 420 nm. Optimum working conditions were established and the method was validated. **Results**: The method presented a good linearity in the concentration range 5-120 μg/ml (regression coefficient $r^2 = 0.9992$). The RSD for the precision of the method was 1.74 and for the intermediate precision 1.43, and recovery values ranged between 98.25-101.48%. **Conclusions:** The proposed and validated spectrophotometric method for the determination of bisoprolol is simple and cost effective. **Keywords:** SPECTROPHOTOMETRIC METHOD, VALIDATION, BISOPROLOL, PICRIC ACID.

Bisoprolol is a valuable drug used in the treatment of cardiovascular diseases. It has special pharmacokinetic properties demonstrated by numerous clinical studies.

There are few spectrophotometric methods for the assay of beta blockers and fewer for bisoprolol. Most of the published methods are high performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (LC–MS/MS), liquid chromatography–electrospray ionization mass spectrometry (LC–ESI, MS) and voltammetry methods (1-12).

This paper presents a spectrophotometric method for the assay of bisoprolol using picric acid as reagent. The developed method was validated by studying the following parameters: linearity, detection and quantification limit, precision and accuracy (13-16).

MATERIAL AND METHODS

Reagents and apparatus:

- bisoprolol fumarate - 100.07% pure, reference substance produced by UniChem Laboratories, LTD, India;
- all the other used reagents were produced by TunicProd, Bucharest, Romania;
- Kern 770 Analytical balance;
- Hewlett Packard 8453 UV-VIS spectrophotometer;
- Ika Vibrax VXR Basic ultrasonic bath.

Solutions:

- 100 μg/ml bisoprolol stock solution:

10 mg bisoprolol were dissolved in chloroform in 100 ml volumetric flask;

- Working solutions containing 5 to 120 µg/ml bisoprolol were obtained by diluting the stock solution with chloroform.

- 0.1% picric acid solution prepared in chloroform.

Principle of the method: bisoprolol fumarate forms with picric acid a complex combination soluble in chloroform with maximum absorbance at 430 nm, proportional with bisoprolol concentration.

In order to establish the optimum wavelength for the detection, 1 ml of the 5 µg/ml and 120 µg/ml working solution were mixed with 1 ml 0.1% picric acid solution. The UV-VIS absorption spectrum was recorded using a 1 cm cell after 25 minutes.

In order to establish the optimum working conditions, two solutions of 5 µg/ml and 120 µg/ml were used (the minimum and maximum concentration of the linear range), while the parameters of the method were changed. The optimum concentration of picric acid solution and the stability of the reaction product were determined.

Procedure: 1 ml of 0.1 % (w/v) picric acid solution was added to 1 ml of bisoprolol in a concentration range of 5 - 120 µg/ml and then stirred for 5 minutes. The absorbance was measured at 430 nm in a 1 cm cell versus a blank solution prepared in similar conditions.

Validation of the method

Detection limit (LOD) and quantification limit (LOQ) were calculated using the following formulas: LOD = 3 x standard error / slope, and LOQ = 10 x standard error / slope (7).

Precision of the method was investigated through its repeatability and reproducibility. Three solutions of 20 µg/ml, 60 µg/ml and 80 µg/ml bisoprolol were used. Three assays were performed for each concentration on the same day in order to assess repeatability. Three sets of assays were performed on different days in order to evaluate reproducibility.

In order to establish the accuracy of the method, bisoprolol solutions of 20 µg/ml, 60 µg/ml and 80 µg/ml were analyzed. For each concentration, three determinations were performed.

RESULTS AND DISCUSSION

The analysis of absorption spectra showed a maximum absorbance for the reaction product at 420 nm (fig. 1). This value was used for all determinations.

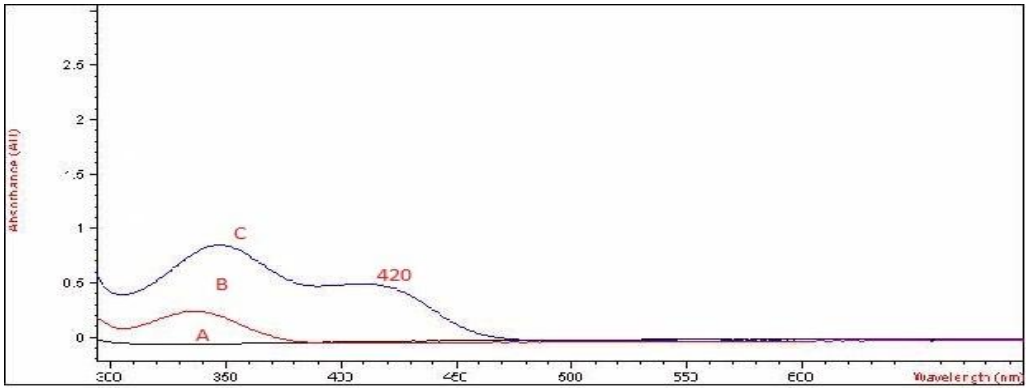

Fig. 1. Absorption spectra for bisoprolol (A), picric acid (B), and reaction product (C)

The optimum concentration of picric acid solution was found to be 0.1 % (w/v) (tab. I).

Absorbance was measured 25 minutes after preparation (tab. II).

Linearity: solutions containing bisoprolol in a concentration range of 5 – 150 μg/ml were used.

The obtained data (tab. III) were analyzed by linear regression (fig. 2) and then a calibration curve was obtained (fig. 3). In conformity with the experimental data, the developed method for bisoprolol determination was linear in 5 - 120 μg/ml concentration range. The parameters of linearity are shown in table III.

TABLE I

Reagent concentration

Picric acid (% w/v)	**Bisoprolol**	
	5 μg/ml	**120 μg/ml**
0.025	0.0319	0.5186
0.05	0.0320	0.5198
0.1	0.0327	0.5284
0.5	0.0326	0.5276
1.0	0.0325	0.5267
1.25	0.0325	0.5255

TABLE II

Stability of reaction product

Bisoprolol (μg/ml)	**Time (minutes)**						
	5	**10**	**15**	**20**	**25**	**40**	**60**
5	0.0320	0.0321	0.0322	0.0324	0.0328	0.0327	0.0326
120	0.5243	0.5259	0.5262	0.5268	0.5289	0.5283	0.5275

TABLE III

Linearity of the method

Bisoprolol (μg/ml)	**Absorbance (λ = 420 nm)**				**Average**
	I	**II**	**III**	**IV**	
5	0.0313	0.0327	0.0324	0.0326	0.0323
10	0.0542	0.0483	0.0480	0.0478	0.0495
20	0.0883	0.0870	0.0880	0.1094	0.0932
40	0.1880	0.1879	0.1860	0.1976	0.1899
60	0.2737	0.2755	0.2725	0.2857	0.2768
80	0.3683	0.3558	0.3625	0.3581	0.3612
100	0.4460	0.4440	0.4396	0.4374	0.4417
120	0.5288	0.5195	0.5288	0.5123	0.5223
130	0.5292	0.5287	0.5296	0.5254	0.5282
140	0.5282	0.5275	0.5288	0.5298	0.5286
150	0.5268	0.5295	0.5301	0.5265	0.5282
Linearity parameters	Range 5 - 120 μg/ml. Intercept = 0.00336 Slope = 0.0113. Correlation and regression coefficient r = 0.9995, r^2 = 0.9992 Detection limit (LOD) = 0.89 μg/ml Quantification limit (LOQ) = 2.97 μg/ml				

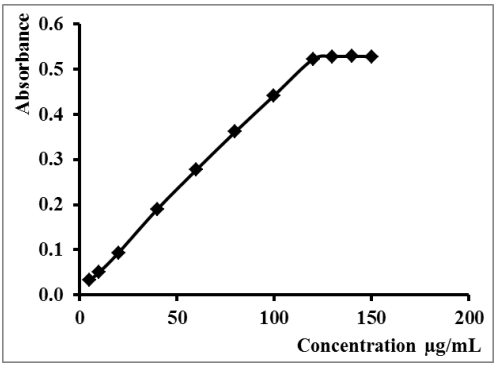

Fig. 2. Linearity

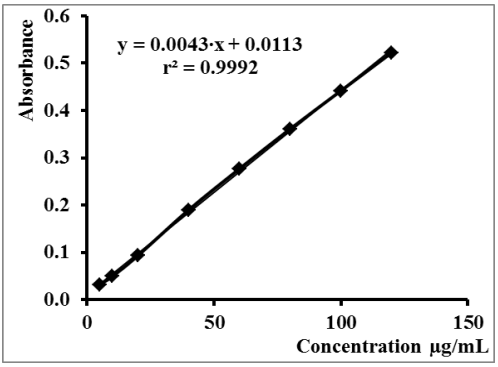

Fig. 3. Calibration curve

The calibration curve equation was: Absorbance = 0.0043 × concentration + 0.0113.

The precision of the method was closely observed through repeatability and reproducibility.

Bisoprolol concentration in the samples was calculated based on the experimental data and calibration curve equation (tab. IV). The relative standard deviation was less than 2%. This fact proves that the proposed method is precise.

Accuracy: sample concentration was calculated based on the experimental absorbance data and the regression curve equation (tab. IV). We noticed that recovery was between 98.64 and 101.08 for the studied concentration range. These values prove that the proposed method is accurate.

TABLE IV

Method precision and accuracy

Bisoprolol (µg/ml)	**Repeatability**		**Reproducibility**		**Accuracy**	
	Absorbance	**Recovery %**	**Absorbance**	**Recovery %**	**Absorbance**	**Recovery %**
20	0.0963	98.51	0.0969	99.20	0.0965	98.74
	0.0980	100.52	0.0985	101.06	0.0979	100.36
	0.0944	97.30	0.0961	98.27	0.0968	99.09
60	0.2737	101.38	0.2728	101.03	0.2749	101.08
	0.2725	100.93	0.2759	102.23	0.2726	100.96
	0.2657	98.27	0.2683	99.30	0.2666	98.64
80	0.3592	100.82	0.3549	99.57	0.3588	100.70
	0.3616	101.51	0.3619	101.60	0.3636	101.05
	0.3581	100.49	0.3602	101.10	0.3587	100.67
Statistical data	RSD% = 1.74		RSD% = 1.43		Mean = 100.34	

CONCLUSIONS

A spectrophotometric method was developed for the assay of bisoprolol using picric acid as reagent. Statistical analysis proved that the method is repeatable for estimation of bisoprolol as bulk drug.

The proposed method was found to be simple, easy to perform, precise and accurate.

It is a novel method and can be used as a more economic alternative to other spectrophotometric techniques for routine analysis in drug quality control or in laboratory.

ACKNOWLEDGEMENTS

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CONSIDERATIONS ON THE EPIDEMIOLOGY, ETIOLOGY AND PROFILE OF PERIODONTAL - DENTAL INJURIES IN PRESCHOOL POPULATION IN IAŞI COUNTY

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CONSIDERATIONS ON THE EPIDEMIOLOGY, ETIOLOGY AND PROFILE OF PERIODONTAL-DENTAL INJURIES IN PRESCHOOL POPULATION IN IAŞI COUNTY (Abstract): In recent years there has been a considerable increase in the number of temporary teeth injuries, this variability being determined by multiple etiologic factors, which we need to be aware of, as well as by predisposing conditions. **Aim:** To characterize the epidemiology, etiology and pathology of specific types of periodontal-dental trauma in a series of preschool children from Iasi County. **Material and methods:** The study included a total of 672 subjects aged 3 to 7 years old, from both urban and rural areas of Iaşi County. Statistical analysis was performed using *STATISTICA* software used in medical research. **Results:** Prevalence of periodontal-dental trauma in the study group was 19.64%. Gender distribution in the study group showed a preponderance of males (62.12%). The risk of periodontal-dental trauma in deciduous teeth was 1.63 times higher in boys compared to girls. Fall accidents (41.67%) closely followed by playground accidents (31.82%) were the most common causes of periodontal-dental trauma in the study group. **Conclusions:** The prevalence of temporary dental trauma in the study group was relatively high, requiring the implementation of oral health promotion strategies and prevention programs, primarily addressed to parents and educators. **Keywords:** PERIODONTAL-DENTAL TRAUMA, TEMPORARY TEETH, PREVALENCE, ETIOLOGY.

Oral and facial traumatic pathology in children is a complex issue with a great impact on cervico-facial and psycho-somatic harmony, its epidemiological, clinical, therapeutic and developmental features differing from traumatic injuries in adults.

Data in the literature on the prevalence of periodontal dental trauma vary considerably from study to study. This large variability could be accounted for by a combination of factors such as trauma classification, type of studied dentition, or differences in geographical area, socio-cultural, and behavioral patterns of the studied groups (1).

In recent years there has been a considerable increase in the number of temporary teeth injuries, this variability being determined by multiple etiologic and predisposing factors which we need to be aware of

and that require preventive, curative and interceptive measures (1).

This paper aims to determine the epidemiological profile, etiology and specific lesions of dental periodontal traumas in a preschool population of Iasi County.

MATERIAL AND METHODS

The study group included a total of 672 subjects selected of the 729 targeted children aged 3 to 7 years old, from urban and rural areas of Iasi County, who have been examined in the Iasi Pediatric Dentistry Clinic between May, 2011- May, 2012.

Children were examined by specialist dentists who have previously participated in a calibration process to minimize potential inaccuracies of diagnosis and classification of traumatic lesions; thus, the classification proposed by Andreasen et al. (1) has been used.

Children legal guardians were informed about this research through oral presentations by the research team. All legal guardians were asked to fill-in a questionnaire including demographic data, circumstances of injury, and provided an agreement to enroll their child/children in the research. Also, a written informed consent was obtained.

Criteria for inclusion in the study were: age (3 to 7 years), obtaining informed consent from parents or legal guardians, absence of severe anterior teeth caries. Exclusion criteria were: refusal to provide informed consent, lack of child cooperation, eruption of permanent incisors.

The approval of the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy - Iasi and Iasi School Inspectorate were as obtained.

For statistical data processing we used *STATISTICA* software. Specific tests (ANOVA, Pearson Chi - square (χ^2), Spearman, Gamma) were used and the parameters of interest were analyzed. Thus **p**, the calculated reference indicator, reflected the level of significance of the tests, was compared with p = 0.05 corresponding to a 95% confidence interval (CI) with a significant value for p <0. 05.

RESULTS

Prevalence of periodontal dental trauma in the study group was 19.64%.

Sex distribution of our patients was homogeneous (52.53% boys, 47.47% girls). When analyzing the sex distribution of injured children, a predominance of boys (62.12%) was found. This is supported by the nonparametric correlation Chi-square test, which indicates a moderate association between boys and presence of trauma ($\chi 2$ = 6.06, r = -0.438 p = 0.0137, 95% CI).

The risk for dental periodontal trauma in deciduous teeth for boys was 1.63 times higher than for girls (Odds Ratio = 1.63, minimum = 1.08, maximum = 2.45, 95 % CI). Prospectively, by calculating hazard ratio (the relative risk (RR)), it came out that boys have a 1.48 times higher risk of periodontal dental trauma (RR = 1.48, minimum = 1.08, maximum = 2.04, 95% CI). The rural/urban distributions of children with dental trauma revealed a higher prevalence (61.36%) of traumas in urban areas, and a moderate statistical association between the two parameters (tab. I).

Risk of periodontal dental trauma in urban children (calculated by contingency table) was significantly higher than in rural children. The calculated odds ratio was 2.12 (minimum = 1.41, maximum = 3.20, 95% CI) and the risk ratio (RR) was estimated at 1.83 (minimum = 1.34, maximum = 2.51, 95% CI).

TABLE I

Parameters estimated in testing the association of periodontal dental trauma in temporary teeth *vs.* area of origin

df=1	Chi-square χ^2	P *95% Confidence Interval*
Pearson Chi-square - χ^2	14.73144	0.00012
M-L Chi-square	14.75281	0.00012
Correlation Coefficient (Gamma)	-0.359902	0.00012

As to the etiology of periodontal dental trauma in temporary teeth in our study group, fall accidents (41.67%) ranked first, closely followed by playground accidents (31.82%).

Dental lesion profile in our patients was dominated by subluxations (26.52%), enamel fracture and intrusion, identified in 23.48% and 18.18% of cases, respectively. In significantly lower percentages other types of lesions were recorded: enamel and dentin fracture (9.09%), contusion (8.33%), lateral luxation (6.82%), penetrating fracture (4.55%) and avulsion (3.03%).

The mean age of injured children was 53.4 months ± 11.13 DS.

The relationship between dental-periodontal injury pattern correlation and mean age of children reflected a correlation of dental avulsions with the highest mean age (65.50 ± 12.56 DS), and of the penetrating fractures with the lowest mean age (42.67 ± 7.42 DS)) (tab. II).

TABLE II

Age-related indicators for the type of periodontal dental trauma in temporary teeth

Type of trauma	Mean age (months)	Mean		Std. Dev.*	Std. Er.†	Min‡	Max$^{\alpha}$	Q25$^{\beta}$	Median	Q75
		-95%	*-95%*							
Enamel fracture	55.00	51.04	58.96	10.80	1.94	36.00	65.00	49.00	62.00	63.00
Enamel and dentine fracture	61.17	56.44	65.90	7.44	2.15	52.00	68.00	53.00	66.00	68.00
Enamel, dentin and pulp fracture	42.67	34.88	50.45	7.42	3.03	37.00	54.00	37.00	39.00	50.00
Subluxation	50.23	46.11	54.35	12.00	2.03	37.00	75.00	39.00	50.00	56.00
Lateral luxation	47.67	41.70	53.63	7.76	2.59	39.00	57.00	42.00	42.00	56.00
Intrusion	53.71	49.93	57.49	8.96	1.83	43.00	70.00	45.50	55.00	58.00
Avulsion	65.50	45.52	85.48	12.56	6.28	47.00	75.00	58.50	70.00	72.50
Contusion	56.00	48.19	63.81	11.62	3.50	46.00	76.00	47.00	48.00	70.00
Total Groups	53.40	51.48	55.32	11.13	0.97	36.00	76.00	44.50	53.50	63.00
Anova Test: p=0.00115										

*Standard Deviation; †Standard Error; ‡Minimum; $^{\alpha}$Maximum; $^{\beta}$Quartiles.

The analysis of lesional types according to the cause of trauma revealed that playground accidents mostly resulted in enamel fractures. Periodontal-dental trauma due to falls has most frequent resulted in lateral luxation, complicated fractures and subluxations, while bicycle accidents in intrusions and avulsions (fig. 1).

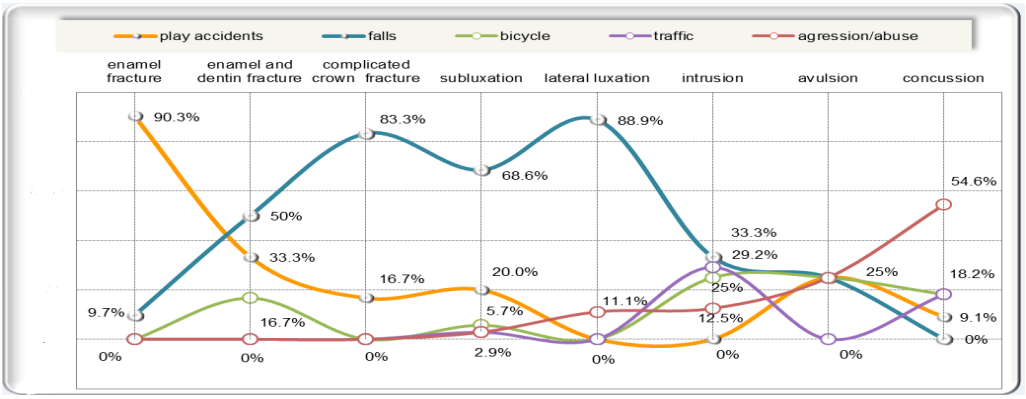

Fig.1. Temporary teeth dental-periodontal trauma etiology vs. type of injury

DISCUSSION

The prevalence of temporary teeth periodontal-dental trauma varies greatly in the literature. Thus, in Brazil (2, 3) it ranges between 9.4% and 41.6%, while in the US (4) it reaches 23.00% compared with 18% in Belgium (5). It should however be noted that many of these studies report the prevalence of different age groups (1), from preschool environments, both public and private, with or without socioeconomic differences, so they are unlikely to be comparable. As mentioned, this study was conducted on a age-limit population, and the traumatic events occurring before the age of 3 years, when there is an increased risk of dental injuries, were not recorded.

The 19.64% prevalence of traumatic injuries in temporary dentition found in our study group is close to that reported by Carvalho (5). This value can be close to the real one, but our study group being a small one it may not be representative for the entire pediatric population in the area of interest.

Although recent studies (4, 6) show that the distribution of periodontal dental trauma in temporary dentition is less influenced by sex, some authors (7) report that there is a higher frequency of occurrence in boys, as supported by the present research, which revealed a moderate association between trauma and boys.

The main etiological factor of periodontal-dental traumatic accidents in our study was falls, as found in most studies (5, 8, 9). Especially in younger age groups, poor motor coordination, specific curiosity and carelessness, may make children to perform tasks at high risk of trauma.

Data of various studies on periodontal dental injuries are difficult to compare due to the different classification criteria found in the literature; nevertheless, coronal fractures, simple or penetrating, are often reported as lesions with the highest prevalence by many authors (10, 11, 12). On the other hand, there are a significant number of studies which concluded that dental

luxation proved to be the most common type of injuries seen in primary dentition (13, 14). In our study subluxations and enamel fractures are the most common injuries. These differences can be accounted for by variations in study group selection, screening method, and researchers.

CONCLUSIONS

Prevalence of temporary teeth periodontal – dental trauma in our study group was relatively high (19.64%), requiring the implementation of strategies aimed at promoting oral health and prevention programs for parents and educators. To establish a more accurate profile of pediatric periodontal-dental trauma in our region and for their correct management, all medical and social institutions of interest in the field should become involved.

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SÜDECK´S POST-TRAUMATIC OSTEODYSTROPHY

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SÜDECK´S POST-TRAUMATIC OSTEODYSTROPHY (Abstract): The diversity that exists in the types of trauma, in the investigated anatomical structures, in the sites of trauma, in the outcomes of traumatic injuries, and in the general reaction of the body lead to a symptomatological polymorphism of post-traumatic sequelae. Therefore it is impossible to establish a well-defined nosological entity of these sequelae. The damaged tissues react under a very similar scheme, irrespective of the type of tissue, trauma or area, namely, through an inflammatory process, that causes various sequelae depending on certain parameters. Südeck post-traumatic osteodystrophy may be defined as a pathological entity based on some well-defined clinical features, on the development of its own therapy and on its more or less ubiquitous character. Südeck`s post-traumatic osteodystrophy of the hand is rarely found isolated. This "acute bone atrophy" is usually included in the "pathological syndrome of the hand" and is caused by circulatory disorders that occur on a traumatized hand or on a hand with injury "at a distance", especially when nerve damage is involved. **Keywords**: ALGONEURODYSTROPHY, POST-TRAUMATIC SEQUELAE, REFLEX SYMPATHETIC DYSTROPHY.

Vandenabelle (1) gave in 1976 an historical overview of algoneurodystrophy (AND) including description, outcomes and the names under which this disease was known: post-traumatic osteoarthropathy, acute bone atrophy, post-traumatic painful osteoporosis, reflex sympathetic dystrophy, neurotrophic rheumatism, shoulder-hand syndrome, Südeck-Leriche syndrome, neurotrophic syndrome, sympathetic algodystrophy of the limbs). Etiopathogenic and pathophysiological mechanisms were also chronologically reviewed.

Algoneurodystrophy is a form of disease that has a great variety of clinical aspects (2). That is why there are many controversies surrounding the different issues that define its nosological relations. One of them is “the acute inflammatory bone atrophy” (1900, Südeck) (3), which was seen as a clinically well-defined post-traumatic sequel. The anatomical substrate is the osteoporosis, that was called "acute inflammatory bone atrophy" by Südeck and was seen as a clinically well-defined post-traumatic sequela, but Südeck considered actually the inflammatory process as dominant.

The first relatively complete anatomoclinical description of algoneurodystrophy is attributed to Südeck (1900). The term “anatomical substrate” was used

for the first time by Südeck and this was the premise for describing the radiological changes. The anatomical substrate is the osteoporosis that was called "acute inflammatory bone atrophy" by Südeck and was seen as a clinically well-defined post-traumatic sequela.

This disease was later called "post-traumatic painful osteoporosis" by Leriche (3). He considered it as a local disturbance of the neurovegetative system and, as the sympathetic nervous system plays a main role, it was also called "reflex sympathetic dystrophy" in the Anglo-Saxon speaking world. The disease was later known as Südeck-Leriche syndrome (3).

Some neurotrophic disturbances may occur at the level of the hand after the injuries of this segment, as well as after the injuries "at a distance" of the upper limb (4).

These disturbances are not consistent with the severity of the primary traumatic lesions, but rather with the subsequent complaints experienced by the injured person, with the excitability of his nervous system, with the administered treatment and with the poor outcome of treatment of lesions.

Definition of Südeck´s post-traumatic osteodystrophy. Osteodystrophy is a generalized disease of the skeleton, characterized by decreased bone mass and deteriorated micro-architecture of the bone tissue. Osteoporosis leads to increased bone fragility and risk of fractures that may occur in minor trauma or even spontaneously. Osteoporosis is the most frequent metabolic bone disease and affects women more than men. It decreases the bone strength, which brings a greater risk of fractures. The major issue in the management of osteoporosis lies in the prophylaxis of this disorder, and the most economically advantageous and the easiest to apply method in the pacient self-care is the kinetotherapy prophylaxis.

Pathophysiological syndrome of the hand. The disorders of this syndrome may be designated as "syndrome of post-traumatic nervous irritation" or as "post-traumatic reactive syndrome"(5).

These post-traumatic trophoneurotic disturbances of the upper limb and of the hand in particular represent a disputed issue, both in terms of etiopathogenesis and treatment. The trophic disturbances have a progressive onset, from fingers to shoulder, when the lesion is distal, and from top to bottom, when the lesion is situated at distance from hand. No tissue is spared and there are to be seen alterations of the structure of skin, hair, nails, subcutaneous cellular tissue, aponeuroses, muscles and bones. The affected hand does not look like a normal hand anymore (6).

In milder cases, the tissue alterations suggest a simple atrophy caused by inactivity. In other cases, the extent of tissue alterations, the rapidity of their development, the associated vasomotor and motor disturbances, as well as the unusually high degree of function loss are due to the dynamic processes.

The pathophysiological syndrome of the hand may take various forms, depending on its intensity: from the simple atrophy caused by inactivity to the complex forms, associated with acute pain. The nervous mechanism that acts on the veins, capillaries, arteries and arterioles, causing nutritional disturbances in all tissues of distal segment of the upper limb, triggers also most of the alterations. Once this mechanism has been activated, it usually maintains a hyperemia with venous stasis, that

deeply disturbs the trophicity of the tissues as it becomes chronic.

The physiopathic syndrome of the hand is always associated with severe forms of Südeck`s osteoporosis. Most often the symptoms of these syndromes overlap and may be confounded: persistent pain that is exaggerated as compared to intensity of trauma, loss of hand function, edema and atrophy of subcutaneous cellular tissue, trophic disturbances of the skin, severe bone demineralization.

The topography of pain and of hyperesthesia does not correspond to the territory of any somatic nerve, as it is less localized and it is unbearable "as a visceral pain". The lesions of arteries or the injuries of the median nerve predispose frequently to nerve irritation syndrome; on the other hand, the median nerve has a much larger number of fibers that travel to the blood vessels as compared to the radial or the cubital nerve.

The centrifugal impulses that origin in the central nervous system and travel to the muscles, sudoriferous glands and blood vessels, as well as those that control vital activities of tissue cells are continuously dependent on the centripetal impulses coming from periphery. If these afferent messages are normal, then the efferent impulses are also normal. But if the afferent messages develop a harmful intensity and cause a permanent irritation, they disrupt the harmony of this automatic regulation, and the efferent impulses become also pathological and generate various disturbances (motor and vasomotor disturbances).

The cortex plays an important role in all these neurovascular disorders. In persons with labile nervous system, the nociceptive stimuli that come from the site of trauma through exteroceptors and proprioceptors act employing the cortex on the neurovascular system of the upper limb and of the hand in particular. This system is disturbed for a more or less long period of time, but sometimes it is chronically disturbed.

Any trauma, no matter how mild it may be, generates an irritative focus, that acts on the surrounding proprioceptors, that are very well developed at the level of fingers and in hand joints. The impulses that come from these areas and travel to the cerebral cortex generate changes in brain dynamics. If the signals that originate in the proprioceptors coincide in time with the excitations of the exteroceptors, conditioned reflexes can be produced. These conditioned reflexes may be sometimes fixed and lasting and may exert multiple effects on the function and trophicity of the tissues.

Clinical aspects. The symptomatology shows three clinical stages of evolution. Stage 1 emerges immediately or sometimes within a few weeks after the injury. It is characterized by acute pain, that increases with movement, skin hyperemia (the skin is warmer and more moist than normal), muscular hypotonia, edema of skin, of connective tissue and of muscle, early osteoporosis (7). Stage 2 develops in the next three months. The pain persists, the skin is cyanotic, cold, moist, and the hair in the area breaks off or gets lost. The edemas persist, joint stiffness occurs and the radiographic diagnosis is represented by the characteristic spotted osteoporosis. Stage 3 is generally regarded as irreversible and it is characterized by atrophic processes of skin, muscles, by having aponeurotic retraction and tendon retraction, severe osteoporosis (in some cases marked bone resorption), functional impotence of joints. At this stage the pain is

decreased, but it disseminates to the limb root. The skin becomes dry and cold.

Hyperemia may explain the osteoporosis, the edema of soft tissues, but it cannot fully motivate the pain, the atrophy and the sclerosis.

Thus, the syndrome is clinically characterized by hyperemia and cyanosis of the skin, that are usually associated with subcutaneous edema and with impairment of finger movements. The joint stiffness and the muscle atrophy also occur. The persistence of pain, the hard edem, the atrophy of subcutaneous tissue and of muscles, the thickening of the joint capsule, the deformation of the fingers in flexion, the progression of osteoporosis may also indicate this syndrome.

The evolution shows various forms of onset. *Acute onset* – in full health, weeks and rarely months after a traumatic episode or after an organic disorder, an algoneurodystrophic syndrome and its characteristic symptoms develop in 24 hours or in 30 days at most. In the cases with acute onset, the local symptoms are very intense and the general repercussion on the body is significant. *Subacute onset* – is the most common form. In 6-7 days the painful and vasomotor syndrome occurs. The local and general symptoms are less obvious than in the acute dramatic form of the acute onset. *Chronic onset* – is found usually at an older age, in neurological patients and in less reactive persons. In 15 days at most a painful and vasomotor syndrome gradually emerges, but it is frequently attenuated and therefore difficult to diagnose (8).

Diagnosis. The inflammation tests are negative and ESR may have values of 30 mm /h. The radiography reveals a local demineralization and shows a spotted osteoporosis of spongiosa. The compact bone of the diaphyses is spared for some time, but then it also become thin. Nevertheless joint space narrowing or cartilage thinning are not found.

The bone scintigraphy shows characteristic uniform uptake. If the traumatic lesions are peripheral (fingers), the atrophy progresses in a centripetal direction. If the lesions are "at distance" from hand (e.g. lesions of the nervous trunks), the bone atrophy progresses in a centrifugal direction, towards the periphery of the upper limb.

Osteoporosis occurs in closed or open mechanical trauma, in burns, frostbites, nerve damage and especially in the latent inflammations that follow these injuries.

The histopathological examination shows a discrete synovial and subsynovial edema. There are also perivascular cellular infiltrations with lymphocyte predominance.

The treatment is developed with regard to the triggering event, to the severity of trauma, to the stage of disease and to the prophylaxis.

If the syndrome begins to develop, the treatment involves the suppressing of all irritations that originate in the traumatic focus, balancing the nervous system, recovering the normal activity of the hand (active kinetotherapy).

The suppression of irritations is made by complementing the surgery of traumatic focuses and of their complications, namely, unreduced fractures, non-eliminated sequestra, irritating foreign bodies, painful nerve injuries, subacute infections, etc.

One of the best means of suppressing irritations of the traumatic focuses is the proper immobilization of the injured segment (immobilization depending on posi-

tion The balancing of the nervous system is also important and it is achieved by gaining patient`s confidence and by obtaining the patient`s full compliance to treatment, as well as through novocaine infiltrations at various levels, especially on the upper thoracic ganglia.

The somatic nerve infiltrations have a good outcome. They may be performed at distance from hand, on the nervous trunks or even on the brachial plexus or they may be locally applied, around the lesion, in the area of hyperesthesia. Thus, they act for the suppression of the nociceptive irritations. Local infiltrations with novocaine have a better outcome when the point from where the afferent nociceptive stimuli originate is precisely identified and injected. The infiltrations on the regional sympathetic nervous system act on the afferent pathways.

The recovery of the normal activity of hand (when possible) leads to healing in the shortest possible time. All procedures should be always performed carefully, skillfully and with full compliance of the patient, in order to achieve simultaneously the immobilization needed for the suppression of the irritations of the traumatic focus and the active mobilization of the uninjured segments. This mobilization should be performed carefully and persistently, progressively, as the movements must always be made below the threshold of pain.

The concomitant upper extremity suspension that reduces the venous stasis and edema, contributes to the implementation of the active kinetotherapy.

The edema of the back of the hand may represent one of the components of the physiopathic syndrome, especially when this edema becomes chronic as in hard edemas. However, the chronic edema of the back of the hand may also occur isolated, without the other components of the syndrome or without having components so marked as the edema. The edema requires surgery when it becomes chronic and hard.

Curative treatment – has the following aims: to eliminate pain, to intercept the pathogenic line, to recover the function of the affected segment. Combining drug therapy with kinetotherapy and with orthopedic surgery may result in cutting off the reflex circle, the restoring of a normal active vascularization and not least the pain relief.

The therapeutic means that pursue the specific blockade of the sympathetic nervous system represent the most reasonable strategy. This blockade alleviates pain, because the pain impulses that are produced as a reflex in the somatic C-fibers by the excitation of the adjacent sympathetic postganglionic fibers disappear. Thus, the pathological afferentation towards the intercalary neurons is interrupted (or reduced) and results in the reduced activity of these neurons. But the blockade does not completely eliminate pain, because the pain impulses continue to be triggered from the hyperemia area of the affected joints and they travel through the common thick somatic fibers towards the spinothalamic pathway.

Vasodilators are frequently used. Calcitonin reduces bone resorption, decreases the activity and the number of osteoclasts, but they do not repair what has been lost. It also decreases rapidly the pseudo-inflammatory phenomena and it has an analgesic effect through a double mechanism (peripheral and central). Calcium preparations, synthetic anabolic substances and psychotropic drugs may also be used.

The kinetotherapy treatment aims to

restore joint mobility and the muscle tone and represents the mainstay of recovery, but when associated with physiotherapy can give in some cases even better results (1, 10). Through the used methods and procedures, kinetotherapy plays a very important role both in primary and in secondary prevention, as the use of a specially structured and assembled physical exercise has a beneficial effect on disease prevention, as well as on prevention of deficiencies and dysmorphisms caused by sequelae of medical conditions or injuries (9).

Südeck and Böhler (cit. 10) suggested that this syndrome may be prevented if the nociceptive stimuli are suppressed. Although bone atrophy is thought to be "inevitable" in risk populations, the complete syndrome develops only if the rules of proper immobilization of the traumatic focus are not followed. This immobilization should be associated with the active mobilization of all uninjured segments. "No mobilization exercise should cause pain", because the pain is the most important nociceptive stimulus in such cases. This stimulus leads to neurovascular disturbances that maintain or aggravate Südeck`s syndrome. If the syndrome is installed (at the stage of spotted atrophy of spongiosa), the safest means of stopping its development is the proper immobilization itself, namely, uninterrupted, in the passive posture of the affected segment, with the active, careful mobilization of the mobilized segments, as long as the pain does not arise (11).

In order to combat the venous stasis and the edema, the suspension of the upper limb or at least of the hand can be simultaneously used. The following physiotherapeutic measures may also be recommended for accelerating the healing: short warm baths (for 10 minutes) followed by the suspension of the upper limb, circulatory gymnastics through warm baths alternating with cold baths, cold air baths (for 15-20 minutes), and diathermy may also have beneficial effects (12).

In bone atrophies with finger stiffness, the perseverance of kinetotherapists and the full and conscious compliance of the patient may result in the partial recovery of finger motility (13).

**Prognosis of Südeck`s syndrome.** The post-traumatic Südeck`s syndrome has usually a favorable prognosis, as the acute bone atrophy disappears progressively after the suppression of irritative noxae.

If the bone atrophy persists as a result of maintain the continuous irritations, it may become chronic and a marked osteoporosis of the whole upper limb develops. These bones have a "glassy" appearance.

The acute "spotted" atrophy may develop into chronic bone atrophy if the primary lesion and the irritations that arise from it are not managed in time.

The evolution of the disease is unpredictable, and remains unpredictable even when a complex pathogenic treatment is administered. Many forms that were treated with complex therapies evolve arbitrarily, and the vasomotor and painful symptoms may return after they have regressed as a result of the treatment (14, 15, 16).

CONCLUSIONS

The best management of Südeck`s disease is represented by the consideration for improving of the kinetotherapeutic strategies, for pain relief, for reducing vascular stasis, for avoiding contractures and capsular retractions, as well as for combating anxiety and depressive moods in patients.

By means of an educational therapeutic rehabilitating process, kinetotherapy aims to improve the physical and mental health of persons with special health needs, in order to facilitate them the social and occupational integration or reintegration.

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HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL DYSPLASIA

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HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL DYSPLASIA (Abstract): Pap testing is considered to be the best screening tool for cervical cancer but there is currently great interest in the possible application of Human Papilloma virus (HPV) testing to supplement Pap screening for cervical cancer. **Aim:** To determine the prevalence of high-risk HPV types in the studied population and to explore the association between high-risk HPV types and cervical dysplasia. **Material and methods**: Cross-sectional study conducted at the Iasi "Cuza Voda" Obstetrics-Gynecology Hospital and Suceava County Hospital. 332 women who underwent colposcopy for cervical lesions between 2006 and 2011 were included in this study. **Results**: The overall prevalence of HPV was 57.23%. HPV prevalence differs significantly in the three age groups up to 50 years. It was highest in patients below the age of 40 and progressively lower with advancing age. The overall prevalence of cervical dysplasia was 56.62%. The prevalence of cervical dysplasia was highest in the age groups up to 40 years. The most important determinant of HPV infection is age. **Conclusions**: Persistence of HPV appears to be associated with progression to squamous intraepithelial lesion. Dysplasia is often missed in a cervical sample either because of human error in screening and interpretation, or because of suboptimal quality of Pap smear. Incorporation of HPV testing into the present Pap screening program has the potential of making screening for cervical cancer more effective, and a necessary prelude to assessing this is by determining the prevalence of the high-risk types. **Key words:** INVASIVE CERVICAL CANCER, PAP TEST, HUMAN PAPILLOMA VIRUS

Invasive cervical cancer is a disease that can be prevented by early detection and treatment. Recognizable precursor lesions generally precede it. The introduction of Pap screening programs has accelerated the decline in mortality due to cervical cancer in the past 45 years. Although this indicates a successful screening program, a series of studies revealed the sensitivity of the Pap smear to be only 51% and an unacceptable number of women who have been cytological screened develop invasive cervical cancer.

The objectives of the study were to determine the prevalence of high-risk HPV types and of cervical dysplasia in the studied population and to explore the association between high-risk HPV types and cervical dysplasia.

MATERIAL AND METHODS

In order to achieve these objectives, a cross-sectional study was conducted at the Iasi "Cuza Voda" Obstetrics-Gynecology Hospital and Suceava County Hospital. 332 women who underwent colposcopy for cer-

vical lesions between 2006 and 2011 were invited to participate. Written informed consent was obtained from the women being screened. Subjects were assured that their participation was voluntary and that their regular medical care would not be affected if they declined to participate.

The procedure for sample collection for HPV testing was incorporated into routine Pap testing. Pap samples were collected in a liquid based medium, which allowed both cytological diagnosis and HPV testing to be done on the same sample.

A cervical broom device was used to collect cells from the endocervix. The brush was then swirled immediately into a 20-ml container of buffered fixative to dislodge the cells into the medium. The sample was transported to the cytology laboratory of the Synevo laboratories in Romania. Pap test was always done first. Residual samples were forwarded for HPV testing. Pap smear reporting was based on the Bethesda System and the results were forwarded to the researcher. Cytology results were classified as follows: negative, benign cellular changes (BCC), atypical squamous cells of undetermined significance (ASCUS), low grade cervical squamous intraepithelial lesion (LSIL) and high grade cervical squamous intraepithelial lesion (HSIL). A pathologist routinely reviewed all abnormal Pap smears.

Colposcopy with cervical biopsy was performed in all cases. The findings of cervical biopsy histopathology were defined as HSIL, LSIL, negative (no colposcopic abnormality), others (including condyloma, benign atypia, HPV changes, squamous intraepithelial lesion unqualified, squamous intraepithelial lesion unqualified favoring HSIL or LSIL, immature squamous metaplasia with or without atypical, mature squamous epithelium, acute and chronic inflammation), adenocarcinoma in situ (AIS), and invasive cervical cancer.

Confidence intervals (the least frequency with which the interval will contain the true parameter) do much more than assess the extent to which the null hypothesis is compatible with the data. They provide simultaneously an idea of the likely magnitude of the effect and random variability. In this study 95% confidence intervals were used to assess statistical significance and random variability of the point estimate. Confidence intervals can also show that the data do not contain the information necessary for reassurance about an absence of effect. For a study to provide evidence of lack of an effect, the confidence limits must be near the null value. P-values were reported when analysis of trends were done.

RESULTS

Data on patients'age and educational level were obtained from laboratory requisition form and clinical documentation. Mean age of patients was 36.9 ± 11.57 years with a median of 28 years. Most of the patients (176 – 53.01%) followed a medium education (high school). Age-group prevalence of HPV infection is presented in tab. I.

TABLE I

Age-group prevalence of HPV infection

Age group	Number of subjects	HPV prevalence	95% CI
<30	106 (31.93%)	58.49%	42.6-65.8
31-40	146 (43.97%)	59.59%	52.5-68.6
41-50	50 (15.06%)	54%	50.5-57.9
>51	30 (9.04%)	56.66%	48.9-60.0

HPV prevalence differs significantly in the three age groups up to 50 years. It was highest in the patients under 40 and progressively lower with advancing age.

HPV prevalence did not differ signifi-

cantly with education. Sixty-four patients (19.69%) did not provide information on their education level and prevalence in this group was about the same as for those with known education.

HSIL accounted for 88 (26.5%), LSIL for 92 (27.71%), atypical squamous cells of undetermined significance (ASC-US) for 69 (20.78%) and squamous cell carcinoma (SCC) for 5 (1.5%) of the referral Pap smears in women with cervical dysplasia. Pap smear was negative in 78 cases (23.49%).

Colposcopically directed cervical biopsies reported no pathological abnormality (negative) in 64 (19.28%), HSIL in 105 (31.62%), LSIL in 83 (25%) and other lesions in 80 (24.1%) women. A fair agreement between Pap smear and cervical biopsy was proved by the Kappa test (0.5) when analyzing for squamous intraepithelial lesions (SIL).

The prevalence of cervical dysplasia was highest in the age groups up to 40 years. At age >41, the prevalence was half of that at the younger ages, and dropped even lower among those over 50.

Although not statistically different, prevalence of cervical dysplasia was slightly higher among those with high school education than in those with no or primary education, and higher again among those with a higher education.

In women with the cytological diagnosis of SIL, over 90% had HPV. Twenty-one percent of the women who were negative for cervical dysplasia (including BCC) were positive for HPV.

Women younger than 40 years had the highest odds ratio (OR) for HPV infection. The OR was significantly lower (0.63) in the 41-50 year-old group and dropped even lower (0.22) in those over the age of 51.

When comparing the OR for HPV infection in the groups by education, the lowest association (fully adjusted) was for the group with high school education, while the strongest association was in those with no, primary or gymnasium education had. Those with unknown education had the same OR as those with secondary education.

TABLE II

Odds ratio (OR) and 95% confidence intervals (95% CI) for cervical dysplasia and age, adjusting for the other variables and then additionally adjusting for HPV

Age group	Basal OR	Basal 95% CI	Fully adjusted OR	Fully adjusted 95% CI	HPV adjusted OR	HPV adjusted 95% CI
<30	1.0	Referent	1.0	Referent	1.0	Referent
31-40	1.02	0.6-1.7	1.03	0.6-1.7	1.5	0.8-2.7
41-50	0.45	0.2-0.9	0.43	0.2-0.9	1.3	0.6-2.8
>51	0.33	0.1-0.7	0.32	0.1-0.8	0.66	0.3-2.2

HPV was very strongly associated with cervical dysplasia (OR: 28.6) even after adjustment for age, education and smoking status. Even though the confidence intervals were large (95% CI: 12-76), it was evident that HPV was significantly associated with cervical dysplasia.

Women aged 40 and younger had the highest OR for cervical dysplasia and it was less than half in the women over 41. With age, OR became insignificant in the fully adjusted model and in fact it was HPV that mostly influenced this change.

There seemed to be a pattern of decreasing OR estimates for cervical dysplasia in smokers, with those who were former

smokers having a lower OR (0.70) and current smokers having an even lesser OR (0.62); however, none of these were statistically significant.

The age-adjusted odds of having cervical dysplasia increased significantly and dramatically with increasing viral load. The p-value for this trend is <0.0001. The prevalence of high-risk HPV types was about 55% and did not differ significantly by level of education. However, it did differ significantly by age showing a dramatic decrease in prevalence from 62.1 % in the less than 40 year-olds to 48% in the over 41 year-old.

Prevalence of cervical dysplasia was 56.62% and did not differ significantly by highest level of education in the household. It too differed significantly by age between under 40 (59.7%) and over 41 year-olds (53.9%). Over 90% of women with a cytological diagnosis of SIL had high-risk HPV types.

Basal and age adjusted OR's between HPV and the five selected variables showed age as the only variable significantly associated with HPV. Age was also significantly associated with cervical dysplasia but the OR estimates shifted toward the null when controlling for HPV and the association changed to become insignificant after that adjustment HPV was strongly associated with cervical dysplasia with a fully adjusted OR of 28.6 (14.7, 55.5). The association between cervical dysplasia increased markedly with increasing viral load ($p<0.0001$).

DISCUSSION

Cervical dysplasia is a broad term describing the precursor lesions that are considered to be indicators of the progression to cervical cancer. These lesions were classified as cervical intra-epithelial neoplasia (CIN).

Numerous studies have consistently reported the association between sexual behavior and cervical cancer, and since the 1980's, attempts have been made to try to understand the role of HPV as the sexually transmitted agent responsible. The poor sensitivity and specificity of the methods used for HPV testing have led to varying estimates of the relationship between HPV infection and cervical neoplasia and this variability had led to considerable skepticism about the clinical role of HPV. A new generation of HPV testing has shown more consistent associations, and HPV DNA has been detected in over 99.7% of tumors from patients with invasive cervical cancer. HPV - negative cancers are virtually non-existent (5, 6).

This study found a strong association (OR: 28) between high-risk HPV types and cervical dysplasia. Comparison with other studies has been difficult because of the different techniques used for detection and the different combinations of strains being tested for, nonetheless, OR's of over 15 have been reported in a review of case-control studies that used reliable methods for HPV DNA detection. Cigarette smoking has been described as an independent risk factor for cervical neoplasia however this was not evident in this study. The OR estimates were actually lower in smokers but these were not statistically significant.

In examining indicators of socio-economic status, one study used low educational achievement and found it associated with increased odds ratios (OR's) of 3.4 (1.2-10.1) for CIN. Bosch et al also found a low level of education to be associated with cervical cancer (1).

The role of smoking is controversial. Some studies have shown a significant association between smoking and invasive cervical cancer; however, there has been uncertainty about whether the reported association

was a causal or a confounding phenomenon reflecting such factors as sexual habits and infection with an unidentified infectious sexually transmitted agent (7).

Other risk factors that have been examined in the literature are oral and barrier contraceptives. Studies on oral contraceptives have shown protective, null, or weak associations with cervical neoplasia. Most recent studies have shown no association with barrier contraceptives have been shown to have a protective effect against cervical cancer: vaginal spermicides (OR = 0.28), diaphragms (OR = 0.67) and condoms (OR = 0.53) (8).

A series of studies have revealed that a unique feature of HPV testing is the ability to predict the subsequent development of cervical cancer precursors among cytologically negative women (9).

CONCLUSIONS

Persistence of HPV appears to be associated with progression to SIL. The high rates of sexually transmitted diseases (STD) imply that a potentially large number of women in Romania are at high risk for development of cervical cancer. Early detection could mean earlier treatment and a reduction of the number of women at risk for progression to invasive cancer.

Among patients with an abnormal Pap smear, women with HSIL require immediate colposcopy and histologic diagnosis. For patients with ASCUS or LSIL, women can be followed by either performing colposcopy immediately or by repeating the smears every six months for two years, or then performing colposcopy if LSIL persists.

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PREVALENȚA VIRUSURILOR PAPILLOMA UMANE IZOLATE DIN LEZIUNILE COLULUI UTERIN LA O POPULAȚIE FEMININĂ DIN TRANSILVANIA

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PREVALENCE OF HUMAN PAPILLOMA VIRUS ISOLATED FROM CERVIX LESIONS IN A FEMALE POPULATION FROM TRANSILVANIA (Abstract): Between the years 2008-2011 reverse hibridisation (INNO-LiPA HPV Genotyping Extra test) and genotyping la Roche (the kit: Linear array HPV genotyping test) were used for detection of Human Papilloma Virus (HPV) in the cervix secretions of 182 female patients aged 16-63 years, predominantly of urban origin. 99 patients (54.4%) were identified as being infected with various types of HPV, prevalent in urban (53 single infections and 46 multiple infections). HPV infection was not detected in 83 (45.6%) patients. Only 7 females from rural areas were tested (5 females had single or multiple HPV infections). 32 types of HPV were identificated: 15 HPV types with high risk (51, 82, 56, 18, 39, 45, 59, 68, 16, 31, 33, 35, 52, 58, 73), 14 types with low risk (42, 61, 62, 72, 81, 83, 84, CP6108, 70, 6, 11, 55, 74, 54), and 3 types with possible high risk (26, 53, 66). The type of HPV could not be identified in other two cases. The most frecvent types of HPV with high risk isolated were: the type 16. The types 51 and 58 of HPV with high risk and the type 84 with low risk are detected in single infections in urban and in rural. HPV clades involved in single infections are: 1 (1 case), 3 (5 cases), 5 (4 cases), 6 (5 cases), 7 (5 cases), 9 (21 cases), 10 (7 cases). The clades 11 (7 cases) and 13 (6 cases) were involved only in multiple infections detected in urban. The types 35, 39, 59, 68 of HPV with high risk were isolated from multple infections. In rural, multiple infections with two HPV were detected. The citological screening by Babeş-Papanicolaou examination was made only in 9 cases: HPV was not detected in 4 cases (one female had ASC-US: atypical squamous cells of "undetermined significance"); in 5 positive cases were detected HPV 16, 31, 58, 6. **Key words:** PCR, HPV, INFECTIONS, URBAN, RURAL, CITOLOGICAL EXAMINATION

Infecția cu HPV este considerată cea mai frecventă dintre infecțiile cu transmitere sexuală; se estimează că 75% dintre persoanele active sexual au fost infectate cu HPV la un moment dat în viață.

Cancerul colului uterin este a doua cauză frecventă de deces prin cancer la femei în întreaga lume. Epiteliul scuamos este cel

mai susceptibil la infecţia cu HPV. Este important să se stabilească prevalenţa infecţiei cu HPV în diferite tipuri de leziuni cervicale şi să se evidenţieze relaţia dintre HPV şi gravitatea leziunilor cervicale (1). Rolul HPV în patogeneză a fost recunoscut pentru mai multe tipuri. Unele virusuri, specifice pentru mucoasa genitală, în special HPV 6 şi 11 (tipuri benigne), pot infecta şi alte mucoase (respiratorie, bucală, conjunctivală) (2, 3). Astfel, HPV 16 este implicat în cancerele cervicale invazive (4). Sensibilitatea şi specificitatea screeningului pentru cancer cervical creşte prin combinarea citologiei şi a hibridizări (5). Genotiparea HPV permite o clasificare mai bună a femeilor cu anormalităţi cervicale în diferite grupe de risc şi poate fi utilă în orientarea tratamentului (6).

MATERIAL ŞI METODE

În perioada 2008-2011 au fost investigate secreţiile cervicale recoltate de la 182 de femei în vârstă de 16-63 de ani, provenind din mediul urban şi rural, în scopul depistarii virusurilor Papilloma umane **(**HPV), utilizând tehnica PCR:

1. Tehnica PCR de hibridizare inversă (testul INNO-LiPA HPV Genotyping *Extra*) (INNOGENETICS) a fost utilizată în anul 2008. Testul permite decelarea a 28 de genotipuri HPV (având 28 de sonde moleculare specifice şi 4 sonde de control): HPV cu risc crescut (High risk): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82; HPV cu risc probabil crescut (Probable high risk): 26, 53, 66; HPV cu risc scăzut (Low risk): 6, 11, 40, 43, 44, 54, 70; HPV neclasificate din punct de vedere al riscului oncogen (Additional types): 69, 71, 74 (7).

2. Tehnica PCR de genotipare Roche – kitul Linear array HPV genotyping test, folosind aparatul PCR–Golden Plated GeneAmp® PCR System 9700 de la Applied Biosystems, a fost utilizată în perioada 2010-2011. Această metodă poate detecta 37 de tipuri de HPV: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108 (8).

Prelucrarea statistică a rezultatelor a apelat la compararea a două medii cu o distribuţie de probabilitate normală prin testul Student, iar în cazul în care nu a existat o distribuţie normală a fost utilizat testul Mann-Whitney. Pentru compararea a mai mult de două medii cu o distribuţie de probabilitate normală a fost utilizat testul Anova. În cazul în care nu a existat o distribuţie normală s-a utilizat testul Kruskal-Wallis. Pentru testarea distribuţiei normale a fost folosit testul Kolmogorov-Smirnov. Pentru compararea a două frecvenţe a fost utilizat testul hi-pătrat sau Fisher-exact în cazul în care frecvenţele teoretice sunt sub 5. Analiza de corelaţie între variabile continue a fost efectuată prin calcularea coeficienţilor de determinare şi ecuaţia dreptei de regresie. Pragul de semnificaţie pentru testele folosite a fost luat $\alpha = 0,05$. Calculele statistice au fost efectuate cu ajutorul aplicaţiilor *SPSS 13.0*.

REZULTATE

Din cele 182 de testări efectuate, în 99 de cazuri (54,4%) a fost detectat HPV (53 de cazuri: 53,5% - infecţii unice şi 46 de cazuri: 46,5% - infecţii multiple), fiind identificate 32 de tipuri diferite de HPV:

- 15 tipuri de HPV cu risc crescut (C) (HPV: 51, 82 (grup filogenetic α5), 56 (grup filogenetic α6), 18, 39, 45, 59, 68 (grup filogenetic α7), 16, 31, 33, 35, 52, 58 (grup filogenetic α9), 73 (grup filogenetic α11);
- 14 tipuri de HPV cu risc scăzut (S):

42 (grup filogenetic α1), 61, 62, 72, 81, 83, 84, CP6108 (grup filogenetic α3), 70 (grup filogenetic α7), 6, 11, 55, 74 (grup filogenetic α10), 54 (grup filogenetic α13);

- 3 tipuri de HPV cu risc probabil crescut (PC): HPV 26 (α5) şi 53, 66 (α6).

La alte 2 cazuri nu s-a putut preciza tipul de HPV (HPV X).

Examenul citologic Babeş-Papanicolaou a fost efectuat numai la 9 paciente. La un caz (femeie de 32 de ani) s-a evidenţiat ASC-US, atipii ale celulelor pavimentoase intermediare. La celelale 5 cazuri (4 din mediul urban şi 1 din mediul rural) s-a identificat prin PCR HPV de tip 16 în două cazuri (asociat cu ASC-US sau ASC-H) şi respectiv tipurile 31, 58 şi 6.

Din cele 182 de femei, în 83 de cazuri nu am găsit infecţie cu HPV, 53 de cazuri aveau infecţii unice, 26 de cazuri prezentau infecţie cu două tipuri de HPV şi la 20 de cazuri identificate in mediul urban am diagnosticat asocieri de 3, 4, 5 sau 6 HPV (fig. 1).

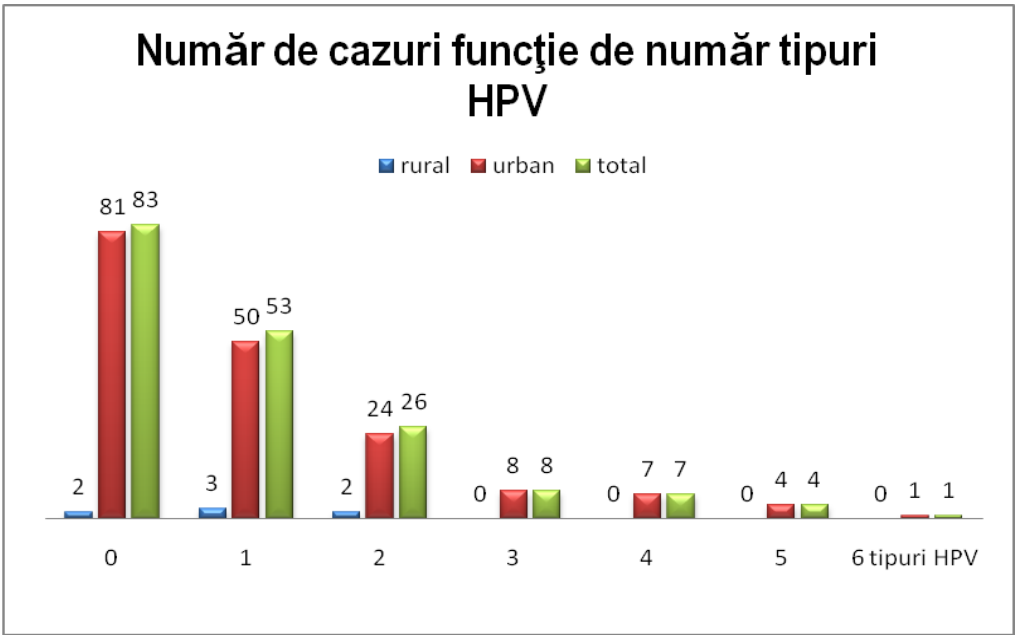

Fig. 1. Numărul de cazuri funcţie de numărul de tipuri de HPV

Din cele 99 de infecţii cu HPV, 94 de cazuri (94,9%%) au fost detectate *în mediul urban* şi dintre acestea *50* (53,2%) sunt *infecţii unice* (produse de un singur HPV) şi *44 (46,8%) infecţii multiple* (cauzate de mai multe tipuri de HPV).

Tipurile de HPV implicate în *infecţii unice în mediul urban* şi numărul total de cazuri detectate, mai ales la femei cu vârsta de 20-30 de ani sunt prezentate în tabelul I.

Tipurile de HPV 51, 58 cu risc crescut şi tipul 84 cu risc scăzut sunt implicate în infecţii unice atât în mediul urban cât şi în mediul rural.

În infecţiile multiple cu HPV detectate *în mediul urban* se pot asocia două, trei, patru, cinci, chiar şase virusuri (tab. II, III).

TABELUL I

Tipurile de HPV implicate în infecţii unice la femei în mediul urban

Tipuri de HPV	**Riscul HPV**	**Grup filogenetic**	**Număr total de cazuri**	**Număr de cazuri din total între 20-30 de ani**
16, 31, 33, 52, 58	C	α9	21	13 (6 cazuri cu tipul 16; 3 cazuri cu tipul 31; 1 caz cu tipul 33; 2 cazuri cu tipul 52; 1 caz cu tipul 58)
45, 18	C	α7	5	1 caz cu tipul 45 la 26 de ani; 1 caz cu tipul 70 la 20 de ani
70	S			
51, 82	C	α5	4	4 (tipul 51: 2 cazuri la 30 de ani, 1 caz la 21 de ani; tipul 82: 1 caz la 25 de ani)
56	C	α6	5	2 cazuri cu tipul 66 la 24 şi la 25 de ani
66	PC			
6, 55	S	α10	7	5 cazuri cu tipul 6 (cate 2 cazuri la 21 si 22 de ani; 1 caz la 23 de ani)
42	S	α1	1	-
62, 81, 83, 84, CP6108	S	α3	5	1 caz cu tipul 83 la 23 de ani
HPV X **(neprecizat)**	-	-	2	1 caz la 21 de ani

TABELUL II

Asocieri de câte două tipuri de HPV /număr de cazuri în mediul urban

Asocieri de câte două tipuri de HPV/număr de cazuri/vârstă						
16, 52 (α9/α9) 1 caz la 20 de ani	16, 54 (α9/α13) 1 caz la 44 de ani	16, 55 (α9/α10) 1 caz la 29 de ani	16, 39 (α9/α7) 1 caz la 27de ani	16, 35 (α9/α9) 1 caz la 32 de ani	16, 61 (α9/α3) 1 caz la 42 de ani	16, 6 (α9/α10) 1 caz la 21 de ani
6, 11 (α10/α10) 1 caz la 16 ani	6, 74 (α10/α10) 1 caz la 38 de ani	6, 35 (α10/α9) 1 caz la 25 de ani	6, 68 (α10/α7) 1 caz la 25 de ani	6, 31 (α10/α9) 1 caz la 22 de ani	6, 66 (α10/α6) 2 cazuri: la 22 şi 31 de ani	51, 82 (α5/α5) 1 caz la 33 de ani
66, 51 (α6/α5) 1 caz la 23 de ani	66, 83 (α6/α3) 1 caz la 23 de ani	62, 58 (α3/α9) 1 caz la 29 de ani	62, 73 (α3/α11) 1 caz la 20 de ani	52, 11 (α9/α10) 1 caz la 35 de ani	52, 31 (α9/α9) 1 caz la 29 de ani	52, 53 (α9/α6) 1 caz la 43 de ani
53, 70 (α6/α7) 1 caz la 44 de ani	54, CP6108 (α13/α3) 1 caz la 25 de ani					

În mediul rural, din cele 7 cazuri investigate, la 3 cazuri au fost detectate *infecţii unice* cu HPV tipul 51 cu risc crescut (C) la o pacientă de 39 de ani (1caz), HPV tipul 58 cu risc crescut la o pacientă de 63 de ani (1caz) şi HPV tipul 84 cu risc scăzut la o

pacientă de 24 de ani (1 caz). *În mediul rural, infecțiile multiple cu HPV* au fost produse numai de asocieri de două HPV: tipul 16 cu risc crescut şi tipul 61 cu risc scăzut (la o pacientă de 23 de ani), iar la o pacientă de 20 de ani – tipul 18 (risc crescut) şi CP 6108 (risc scăzut).

TABELUL III

Asocieri de 3, 4, 5 şi 6 HPV detectate în mediul urban

Asocieri de HPV	**Tipuri de HPV asociate/număr de cazuri/vârstă**						
Asocieri de 3 HPV	16, 51, 52 (α9/α5/α9)	58, 81, CP6108 (α9/α3/α3)	52, 59, 62 (α9/α7/α3)	51, 66, 52 (α5/α6/α9)	16, 53, 73 (α9/α6/α11)	55,72,73 (α10/α3/α11)	52, 53, 82 (α9/α6/α5)
	1 caz la 20 de ani	1 caz la 22 de ani	1 caz la 28 de ani	1 caz la 22 de ani	2 cazuri: la 25 şi 36 de ani	1 caz la 22 de ani	1 caz la 23 de ani
Asocieri de 4 HPV	53, 31, 52, 54 (α6/α9/ α9/α13)	53, 66, 16, 54 (α6/α6/ α9/α13)	39, 31, 52, 54 (α7/α9/ α9/α13)	45, 51, 52, 62 (α7/α5/ α9/α3)	42, 52, 55, 81 (α1/α9/ α10/α3)	16, 35, 56, 61 (α9/α9/ α6/α3)	31, 53, 62, 81 (α9/α6/ α3/α3)
	1 caz la 31 de ani	1 caz la 20 de ani	1 caz la 24 de ani	1 caz la 34 de ani	1 caz la 27 de ani	1 caz la 26 de ani	1 caz la 42 de ani
Asocieri de 5 HPV	6, 16, 54, 73, 84 (α10/α9/ α13/α11/α3)	6, 31, 35, 39, 73 (α10/α9/α9 /α7/α11)	11, 18, 31, 33, 61 (α10/α7/ α9/α9/α3)	33, 53, 73, 82,CP6108 (α9/α6/α11 /α5/α3)			
	1 caz la 19 ani	1 caz la 23 de ani	1 caz la 21 de ani	1 caz la 22 de ani			
Asociere de 6 HPV	16, 26, 39, 42, 51, 59 (α9/α5/α7/ α1/α5/α7)						
	1 caz la 36 de ani						

Numai în cazurile cu infecții multiple, nu şi în cele cu infecții unice, am izolat următoarele HPV cu risc crescut: tipul 35 (4 infecții în mediul urban), tipul 39 (4 infecții în mediul urban), tipul 59 (2 infecții în mediul urban), tipul 68 (1 infecție în mediul urban), precum şi tipuri de HPV cu risc scăzut: 61 (3 infecții în mediul urban, 1 infecție în mediul rural), 72 (1 infecție în mediul urban), 54 (6 infecții în mediul urban), 11 (3 infecții în mediul urban), iar HPV cu risc probabil crescut sunt tipurile: 53 (9 infecții în mediul urban), 26 (1 infecție în mediul urban).

Am detectat 59 de infecții la paciente între 20 şi 30 de ani: 29 de infecții unice (dintre care 1 infecție în mediul rural) şi 30 multiple (dintre care 2 în mediul rural).

HPV 16 (grupul α9) cu risc crescut este implicat cel mai frecvent în infecții unice (10 cazuri) şi în infecții multiple: 14 în mediul urban (o pacientă infectată cu 6 virusuri, una cu 5 HPV, 2 paciente cu 4 HPV, 3 paciente cu 3 HPV, 7 paciente cu 2 HPV asociate) şi un caz cu 2 HPV asociate în mediul rural. HPV 52 (grupul α9) cu risc crescut a fost evidențiat în 16 cazuri (4 infecții unice, 8 cazuri cu infecții multiple

cu 2 sau 3 sau 4 HPV şi 4 cazuri cu 2 HPV asociate), predominant tot între 20 şi 30 de ani şi în infecţii multiple, fiind al doilea HPV izolat din infecţii, ca frecvenţă, după tipul 16. Tipul 6 cu risc scăzut este implicat în 6 infecţii unice. Tipurile cu risc crescut 31, 52 şi tipul 66 cu risc probabil crescut sunt implicate fiecare în câte 4 infecţii unice. HPV 51 cu risc crescut este izolat în 10 cazuri: 2 infecţii multiple cu 2 şi 3 HPV asociate şi într-un caz de infecţie multiplă cu 4 HPV asociate. HPV 55 (cu risc scazut) şi HPV 58 (cu risc crescut) sunt implicate fiecare în câte o infecţie unică şi în câte o infecţie multiplă cu 2, respectiv cu 3 HPV asociate. Tipul 55 se identifică şi într-o infecţie cu 4 HPV asociate. HPV 70 şi HPV 83 au fost identificate fiecare în câte o infecţie unică şi în câte o infecţie multiplă cu 2 HPV asociate. HPV CP6108 (cu risc scăzut) a fost implicat într-o infecţie unică şi în 3 infecţii multiple cu 2, 3, respectiv 5 HPV asociate. HPV 26 cu risc probabil crescut a fost izolat numai de la un caz cu infecţie multiplă cu 6 virusuri asociate, datorită posibilităţii de a fi rar implicat în infecţii; nu este exclus să nu fi fost depistat datorită numărului mic de cazuri investigate. Tipul 6 cu risc scăzut a fost implicat in 16 cazuri, fiind al treilea tip de HPV identificat ca frecvenţă.

Nu există diferenţe semnificative ale mediei de *vârstă* raportat la grupul filogenetic al HPV (p=0,26) (tab. IV). Grupele filogenetice din care fac parte HPV implicate în infecţii unice sunt: α1 (1 caz), α3 (5 cazuri), α5 (4 cazuri), α6 (5 cazuri), α7 (5 cazuri), α9 (21 cazuri), α10 (7 cazuri). Grupele filogenetice α11 şi α13 au fost implicate numai în infecţii multiple în mediul urban (HPV din grupul filogenetic α11 în 7 infecţii multiple şi α13 în 6 infecţii multiple).

TABELUL IV

Grupul filogenetic al HPV corelat cu vârsta

p=0,26	N	Media vârstei	Deviaţie standard	Eroare standard	Intervalul de confidenţă pentru medie 95%		Vârsta minimă	Vârsta maximă
HPV X	2	26,50	7,78	5,50	- 43,38	96,38	21	32
α9	64	29,13	8,92	1,11	26,90	31,35	19	63
α1	3	32,00	4,58	2,65	20,62	43,38	27	36
α10	24	25,58	7,08	1,45	22,59	28,57	16	42
α3	25	29,04	9,03	1,81	25,31	32,77	19	45
α5	15	29,13	6,14	1,59	25,73	32,53	20	39
α6	21	29,00	8,02	1,75	25,35	32,65	20	44
α7	16	30,00	8,57	2,14	25,43	34,57	20	49
α11	7	23,86	5,70	2,15	18,59	29,13	19	36
α13	6	27,17	9,28	3,79	17,43	36,91	19	44
Total	183	28,46	8,21	0,61	27,27	29,66	16	63

Legendă: N = număr de HPV dintr-un grup filogenetic

DISCUŢII

Se consideră că utilizarea metodei hibridizării pentru detectarea HPV ca metodă de screening este mai sensibilă decât utilizarea numai a frotiului pentru detectarea koilocitelor (9), aspect care concordă cu rezultatele noastre.

În studiul prezent, HPV 16 a fost cel

mai frecvent izolat din infecții unice şi multiple în mediul urban şi rural (25 de cazuri = 13,7%), la fel cum rezultă şi din alte cercetări (10, 11). HPV 52 (grupul α9) cu risc crescut are incidență crescută în Asia şi Africa, unde determină 2 – 3 % din totalul cancerului cervical din lume (12); noi am constatat prevalența izolării acestui tip de HPV după tipul 16, situație relatată şi în alte studii (13, 14, 15, 16). Şi alți autori au depistat tipurile de HPV identificate în această lucrare, cum sunt HPV 31, 52, 51, 58, 84 (17) şi respectiv tipurile 26, 6, 55, 70, 83, CP6108 (18).

Numărul mic de cazuri investigate în mediul rural (7 cazuri) se poate datora gradului scăzut de educație; femeile nu se prezintă la medic pentru investigații atât de frecvent ca în mediul urban (19). Totuşi, rata de expunere a femeilor tinere pare a fi mai mare, cu implicarea mai multor tipuri de HPV, ceea ce sugerează deficiențe de educație sexuală.

Nu se constată diferențe semnificative ale mediei de vârstă ale pacientelor raportat la grupul filogenetic al HPV, la fel ca şi în alte cercetări (20). Grupul filogenetic α9 a fost cel mai frecvent implicat în infecția cu HPV, concordant cu alți autori (14).

În studiul din lucrarea prezentă, numai la 9 cazuri a fost efectuat examenul citologic Babeş-Papanicolaou, dintre care la un caz (femeie de 32 de ani din mediul urban) s-a evidențiat ASC-US şi atipii ale celulelor pavimentoase intermediare fără a se identifica vreun tip de HPV. Examenul citologic Babeş-Papanicolaou, deşi se ştie că are sensibilitate limitată, necesită repetare şi determină rate crescute de rezultate fals pozitive; el rămâne metoda standard pentru detectarea LSIL (leziuni intraepiteliale scuamoase de grad scăzut) la femei sub 35 de ani. Testarea prezenței HPV cu risc crescut nu este atât de importantă pentru detectarea LSIL la acest grup de femei. Deşi testarea HPV cu risc crescut este utilă pentru predicția riscului de progresie a bolii, pozitivarea HPV cu risc crescut în LSIL nu indică aplicarea unui tratament chirurgical (conizație) la aceste femei cu vârsta sub 35 de ani (21). Scăderea mortalității legate de cancerul cervical este atribuită implementării programelor de screening, care se recomandă să fie inițiat la aproximativ 3 ani după începerea vieții sexuale, dar nu mai târziu de vârsta de 21 de ani şi să se continue până la vârsta de 65 sau 70 de ani. Utilizarea atât a examenului citologic cât şi a testării HPV la intervale de 3 ani la femei în vârstă de 30 sau peste 30 de ani este comparabilă ca sensibilitate cu efectuarea anuală a examenului citologic pentru detectarea leziunilor precursoare ale cancerului cervical (22).

CONCLUZII

Frecvența infecției genitale cu HPV în populația feminină din arealul abordat este insuficient supravegheată şi cunoscută, fiind investigate eventual paciente cu probleme de sănătate în sfera genitală. Aspectul este mai grav în mediul rural.

Nu se constată diferențe semnificative ale mediei de vârstă funcție de grupul filogenetic al HPV.

HPV tipul 16 (grup α9) apare ca fiind prevalent în infecțiile unice şi multiple diagnosticate la pacientele investigate.

Proporția cazurilor cu infecție unică sau multiplă la femei active sexual din mediul urban este aproximativ egală, sugerând deficiențe generale de educație şi comportament sexual.

Răspândirea HPV – aşa cum este cunoscută acum – este un argument suficient în favoarea vaccinării HPV de masă.

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IMPLICAŢIILE DIFUZIEI INTERNE ÎN BIODEGRADAREA LIPIDELOR CU CELULE IMOBILIZATE DE *BACILLUS SP.* DISPUSE ÎN STRAT FIX TIP "BASKET"

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INFLUENCES OF INTERNAL DIFFUSION ON THE LIPIDS BIO-DEGRADATION WITH IMMOBILIZED *BACILLUS SP*. CELLS IN FIXED BED OF BASKET TYPE (Abstract): **Aim:** This paper develops the previous studies on the transfer processes and biochemical reaction kinetics involved in the lipids biodegradation. **Material and methods**: The experiments were carried out in 10 l (8 l working volume) laboratory bioreactor with computer-controlled and recorded parameters. The basket was placed centered around the stirrer and was filed with *Bacillus sp.* cells immobilized in alginate. The optimum impellers combination was found to be of two Rushton turbines, the superior one placed outside the basket and the other inside the basket at its inferior extremity. **Results:** In the investigated case, the influences of the substrate internal diffusion on physical and biochemical processes have been quantified by means of the Biot number, Thiele modulus and reduction factor λ. Compared to the system with free bacterial cells, biochemical processes rate is reduced inside the biocatalyst particle for about 150 - 200 times, this effect being more important than in the case of the bioreactor with mobile bed of immobilized yeast cells. **Conclusions:** The utilisation of immobilized biocatalysts offers many advantages, but allows the unfolding of the internal diffusion of substrate or product, with negative effect on the overall biochemical process evolution. **Key words:** *BACILLUS SP.*, LIPIDS, BIOREACTOR, DIFFUSION.

Lipidele sunt biomolecule organice, de proveniență microbiană, vegetală sau animală, insolubile în apă, însă solubile în solvenți nepolari. Lipidele pot fi complexe (lipide saponificabile), care conțin în structură acizi graşi, incluzând acilgliceroli, fosfogliceride, sfingolipide şi ceruri, sau simple (nesaponificabile), care nu conțin în structură acizi graşi (1).

Dintre lipidele complexe, o clasă aparte o reprezintă triacilglicerolii sau trigliceridele. Această clasă este cea mai cuprinzătoare şi include grăsimile (lipidele în stare solidă la temperatura ambiantă) şi uleiurile. Circa 3% din producția modială de uleiuri vegetale o reprezintă uleiul de măsline, 82% dintre consumatorii săi provenind din Uniunea Europeană. Apele reziduale provenite din industria uleiului de măsline reprezintă un poluant important, datorită

încărcării sale organice ridicate, reprezentate de lipide (0,2-1%), acizi carboxilici (0,5-1,5%), zaharuri (1-8%), polifenoli şi pectine (1-1,5%), compuşi cu azot (0,5-2,4%), taninuri şi polialcooli (2). Majoritatea problemelor asociate cu poluarea generată de aceste ape sunt determinate de fracțiunea fenolică care afectează sever activitatea microorganismelor ecosistemului (3). Pentru epurarea acestor ape reziduale, au fost propuse metode fizice şi chimice (decantare, concentrare, ultrafiltrare, osmoză inversă, flotație, absorbție, oxidare şi fotooxidare), iar, mai recent, şi biologice (4).

Tratarea biologică a apelor cu conținut de ulei s-a realizat în condiții anaerobe sau aerobe, folosind microorganisme din clasa bacteriilor (*Burkholderia cepacia, Phormidium sp., Oscillatoria sp., Chroococcus sp., Enterobacter aerogenes, Mucor racenosus*), drojdiilor (*Candida oleophila, Candida tropicalis, Yarrowia lipolytica*), fungi (*Apergillus niger, Phanerochaete crysosporium, Lentinus edodes, Pleorotus ostreatus, Funalia trogii, Geotrichum candidum, Mucor rouxii, Absidia coerulea, Penicillium restrictum, Penicillium verucosum*) şi *lipaze* libere sau imobilizate. In acest sisteme, trigliceridele sunt biodegradate la acizi graşi cu catenă lungă, care sunt apoi oxidați în poziția β la acetat sau propionat (5). Coexistența unei activități metanogene a microorganismelor implicate în biodegradarea lipidelor manifestă un efect favorabil asupra hidrolizei trigliceridelor.

În acest context, în lucrare au fost analizate performanțele unui sistem de tratare biologică anaerobă a apelor reziduale provenite de la obținerea uleiului de măsline, prin folosirea celulelor de *Bacillus sp.* imobilizate. In acest scop, s-au investigat cinetica biodegradării lipidelor, simultan cu efectul difuziei interne a acestora în granulele de biocatalizatori dispuse într-un bioreactor cu strat fix de tip *"basket"*.

MATERIAL ŞI METODĂ

Echipamentul experimental utilizat a constat dintr-un bioreactor de laborator de 10 l (8 l volum util), de tip Fermac 310/60, Electrolab, prevăzut cu un sistem computerizat de monitorizare, control şi înregistrare a datelor. Stratul tip *„basket"* de biocatalizatori imobilizați a avut o configurație cilindrică, cu diametrul interior de 100 mm, înălțimea de 100 mm şi grosimea de 10 mm. Cilindrul staționar a fost plasat central în jurul axului agitatorului, la distanța de 100 mm de baza bioreactorului (fig. 1).

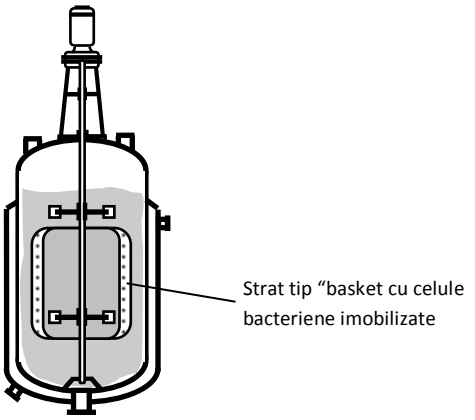

Fig. 1. Bioreactorul experimental tip *"basket"*

Stratul fix a conținut celule de *Bacillus sp.* (*Bacillus subtilis, Bacillus megaterium, Bacillus litheniformis, Bacillus ortoliquefaciens* în proporții egale) imobilizate în alginat de sodiu. Imobilizarea s-a realizat prin tehnica includerii prin reticulare, conform rețetelor existente în literatură (6). În experimente s-au utilizat granule de biocatalizator având diametrul de 3,6 mm. Fracția volumică a biocatalizatorilor a fost de 0,1.

Sistemul de amestecare a constat dintr-un agitator dublu tip turbină Rushton. Turația agitatorului a fost de 150 şi, respectiv,

300 rpm. Durata procesului de biodegradare a fost dictată de viteza de consum a lipidelor totale. Evoluţia procesului s-a realizat prin dozarea lipidelor totale, folosind metoda spectrofotometrică cu trioleină (7).

REZULTATE ŞI DISCUŢII

Pentru stabilirea valorii şi a distribuţiei concentraţiei lipidelor atât la interfaţa mediu-granulă de biocatalizator, cât şi în interiorul granulei de biocatalizator, se consideră bilanţul de materiale al acestora pentru o singură granulă de celule de *Bacillus sp.* imobilizate în alginat. În acest context, variaţia concentraţiei lipidelor cu raza particulei de biocatalizator este redată de expresia:

$$\frac{dC_{LT}}{dt} = D_{LTe} \cdot \left[\frac{1}{r^2} \cdot \frac{d}{dr} \left(r^2 \cdot \frac{dC_{LT}}{dr} \right) \right] - k_{dC} \cdot C_{LT} \qquad (1)$$

în care:

C_{LT} - concentraţia lipidelor totale din mediu, g/l

C_{LTi} - concentraţia lipidelor totale la suprafaţa biocatalizatorilor, g /l

C_{LTP} - concentraţia lipidelor totale în granula de biocatalizator, g/l

D_{LTe} - coeficientul efectiv de difuzie al lipidelor, m^2/s

k_{dC} - viteza specifică de biodegradare a lipidelor, h^{-1}

v_d - viteza de degradare a lipidelor, g/l.h

r - distanţa din centrul granulei de biocatalizator la punctul considerat, m

R_P - raza granulei de biocatalizator, m.

Bi reprezintă criteriul Biot, definit ca măsură a importanţei relative a rezistenţei la difuzie a unui compus prin filmul de lichid de la suprafaţa unei particule, comparativ cu difuzia internă în particulă. In cazul analizat, criteriul Bi se calculează cu expresia (8):

$$Bi = \frac{k_L \cdot R_P}{D_{LTe}} \qquad (2)$$

Variaţia criteriului Bi pentru acest sistem de biodegradare a lipidelor este descrisă grafic în figura 2. Se observă că valoarea acestui criteriu scade pe direcţie radială, datorită dependenţei directe dintre acest criteriul Bi şi coeficientul de transfer de masă al lipidelor prin filmul de lichid de la suprafaţa biocatalizatorilor. Din acelaşi motiv, valorile înregistrate pentru turaţia de 150 rpm sunt semnificativ mai reduse decât cele corespunzătoare turaţiei de 300 rpm.

Diferenţa înregistrată este consecinţa reducerii coeficientului de transfer de masă al lipidelor prin filmul de lichid de la suprafaţa biocatalizatorilor, datorată diminuării turbulenţei în stratul fix şi al creşterii rezistenţei suplimentare corespunzătoare difuziei prin strat.

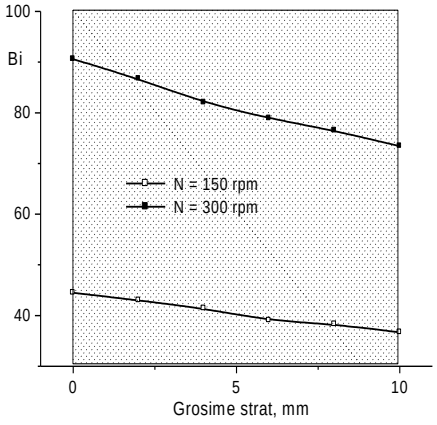

Fig. 2. Variaţia criteriului Biot pe direcţie radială în stratul de biocatalizatori

Un alt parametru ce descrie modul în care difuzia internă influenţează procesul biochimic de consum al lipidelor este modulul

Thiele, φ, definit în acest caz cu relația:

$$\varphi = \frac{R_P}{3} \cdot \sqrt{\frac{k_{dC}}{D_{LTe}}} \qquad (3)$$

Valoarea acestui modul pentru sistemul studiat este 4,10, fiind unică pentru o anumită dimensiune a biocatalizatorilor, indiferent de turația agitatorului. Această valoare, superioară nivelului de referință de 0,1 (9) şi mult mai mare decât în cazul celorlalte sisteme studiate, indică o limitare semnificativă a vitezei reacției biochimice de degradare a lipidelor datorată difuziei interne a acestor compuşi voluminoşi.

Pentru redarea cât mai exactă a efectului generat de difuzia internă asupra biodegradării lipidelor, se utilizează factorul λ, definit ca raportul dintre viteza reacției biochimice în prezența difuziei interne şi viteza aceleaşi reacții în sisteme cu celule libere de *Bacillus sp.* (9). În condiții de staționaritate, se poate considera că viteza reacției biochimice de consum a substratului în interiorul bio-catalizatorilor este egală cu viteza internă de difuzie a substratului. Astfel, în cazul specific al sistemului studiat de biodegradare a lipidelor, relația de calcul a factorului λ devine:

$$\lambda = \frac{4\pi \cdot R_P{}^2 \cdot D_{LTe} \cdot \left.\frac{dC_{LTP}}{dr}\right/_{r=R_P}}{\frac{4}{3}\pi \cdot R_P{}^3 \cdot k_{dC} \cdot C_{LT}} \qquad (4)$$

Deoarece concentrația lipidelor totale în granulele de biocatalizator, C_{LTP}, se calculează cu expresia:

$$C_{LTP} = \frac{Bi \cdot (C_{LT} - C_{LTi})}{\left[e^{-\left(\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} + R_P\right)} - e^{\left(\sqrt{9\cdot\varphi\cdot D_{LTe}^2 + R_P^2} - R_P\right)} \right]} \cdot \left[\frac{e^{\left(\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} - R_P\right)\cdot\frac{r}{R_P}}}{\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} - R_P} - \frac{e^{-\left(\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} + R_P\right)\cdot\frac{r}{R_P}}}{\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} + R_P} \right] \qquad (5)$$

prin combinarea cu ecuația (4) şi efectuarea simplificărilor se obține:

$$\lambda = \frac{3 \cdot D_{LTe} \cdot Bi \cdot (C_{LT} - C_{LTi})}{R_P{}^2 \cdot k_{dC} \cdot C_{LT}} \cdot \frac{e^{-\left(\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} + R_P\right)} + e^{\left(\sqrt{9\cdot\varphi\cdot D_{LTe}^2 + R_P^2} - R_P\right)}}{e^{-\left(\sqrt{9\cdot\varphi^2\cdot D_{LTe}^2 + R_P^2} + R_P\right)} - e^{\left(\sqrt{9\cdot\varphi\cdot D_{LTe}^2 + R_P^2} - R_P\right)}} \qquad (6)$$

Variația factorului λ în interiorul particulei de biocatalizator este indicată în figura 3.

Din analiza alurii dependențelor obținute pentru fiecare turație se poate concluziona că factorul λ variază lent în apropierea suprafeței şi a centrului granulelor. In apropierea suprafeței granulelor de biocatalizator, concentrația mai ridicată a lipidelor, apropiată de cea superficială, conduce la valori ale factorului λ apropiate de 1. Însă, grosimea acestei regiuni superficiale a granulelor se reduce la turația inferioară, datorită diminuării concentrației superficiale a lipidelor şi, implicit, al unei reduceri mai accentuate a concentrației sale pe direcție radială. Variația lentă a factorului λ în regiunea centrală a granulelor se datorează menținerii unui nivel constant redus al concentrației lipidelor în această regiune.

Pe baza acestor rezultate se poate afirma că prin imobilizarea celulelor de *Bacillus sp.* şi dispunerea lor într-un strat fix tip *basket* viteza procesului biochimic de degradare a lipidelor scade considerabil, de 1/λ ori comparativ cu viteza reacției corespunzătoare sistemului conținând celule libere de bacterii. Magnitudinea acestui efect este corelată cu poziția granulelor în interiorul stratului fix şi cu intensitatea amestecării mediului (fig. 4).

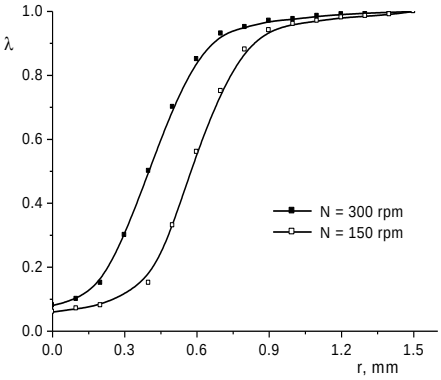

Fig. 3. Variaţia factorului λ cu distanţa de la centrul particulei de biocatalizator

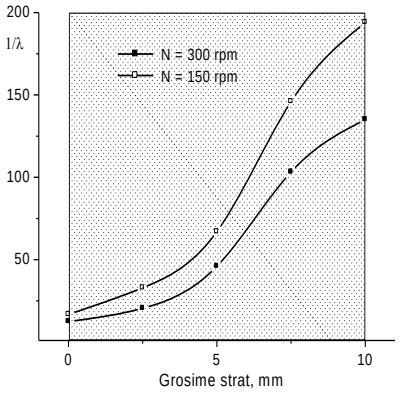

Fig. 4. Variaţia parametrului 1/λ în centrul biocatalizatorilor pe direcţie radială în stratul de biocatalizatori

În acest sens, din motivele discutate anterior, în centrul granulelor, parametrul 1/λ creşte de la suprafaţa interioară a cilindrului la cea exterioară de 10,8 - 11,6 ori, valori superioare fiind înregistrate pentru turaţia de 150 rpm.

CONCLUZII

Utilizarea celulelor imobilizate oferă numeroase avantaje, însă permite apariţia difuziei interne a substratului sau produsului, cu efecte negative asupra evoluţiei procesului biochimic în ansamblul său. In cazul biodegradării lipidelor cu celule de *Bacillus sp.* imobilizate, într-un bioreactor cu strat fix tip „basket", efectul difuziei interne s-a analizat prin intermediul criteriului Biot, al modulului Thiele şi al factorului de reducere λ. Astfel, comparativ cu sistemul conţinând celule libere de *Bacillus sp.,* viteza procesului de biodegradare a lipidelor cu celule imobilizate a scăzut de circa 150 - 200 de ori (valori mai reduse fiind înregistrate pentru turaţii mai ridicate).

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EFECTELE UNUI EXTRACT DIN *CETRARIA ISLANDICA* ÎN MONOTERAPIE ŞI ÎN ASOCIERE CU MAGNEZIUL PE UN MODEL DE HEPATOPATIE INDUSĂ EXPERIMENTAL

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EFFECTS OF A *CETRARIA ISLANDICA* EXTRACT IN MONOTHERAPY AND IN ASSOCIATION WITH MAGNESIUM IN AN EXPERIMENTAL-INDUCED HEPATOPATHY MODEL (Abstract): **Aim**: To investigate the effects of a *Cetraria islandica* extract in an animal model of hepatopathy. **Material and methods**: The experiments were carried out on Wistar rats, treated intraperitoneally (excepting carbon tetrachloride which was administered orally by esogastric tube), at single daily dose, for 28 days and divided in eight groups of 6 rats each: Group 1: Saline solution (0.5ml/100g bw/day); Group 2: $MgSO_4$ (5mg/kbw/day); Group 3: *C. islandica* (Ci) extract (21.56mg/kbw/day); Group 4: Ci+$MgSO_4$; Group 5: carbon tetrachloride (CT) (0.1ml/100g bw/day); Group 6: CT+$MgSO_4$; Group 7: CT+Ci; Group 8: CT+Ci+$MgSO_4$. At the end of the experiment blood samples were taken to assess the effects on the blood parameters,TGO, TGP, GGT, LDH, serum total billirubin, neutrophil phagocytic function (Nitroblue tetrazolium test) and serum complement activity. This parameters were determined with VITROS 750 XRC device, using Johnson&Johnson kits. The results were statistically processed with the help of „t-Student" test. $p<0.05$ was considered statistically significant. **Results**: In this carbon tetrachloride-induced hepatopathy animal model, *C. islandica* extract demonstrated hepatoprotective and immuno-stimulating effects (proved by normalization of glutamic-pyruvic transminase, glutamic-oxalocetic transminase, serum total billirubin, stimulation of neutrophils percentage and phagocytic functions, decreasing the carbon tetrachloride-induced basophilia and monocytosis, especially in association with magnesium). **Conclusions**: Further studies are required to confirm the benefits of this association in hepatoprotective and immunomodulating therapies.
Key words: *CETRARIA ISLANDICA*, MAGNESIUM, CARBON TETRACHLORIDE.

S-a demonstrat că un număr tot mai mare de principii active de origine vegetală prezintă efecte imunomodulatoare sau/şi antiinflamatoare, motiv pentru care în medicina tradiţională a unor ţări, constituie adjuvanţi de bază în tratamentul unor boli (medicina tradiţională indiană, chineză, japoneză, etc.) (1).

Dintre imunomodulatoarele vegetale, „adaptogenii" măresc capacitatea organismului de a se adapta la stres şi la alte situaţii cu risc pentru sănătate. Mecanismele prin care aceştia acţionează sunt încă neclare, dar

se pare că este vorba de o interacțiune a efectelor diverselor principii active (2).

Cetraria islandica face parte din familia *Parmeliaceae* cu denumirea populară: lichen de piatră, mușchi creț de munte, mușchi de piatră, lichenul renilor, lichen de Islanda. Crește în majoritatea țărilor nordice, pe stâncile și platourile din zona alpină. Date din literatură evidențiază acțiunea antitumorală și imunomodulatoare a polizaharidelor din structura acestei specii (3), iar asocierea magneziului ($MgSO_4$) potențează efectele extractului de *C. Islandica* (4, 5).

Scopul studiului a constat în cercetarea experimentală a efectelor unui extract din *C. islandica* în monoterapie sau în asociere cu $MgSO_4$ pe un model de hepatopatie indusă la animale de laborator.

MATERIAL ŞI METODĂ

Au fost utilizate 8 loturi de câte 6 șobolani Wistar, cu greutate cuprinsă între 180-200g, cu repartiție uniformă pe sexe, proveniți de la Institutul de Sănătate Publică din Iași, tratați conform schemei: L1 = SF = Ser fiziologic (0,5ml/100g c/zi); L2 = $MgSO_4$ = $MgSO_4$ (5mg/kg c/zi); L3 = Ci = Extract din C. islandica (21,56mg/kg c/zi, reprezentând 1/80 din DL_{50}); L4 = Ci + $MgSO_4$= Ci (21,56mg/kg c/zi) + $MgSO_4$ (5mg/kg c/zi); L5 = TC = Tetraclorură de carbon (0,1 ml/100g c/zi); L6 = TC+$MgSO_4$ = TC (0,1ml/100g c/zi) + $MgSO_4$ (5mg/kgc/zi); L7 = TC+Ci = TC (0,1 ml/100gc/zi) + Ci (21,56mg/kgc/zi); L8 = TC+Ci+MgSO4 = TC (0,1ml/100g c/zi) + Ci (21,56mg/kgc/zi) + MgSO4 (5mg/kgc/zi). Substanțele au fost administrate în doză unică zilnică, la ora 8 dimineața, timp de 28 zile, pe cale intraperitoneală, cu excepția TC, care a fost administrată pe cale orală, pe sondă esogastrică (6,7).

După 28 zile de tratament s-a recoltat sânge din plexul retroorbitar și au fost determinați următorii parametri: formula leucocitară, capacitatea de fagocitoză a PMN din sângele periferic (testul NBT), activitatea complementului seric, valorile transaminazei glutamice oxaloacetice (TGO), transaminazei glutamice piruvice (TGP), gama-glutamiltransferazei (GGT), lactat dehidrogenazei (LDH), bilirubinei serice totale, colesterolului total, LDL-colesterolului și HDL-colesterolului. Aceşti parametri au fost determinați cu un analizor pentru teste biochimice VITROS 750 XRC, folosind reactivi Johnson&Johnson. Rezultatele au fost prelucrate statistic folosind testul „t-Student". Valoarea $p<0,05$ a fost considerată semnificativă statistic.

Cercetările au fost realizate în conformitate cu recomandările Comisiei de Etică a Universității de Medicină și Farmacie „Gr. T. Popa" din Iași, în strictă concordanță cu reglementările etice internaționale, privind lucrul pe animale de laborator (8).

REZULTATE ŞI DISCUȚII

Tetraclorura de carbon (TC) a determinat o scădere semnificativă statistic a procentelor de PMN din sângele periferic, față de lotul martor tratat cu SF. Mg administrat sub forma $MgSO_4$ a determinat o creştere semnificativă statistic a procentelor de PMN din sângele periferic comparativ cu loturile martor tratate cu SF, similar cu cea determinată de extractul din *C. Islandica*. Asocierea Mg la extractul de *C. islandica* a produs o creştere a efectului stimulator asupra acestui parametru indus de *C. islandica*, dar fără semnificație statistică ($p>0,05$).

TC a determinat o scădere semnificativă statistic a procentelor de limfocite din sângele periferic față de lotul martor tratat cu SF. Mg şi extractul din *C. islandica* nu au determinat modificări semnificative statistic ($p>0,05$) ale procentelor de limfocite din sângele periferic comparativ cu lotul martor tratat cu SF. Asocierea Mg la extractul de *C.*

islandica a diminuat scăderea procentului de limfocite din sângele periferic determinată de TC.

La animalele din toate loturile testate nu au fost constatate modificări semnificative statistic ale procentelor de eozinofile din sângele periferic față de lotul martor tratat cu SF ($p>0,05$).

Administrarea TC a determinat o bazofilie şi o monocitoză semnificative statistic față de lotul martor ($p<0,05$). Atât extractul vegetal din *C. islandica*, la doza utilizată în acest experiment, cât şi Mg administrat sub forma $MgSO_4$, nu au antagonizat efectul produs de TC asupra acestor parametri. În schimb, asocierea Ci cu Mg a determinat diminuarea semnificativă statistic a efectului TC de creştere a procentelor de bazofile şi de monocite din sângele periferic comparativ cu lotul martor ($p<0,05$).

Administrarea TC a determinat o scădere semnificativă statistic ($p<0,05$) a capacității de fagocitoză a PMN din sângele periferic față de SF (fig. 1).

Extractul vegetal din *C. islandica*, la doza utilizată în acest experiment, a determinat o creştere semnificativă statistic a valorilor testului NBT comparativ cu SF, atât în cazul administrării în monoterapie, cât şi în cazul asocierii cu Mg. Efectul s-a menținut şi la animalele cu hepatită toxică indusă experimental, efectul fiind semnificativ statistic mai puternic în cazul asocierii Ci cu Mg ($p<0,01$).

La animalele din toate loturile testate nu au fost constatate modificări ale activității complementului seric (UCH_{50}) comparativ cu SF (fig. 2).

S-a constatat că TC a determinat o alterare hepatică profundă, evidențiată prin creşterea semnificativă statistic ($p<0,01$) a valorilor enzimelor TGP şi GGT, precum şi a bilirubinei serice totale față de lotul martor (fig. 3, 4, 5).

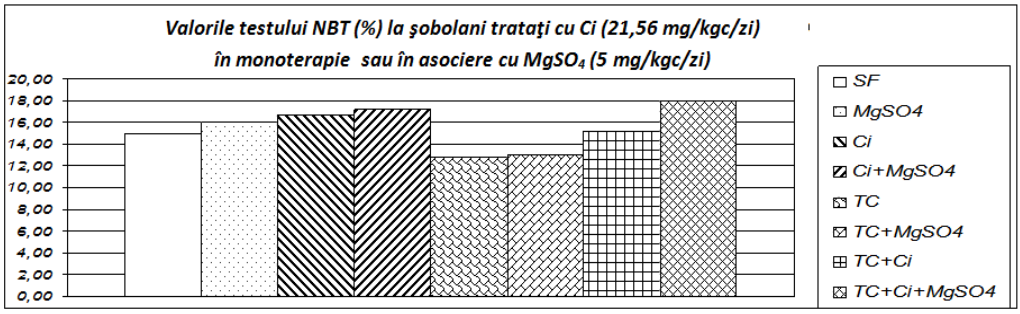

Fig. 1. Valorile testului NBT la 28 zile de la debutul studiului

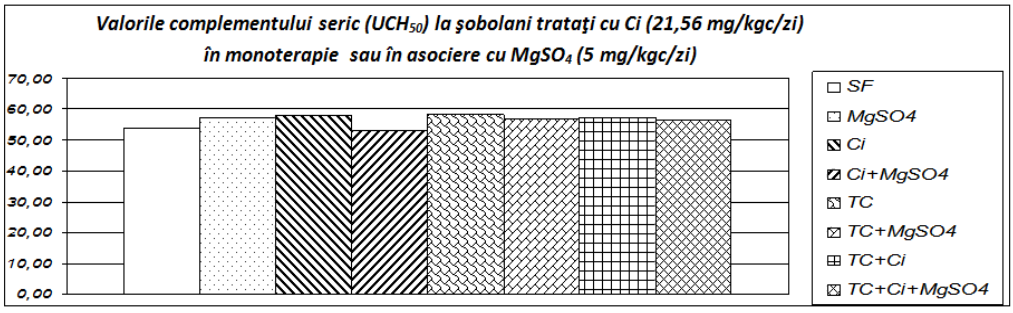

Fig. 2. Activitatea complementului seric la 28 zile de la debutul studiului

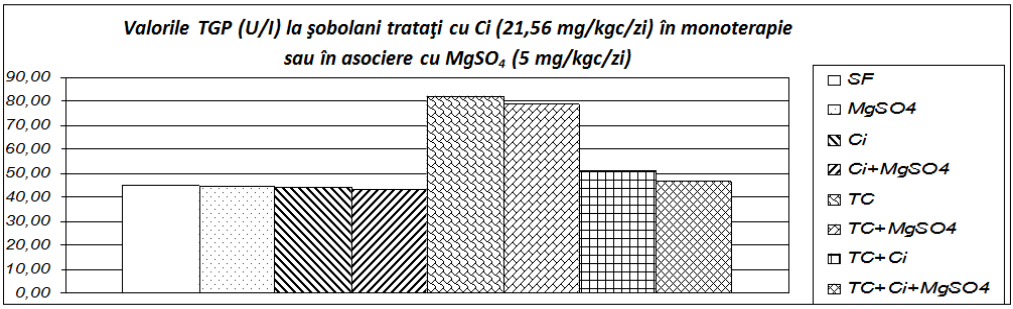

Fig. 3. Activitatea TGP la 28 zile de la debutul studiului

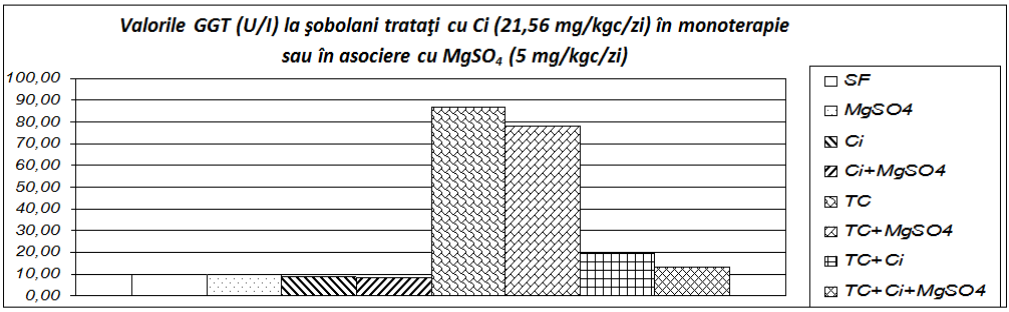

Fig. 4. Activitatea GGT la 28 zile de la debutul studiului

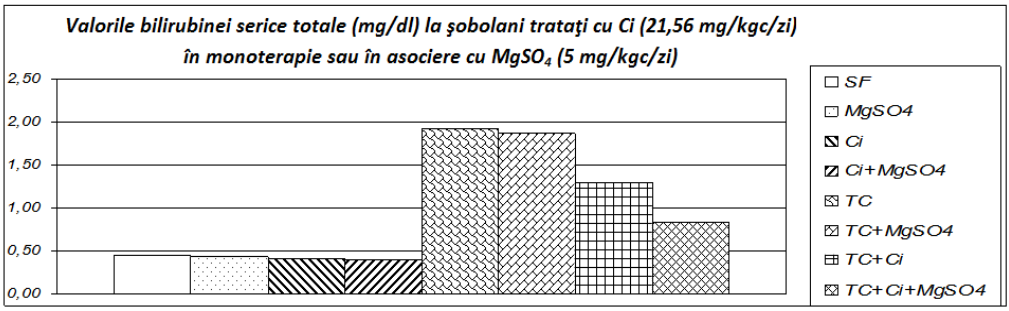

Fig. 5. Valorile bilirubinei serice totale la 28 zile de la debutul studiului

Extractul vegetal din *C. islandica* a determinat o scădere semnificativă statistic a valorilor enzimelor TGP şi GGT, precum şi a valorilor bilirubinei serice totale, atât în cazul administrării în monoterapie, cât şi în cazul asocierii cu Mg, nivelurile revenind la limitele normale. Efectul este semnificativ statistic mai puternic în cazul asocierii Ci cu Mg la animalele cu hepatită toxică indusă experimental, dar Mg în monoterapie nu influenţează aceşti parametri ($p>0,05$).

La animalele din toate loturile testate nu s-au constatat modificări semnificative statistic ($p>0,05$) ale valorilor TGO, LDH, colesterolului total, LDL-colesterolului şi HDL-colesterolului comparativ cu lotul martor tratat cu SF.

Extractul vegetal testat a determinat efecte imunostimulatoare şi hepatoprotectoare, care au fost potenţate prin asocierea cu Mg,

ceea ce sugerează ipoteza utilizării ulterioare a acestei asocieri, în urma unor multiple testări, ca alternativă de medicament sau adjuvant terapeutic hepatoprotector şi imunostimulator (2).

CONCLUZII

Studiile experimentale privind proprietăţile farmacologice ale extractului obţinut din produsul vegetal *C. islandica* (codificat Ci), în doză reprezentând 1/80 din DL_{50} (21,56mg/kgc/zi), administrat în monoterapie sau în asociere cu Mg, sub formă de $MgSO_4$, la animale cu hepatopatie indusă experimental cu tetraclorură de carbon, au condus la următoarele concluzii:

- Ci a determinat o creştere semnificativă statistic a procentului PMN şi a funcţiei fagocitare a acestora, efectul menţinându-se şi la animalele cu hepatopatie indusă experimental;
- în monoterapie, extractul testat nu a influenţat semnificativ statistic scăderea procentului limfocitelor la animalele cu hepatită indusă experimental, dar, în asociere cu Mg, s-a produs o ameliorare semnificativă a acestui parametru în condiţiile experimentale menţionate;
- în asociere cu Mg, extractul vegetal a determinat diminuarea bazofiliei şi a monocitozei la şobolanii Wistar, cu hepatită indusă experimental;
- extractul vegetal, atât în monoterapie cât şi în asociere cu Mg, nu a influenţat semnificativ statistic componenta umorală testată (activitatea complementului seric);
- extractul vegetal, atât în monoterapie cât şi în asociere cu Mg, a ameliorat afectarea hepatică indusă cu TC.

Efectele hepatoprotectoare şi imunostimulatoare au fost semnificative la animalele cu hepatopatie indusă experimental, situaţie ce ar putea fi speculată terapeutic în eventualitatea introducerii ulterioare a acestei asocieri în rândul produselor adjuvante hepatoprotectoare şi al terapiei imunomodulatorilor de generaţia a II-a (imunomodulatori de origine vegetală).

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SITUAȚII CLINICE RARE ALE DIVERTICULILOR COLONULUI

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RARE CLINICAL SITUATONS OF THE DIVERTICULI OF THE COLON **(**Abstract): It is well known that the diverticuli of the colon gets inflamed in10-15% of cases, thus becoming clinical symptomatic as a diverticulitis, while in other 15% they will bleed, with the clinical aspect of an inferior digestive bleeding. Our study presents some clinical observations with diverticuli of the colon, that raised diagnostic and therapeutic problems. **Material and method:** Between 2001-2010, 17 patients were admitted in the 3rd Surgery Clinic, University Hospital "St.Spiridon", Iasi, with asymptomatic diverticuli of the colon, that were put in evidence imagistically , intraoperative or on specimens after removal of the colon for other conditions;meanwhile, there were 15 patients admitted with complications of the diverticuli:5 patients had perforated diverticulitis (4-pericolic abscesses and one with peritonitis in the lower abdomen), 4 had rectal bleeding and the other 6 raised particular problems of diagnostic and treatment, being included in the present study. **Results:** Five out of six underwent operation. One patient refused the surgical treatment. Postoperative evolution was favorable in 4 out of the 5 operated patients. **Conclusions:** The complications of colonic diverticuli present on admission under clinical aspects that usually mimic a colonic cancer. In these situations the imagistic examinations do not offer enough details to elucidate the diagnosis. Our six particular observations strengthen the dictum of avoiding the "mirage" of the first lesion. The extent of the surgical procedure in the case of patients with colonic diverticuli admitted under the clinical aspect of a complication is sometimes disproportionate and encumbered of increased mortality and complication rate. **Key words:** DIVERTICULI, DIVERTICULITIS, PERICOLIC ABSCESS

Este cunoscut faptul că diverticulii colonului, în procent de 10-15%, se pot inflama și se manifestă clinic prin tabloul diverticulitei (1; 2), pentru ca, în alte 15% din cazuri, să se complice cu hemoragie, exteriorizată sub forma hemoragiei digestive inferioare. Pacienții cu diverticulite pot prezenta la data diagnosticului una sau mai multe complicații: abcese peridiverticulare sau extinse în vecinătate, obstrucția colonului, perforația, fistulizarea sau o sângerare importantă. Este adevărat că cei mai mulți pacienți nu prezintă nici o simptomatologie până la internarea în spital, când se prezintă cu o complicație a diverticulozei. Tot atât de adevărat este faptul că un pacient cunoscut cu diverticuloză colică poate avea, la data internării în spital, o altă patologie colonică, după cum sunt posibile complicații rare ale diverticulitei. Lucrarea de față prezintă o serie de observații clinice care au pus probleme de diagnostic și atitudine terapeutică.

MATERIAL ȘI METODĂ

În perioada 2001-2010, în Clinica a III-a Chirurgie Iași, au fost internați 15 pacienți cu diverticulită colonică. Dintre aceștia, cinci pacienti au prezentat diverticulite perforate (cu abcese pericolice-4, și unul cu peritonită submezocolică), patru au prezentat tabloul clinic al unor rectoragii, iar celelalte șase observatii au ridicat probleme particulare privind diagnosticul și tratamentul, făcând obiectul acestei lucrări. În aceeași perioadă au fost internați în clinică, pentru alte afecțiuni (cancere colonice, polipoze colonice, sindroame subocluzive prin aderențe postoperatorii), alți 17 pacienți cu diverticuli colonici asimptomatici și care au fost puși în evidență prin clisma baritată, precum și intraoperator sau pe piesele de rezecție.

Bolnava I.E., 48 ani, se internează în clinică pentru constipație, durere hipogastrică, rectoragii minore, febră 38-39° C., cu caracter intermitent. Tușeul rectal constată ampula rectală goală, cu urme de sânge proaspăt pe mănușa degetului explorator. Rectosigmoidoscopia evidențiază mucoasa rectală edematiată și ușor eritematoasă până la 18 cm de marginea anală, pentru ca, de la acest nivel, să devină intens edematiata, hiperemică, cu îngustarea bruscă a lumenului, impiedicând progresia colonoscopului. Datele de laborator: hematurie microscopică, leucocitoză (16.000/mm³) și anemie moderată (Hb:7,8gr%, Ht:36%). Ecografic se constată o formațiune pelvină, ecogenă, situată retrouterin și deasupra domului vezical, în vecinătatea căreia se găsește o colecție de 4/5 cm. În acest context se formulează diagnosticul de cancer rectosigmoidian cu abces perineoplazic și răsunet septic; se intervine chirurgical, descoperindu-se un bloc pelvin sângerând ușor la manevrele de disecție; pe parcursul acesteia se deschide o colecție purulentă, fetidă, cu caracter de infecție cu E. Coli, care se evacuează și se drenează. Operatorul consideră că diagnosticul este de cancer rectosigmoidian și practică un anus iliac stâng în continuitate. Postoperator se administrează antibiotice conform antibiogramei din secreția evacuată și antiinflamatorii. I se recomandă pacientei control după trei săptămâni de la externare; revine în clinică după șase săptămâni, când se practica o rectosigmoidoscopie cu intenția efectuării unei biopsii, care să certifice diagnosticul de cancer. Surpriza este la examenul rectosigmoidoscopic, care constată lumen rectocolic permeabil până la stoma, cu mucoasa rectală cu discrete caractere inflamatorii. Se efectuează o clismă baritată care pune în evidență multipli diverticuli sigmoidieni; suspiciunea de cancer sigmoidian este infirmată. Se practică colectomie stângă joasă cu colorecto anastomoză, cu evoluție favorabilă. *Particularitatea* cazului: bolnava tânără, internată cu diagnosticul de cancer sigmoidian stenozant, cu abces perineoplazic, sângerând, diagnosticul fiind diverticulită.

Bolnava I.A., 70 ani, purtătoare de diverticuli pe colonul descendent și sigmoid (evidențiați prin clismă baritată), se internează pentru dureri în fosa iliacă stângă, constipație și febră 38°C. Examenul obiectiv constată o formațiune în fosa iliacă stângă, fixă și dureroasă. Radiografia abdominală simplă arată o moderată distensie a cadrului colic, iar din probele de laborator de reținut a fost leucocitoza (15.000/mm³). Clisma baritată pune în evidență o imagine de stop parțial pe bucla sigmoidiană, sugerând diagnosticul de cancer sigmoidian. Se formulează diagnosticul de cancer al colonului stâng cu abces perineoplazic și se intervine chirurgical. Se constată o formațiune cu caractere macroscopice de cancer, situată pe bucla sigmoi-

diană, fixată parietal prin aderențe inflamatorii, care prin liză duc la evacuarea unei colecții purulente. Se drenează abcesul și se practică operația Hartmann. Surpriza apare atunci când piesa de rezecție este secționată: pe peretele sigmoidian se găsesc numeroși diverticuli, dar în mijlocul masei pseudotumorale se află un os de pasăre, ascuțit (clavicula), înfipt în peretele colonului prin baza unui diverticul, pe care-l perforează. Evoluția postoperatorie a fost simplă. *Particularitatea* cazului: corp străin care perforează peretele sigmoidian la nivelul unui diverticul, mimând astfel tabloul clinic al unui cancer sigmoidian perforat, cu abces perineoplazic.

Bolnavul A.P. 66 ani, aflat sub tratament cronic cu anticoagulante dicumarinice pentru o afecțiune valvulară, se internează în clinică pentru rectoragii abundente, paloare, tahicardie (110 bătăi/min), tendință la lipotimie. Din probele de laborator, de reținut: anemie: Hb. 7,8gr.%, un indice de protrombină (I.P.) de 70% . Dupa șase zile de expandare volemică, hemostatice și administrare de plasmă, sângerarea se oprește și face posibilă efectuarea rectosigmoidoscopiei, care decelează un polip pediculat ulcerat lung de 5 cm, care se excizează pe cale endoscopică. Rectul și sigmoidul nu prezintă, aparent, alte leziuni. Pacientul se externează, dar după două luni se reinternează cu rectoragii și sensibilitate dureroasă în fosa iliacă stângă. Se instituie tratament hemostatic cu oprirea hemoragiei și efectuarea ulterioară a clismei baritate care deceleaza multipli diverticuli pe colonul sigmoid, descendent și a trei diverticuli pe colonul transvers. Se propune pacientului intervenția chirurgicală (fiind la a doua sângerare), dar pacientul refuză și se externează. Evolutia ulterioară a fost bună, pacientul fiind pierdut din evidență după un an de la ultima internare. *Particularitatea* cazului: un polip voluminos, pediculat și ulcerat, s-a comportat pentru endoscopist ca sursă sigură a sângerării, neexplorarea restului cadrului colic fiind pusă pe seama "mirajului" primei leziuni, sângerarea fiind dată de diverticulii colonului sigmoid.

Bolnava C.M., 83 ani, diagnosticată cu sindrom de colon iritabil și diverticuloză a colonului stâng, se internează pentru durere în fosa iliacă stângă, anorexie, diaree asociată cu rectoragii minore, stare subfebrilă. În antecedente se remarcă episoade dureroase de intensitate moderată în fosa iliacă stângă. Examenul fizic decelează moderată distensie abdominală și durere moderată la palparea cadranului inferior stâng al abdomenului. Întrucât există în antecedente diagnosticul de diverticuloză, se etichetează suferința în cadrul diverticulitei colonului stâng și se instituie antibioterapia, administrarea de antiinflamatorii, repaos digestiv, expandare volemică și alimentație parenterală timp de șapte zile. Tratamentul instituit duce la remiterea simptomelor de la internare, iar scaunul se normalizează; se reia alimentația per os și pacienta se externează. După cinci săptămâni reapar rectoragiile, cu tabloul clinic al unei anemii secundare, motiv pentru care pacienta se reinternează. Explorarea endoscopică, efectuată atent acum, decelează doi polipi de 17 și respectiv 10 mm, ulcerați și sângerânzi, localizați la 8 și respectiv 9 cm de marginea anală. Se practică polipectomia pe cale transanală; evoluția favorabila, la doi ani de supraveghere. *Particularitatea* cazului: bolnava vârstnică, cunoscută ca purtătoare de diverticuli colonici, sângerează iterativ din doi polipi adenomatoși și nu din diverticuli.

Bolnava S.E. 49 ani, fara un istoric personal de boală, se internează în urgență pentru dureri abdominale difuze, brusc instalate, după o perioada de trei zile de con-

stipație, febră, frisoane, contractură abdominală generalizată, prăbușirea T.A., tahicardie, tahipnee, sugerând diagnosticul de peritonită generalizată. Ecografia decelează lichid abundent în întreaga cavitate peritoneală. Laparotomia efectuată în urgență găsește lichid fecaloid abundent submezocolic și în cantitate mai mică supramezocolic; în hipogastru se găsește un bloc tumoral de aproximativ 20 cm, aparținând colonului sigmoid, care pare că invadează anexa stângă și fața posterioară a uterului. Detașarea acestei formațiuni de uter și anexa stangă fiind imposibil de realizat, se practică ablația monobloc a jumătății inferioare a sigmoidului înglobând tumora (piesa de rezecție: 22 cm), a uterului și a anexelor, cu închiderea bontului rectal, inflamat și îngroșat și colostomie terminală; lavaj și drenaj larg al peritoneului, cu evoluție postoperatorie favorabilă în cadrul terapiei intensive. Surpriza diagnostică e dată de examenul histopatologic la parafină, care constată un important proces inflamator, difuz, cu îngroșarea accentuată a peretelui colonic și prezența a numeroși diverticuli și a unui polip sesil cu displazie epitelială severă, situat la 8 cm de capătul distal al rezecției. Pacienta revine la 2 luni de la operație, când se efectuează colonoscopie pentru restul colonului, prin anusul iliac, și rectoscopie, care exclude existența altor polipi. Se efectuează repunerea în circuit, prin anastomoză mecanică. Evoluție postoperatorie favorabilă. *Particularitatea* cazului: bolnava tânără cu diverticulită perforată ca prim simptom, cu peritonită generalizată, sepsis sever, mimează ca diagnostic un cancer colonic perforat. Interesantă este asocierea polipului cu displazie epitelială severă, care a fost îndepartat odată cu tumora.

Bolnava I.B. 68 ani, se internează în clinică cu febră (38,7°C), frisoane și durere în flancul stâng. Laborator: leucocitoza: 16.000/mm³, uree:1,20gr/l, creatinina: 1,80 g/l. Ecografia decelează o colecție cu caracter purulent, localizată în flancul stâng, iar radiografia abdominală simplă arată o distensie moderată a cadrului colic. Tomografia computerizată (T.C.) cu substanță de contrast nu a putut fi efectuată datorită valorilor ridicate ale ureei și creatininei. Istoricul pacientei e nesemnificativ. Întrucât evoluția bolii s-a agravat, cu toata terapia intensiva aplicată, s-a intervenit chirurgical la 48 ore de la internare, găsindu-se o masă cu caracter tumoral situată pe colonul descendent și o colecție purulentă de vecinătate, fuzată retroperitoneal, în spațiul parietocolic stâng. Se evacuează colecția și, întrucât formațiunea părea stenozantă, destinzând colonul suprajacent, se practică o colectomie stângă înaltă cu colo-colo anastomoza. Piesa de exereză conține o formațiune de 6/4 cm, cu aspect tumoral, iar colonul din vecinătate numeroși diverticuli și important proces inflamator, extins și la ciucurii epiploici, mimând un adenocarcinom al colonului. Evoluția postoperatorie imediată a fost nefavorabilă, prin instalarea dezunirii anastomozei, peritonită și șoc septic. Examenul histopatologic infirmă diagnosticul de cancer și constată un proces inflamator cu importantă îngroșare a peretelui colic, cu tendință la stenoză, precum și prezența a numeroși diverticuli. *Particularitatea* cazului: diverticulita perforată cu abces de vecinătate fuzat retroperitoneal, la o pacientă cu fenomene subocluzive, a sugerat un cancer al colonului stâng, cu abces de vecinătate, diagnostic sugerat și de aspectul macroscopic al piesei de exereza. Examenul histopatologic a infirmat însă diagnosticul de adenocarcinom; evoluția nefavorabilă poate fi prevenită printr-o operație de tip Hartmann.

DISCUȚII

Mulți pacienți cu diverticuloză rămân asimptomatici pe tot parcursul vieții lor, și numai 10-25% vor deveni simptomatici în cadrul diverticulitei (1). Statisticile estimează că un număr de 15-20% dintre pacienți vor face hemoragie diverticulară și, din acest grup, 80% beneficiază numai de măsuri terapeutice vizând expandare volemică, administrare de hemostatice și sânge, fără a necesita tratament chirurgical. Aproape jumătate din pacienți au episoade anterioare de sângerare (2), iar examenul fizic nu poate să precizeze sediul sângerării. Colonoscopia, pentru o serie de autori, ar avea o acuratețe diagnostică de până la 86% din cazuri în privința depistării sursei sângerării (3). Ca și în alte situații întâlnite în explorarea imagistică, colonoscopia trebuie să evite mirajul primei leziuni; depistarea unui polip colonic ulcerat și ablația acestuia nu exclude explorarea ulterioara a întreg cadrului colic, pentru a nu scăpa sursa reală a sângerării, așa cum a fost la una din observațiile noastre. Pe de altă parte, diagnosticul de diverticuloză a colonului, stabilit imagistic cu ani în urmă, nu exclude existența altor cauze de sângerare exteriorizată prin rectoragie sau hematochezis; în acest sens, prezenta unor polipi rectali sau colonici poate fi cauza sângerării, situație întâlnită la o alta observație a noastră. Ca urmare, arteriografia mezenterica selectivă în urgență este o explorare imagistică utilă, permițând identificarea sursei sângerării în pana la 60% din cazuri (4), iar în același timp poate fi și o soluție terapeuticță pemițând oprirea sângerării (instilare de vasopresină sau embolizări). (5). Este adevărat ca la un pacient vârstnic, prezența unei suferințe abdominale acute localizată în fosa iliacă stângă sau flancul stâng sugerează diagnosticul spre o diverticulită, eventual cu abces de vecinătate. Totuși, trebuiesc luate în discuție și alte afecțiuni chirurgicale acute, cum ar fi cancerul colonului stâng perforat, cu abces perineoplazic, un abces perinefretic stâng sau uropionefroză. Din acest motiv trebuie apelat la T.C., care poate defini riscul individual și atitudinea terapeutică. (6). Cea mai mare acuratețe diagnostică o are T.C., cu condiția ca explorarea să se facă cu substanță de contrast, dacă nu există contraindicații, situație întâlnită și la una din observațiile noastre. Explorarea imagistică (colonoscopia, clisma baritată) în diverticulite, are riscul de a rupe abcese peridiverticulare, cu extravazarea bariului ca și a conținutului colonic în cavitatea peritoneală (7). Diverticulitele situate pe partea stângă, la pacienții tineri, s-au dovedit a fi, în cazuistica noastră, mai "virulente" și cu un risc crescut de perforație și recurență, așa cum se citează și în alte studii (8). Starea septică se instalează repede, iar răsunetul sistemic nu permite efectuarea C.T. cu substanță de contrast. Desigur, exista și situații foarte rare când un corp străin se inclavează într-un diverticul al colonului, declanșând un proces inflamator la nivelul peretelui și în final perforează diverticulul, cu apariția unui abces pericolic, așa cum s-a întamplat la una din observațiile noastre. Diverticulitele pot realiza progresiv o obstrucție a colonului, rareori completă. Se pot asocia cu abcese diverticulare, retroperitoneale, mezenterice sau pelvine. Uneori obstrucția colonului se asociază cu un abces pericolic, mimând un cancer, iar explorarea intraoperatorie nu poate face diferența între cele două afecțiuni. În literatura de specialitate nu există studii randomizate care să compare cele două procedee operatorii, operația Hartmann *versus* rezecția colonică cu anastomoză primară, la bolnavii cu diverticulită perforată. Există multiple publicații care compară rezultatele după cele doua procedee și care caută să arate un beneficiu privind scăderea morbidității și a mortalității după

anastomaza primară (9,10,11,12,13), dar pe un număr redus de pacienți, pe loturi neomogene ca leziuni anatomopatologice. Un studiu recent, pe pacienti atent selecționați, cu diverticulite perforate, demonstrează clar că operația Hartmann rămâne opțiunea sigură, în special la pacienții în vârstă, cu multiple comorbidități (14), atitudine la care ne raliem și noi.

CONCLUZII

Complicațiile diverticulozei colonice îmbracă uneori aspecte clinice mimând cel mai frecvent cancerul și la aceste cazuri explorarea imagistică nu oferă date suficiente pentru elucidarea diagnosticului.

Aceste observații particulare întăresc și mai mult dictonul de a evita, pe cât posibil, "mirajul" primei leziuni.

Amploarea intervenției chirurgicale în cazul diverticulilor colonici care se prezintă la internare sub forma complicațiilor este, uneori, disproporționată și grevată de complicații și mortalitate crescute.

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ASPECTE MEDICO-SOCIALE ALE MIGRAȚIEI PERSONALULUI MEDICAL

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MEDICAL-SOCIAL ISSUES OF THE MIGRATION OF THE MEDICAL STAFF (Abstract): The migration of the medical staff represents an increasingly worring reason for romanian health policy-makers. According to the Ministry of Health, in 2009 year, the human resources indicators pointed out 26.5 physicians/ 10,000 inhabitants (whereas the European Union average was 32.8) and 60,4 nurses and midwives (whereas the European Union average was 108.5). Between 2007-2010, over 9000 physicians requested professional certificates in order to practice abroad and the majority have left the country. This phenomenon is related to the Romania's entrance in European Union and it is supposed to continue in the coming years. The direct consequences of the migration of the medical staff consist of significant differences between the country's regions in the health workforce distribution, the most affected by the critical shortage of health service providers being the North-Eastern region, especially in rural area. In order to limit the migration phenomenon, the Ministry of Health elaborated some strategies and workforce policies on medium and long-term, but the results will be difficult to assess. **Key words**: MIGRATION, MEDICAL STAFF, CRITICAL SHORTAGE

După Al Doilea Război Mondial în țările Europei Centrale şi de Est, cu excepția Yugoslaviei, s-a impus modelul sovietic de tip Semaşko de organizare a îngrijirilor de sănătate.

Vreme de câteva decenii în România, ca şi în țările învecinate, s-a reuşit acoperirea întregii populații şi accesul gratuit la îngrijirile de sănătate. Sistemul sanitar era controlat de guvern printr-o planificare centralizată, personalul era remunerat pe bază de salariu, nu exista practică privată, şi totuşi starea de sănătate a populației din Europa Centrală şi de Est era comparabilă, până la mijlocul deceniului şase din secolul trecut, cu cea din Vestul Europei (1). A urmat o deteriorare progresivă a stării de sănătate a populației, consecință a înrăutățirii condițiilor de viață datorită cheltuielilor militare, subfinanțării serviciilor de sănătate şi a unei organizări ineficiente.

Din 1990 şi până în prezent, sistemul de sănătate din țara noastră a parcurs două decenii marcate de numeroase schimbări, prin trecerea de la un sistem centralizat, finanțat integral de la bugetul de stat, la unul bazat pe asigurările sociale de sănătate. În domeniul resurselor umane, România se

confruntă de mulți ani cu un deficit, având una dintre cele mai mici rate europene de acoperire cu personal medico-sanitar (2).

După aderarea României la Uniunea Europeană, începând cu 1 ianuarie 2007, emigrația (sau intenția de emigrare) a personalului medical a devenit o preocupare majoră pentru asociațiile profesionale din domeniu.

SITUAȚIA ACTUALĂ

O analiză a resurselor umane în România, evidențiază în ultimele patru decenii, creşterea treptată a numărului de medici, farmacişti şi asistente medicale (3) (tab. I).

TABELUL I

Evoluția unor indicatori ai resurselor umane din sănătate, 1969-2009

Anul	Nr. mediu de locuitori ce revin la un:			
	Medic*	Stomatolog	Farmacist	Asistent medical
1969	774	5535	4410	211
1974	762	3655	3996	179
1979	705	3247	3553	168
1984	580	3113	3471	171
1989	552	3253	3599	171
1994	567	3844	8078	175
1999	523	2914	3398	197
2004	450	2188	2473	178
2009	426	1718	1790	166

**exclusiv stomatologi*

Statisticile oficiale evidențiază că la sfârşitul anului 2009, în România erau: 50.386 medici (exclusiv stomatologi), din care 12.009 medici de familie; 12.497 medici dentişti (stomatologi); 11.996 farmacişti; 129.673 asistenți medicali şi moaşe (4).

Aceasta înseamnă că, în medie, la fiecare 10.000 locuitori există: 23,5 medici (exclusiv stomatologi); 5,8 medici dentişti (stomatologi); 5,6 farmacişti; 60,4 asistenți medicali şi moaşe.

În prezent, România, comparativ cu alte țări europene, se confruntă cu un deficit de resurse umane, deşi chiar în rândul țărilor industrializate există mari diferențe în ce priveşte indicatorii respectivi de evaluare (5) (tab. II).

Comparativ cu România (23,5 medici/10.000 locuitori), în majoritatea țărilor vest europene, indicele de asigurare cu medici depăşeşte media Uniunii Europene de 32,8, existând unele țări cu peste 40 medici/10.000 locuitori. Pentru asistenții medicali şi moaşe, media Uniunii Europene este de 108,5/10.000 locuitori (din care 79,4 asistenții medicali şi 29,1 moaşe), valori ce contrastează cu cele din România (6).

Libera circulație a forței de muncă şi-a făcut resimțit efectul şi în România, odată cu aderarea la Uniunea Europeană, după care migrația forței de muncă din sănătate a devenit o problemă majoră cu care se confruntă sistemul de sănătate, fiind afectată funcționarea normală şi calitatea serviciilor.

După 1 ianuarie 2007, diploma care atestă pregătirea personalului medical este recunoscută în spațiul UE, iar Ministerul Sănătății eliberează, la cerere, un certificat pentru recunoaşterea diplomei medicilor, asistentelor şi moaşelor. În intervalul 2007-2010, aproximativ 9.000 de medici au obținut certificatele de conformitate pentru a putea munci în străinătate, din aceştia 8000 fiind plecați deja (7,8).

TABELUL II

Resursele umane din domeniul sănătății în unele țări industrializate

Țara	Nr. resurse umane/ 10.000 locuitori	
	Medici	Asistente şi moaşe
Australia	25	97
Austria	37	66
Belgia	42	142
Canada	19	101
Danemarca	36	101
Elveția	40	110
Franța	34	80
Germania	34	80
Grecia	50	36
Irlanda	29	195
Israel	37	62
Italia	37	72
Japonia	21	95
Marea Britanie	23	128
Norvegia	38	162
Spania	33	76
Statele Unite	26	94

Numai în cursul anului 2010, un număr de 2779 de medici au solicitat certificate profesionale curente. Cele mai mari valori au fost înregistrate în Bucureşti – 947, urmat de Cluj – 403, Iaşi – 253 şi Timiş – 202 (8).

Acest fenomen corelat cu intrarea României în Uniunea Europeană, a fost observat în toate țările din Europa de Est şi în multe țări din Europa Occidentală (9).

Un studiu efectuat pe un eşantion reprezentativ la nivel național de asistente medicale, a evidențiat profilul asistentului medical din România, cu un înalt potențial de migrare, astfel: asistent medical generalist, absolvent al unui colegiu de asistenți medicali, cu vârsta cuprinsă între 26 - 35 de ani, care locuieşte într-o zonă urbană, în partea nord-estică sau sudică a țării (10).

Intenția de emigrare a unor cadre medicale este "susținută" şi de faptul că firmele de recrutare din domeniu şi-au intensificat activitatea în ultimii ani, organizând numai în toamna anului 2010 patru ediții (Cluj, Timişoara, Iaşi, Bucureşti) ale Târgului Internațional de Cariere Medicale şi Farmaceutice. Oferta a fost pentru țări ca Marea Britanie, Irlanda, Germania, Suedia, Danemarca, Franța, Elveția, Belgia, Norvegia sau Spania, cele mai mici salarii oferite fiind cuprinse între 80.000 – 120.0000 euro pe an, pentru medicii cu experiență existând şi şansa de a câştiga între 150.000-200.000 euro pe an (11).

Firmele care fac recrutări oferă medicilor cursuri de pregătire, o primă de instalare, dar şi facilități de angajare pentru soții sau soțiile lor, fiind solicitați, în special, medici generalişti, anestezişti şi oncologi. În anul 2010 cele mai multe cereri de certificate profesionale curente au fost pentru specialitatea de medicină generală/ medicină de familie, urmând chirurgia generală şi anestezie şi terapie intensivă.

CONSECINȚE ŞI PERSPECTIVE

Într-un Raport al Comisiei Prezidențiale pentru analiza şi elaborarea politicilor din domeniul sănătății publice din România, se arată că din perspectiva resurselor umane, există dezechilibre importante între diferitele regiuni ale țării. Astfel, în regiunea de Nord-Est a fost înregistrată cea mai slabă acoperire cu medici în mediul rural (2.778 locuitori/1 medic) (12).

Există încă un număr de 98 de comunități fără medic în mediul rural. Populația arondată localităților din mediul rural fără medici de familie era de 145.110 locuitori, reprezentând 1,48 % din totalul populației rurale.

Pentru o treime din teritoriul României, mai mult de 30% din specialitățile medicale nu sunt acoperite cu medici (cardiologie, medicină de urgență, terapie intensivă, diabetologie etc.); o situație similară apare şi în cazul asistentelor medicale. Acest lucru a dus la adâncirea inegalităților dintre populația rurală şi urbană sub aspectul accesului la serviciile de sănătate.

Ca urmare a posibilității personalului medical din România de a lucra în țările UE, se va asista în continuare la o reducere a densității personalului din domeniul medical, mai ales în rândul tinerilor şi a celor înalt specializați. Este de aşteptat ca structura pe vârste a medicilor de familie şi mobilitatea crescândă a forței de muncă, să supună sistemul de sănătate la presiuni considerabile.

De asemenea, în acelaşi Raport al Comisiei Prezidențiale, se arată că majoritatea medicilor de familie au vârsta cuprinsă între 45 şi 55 ani, urmând a se pensiona în 10-15 ani, şi că puțini sunt tinerii care practică medicina de familie. Dacă această tendință va continua, numărul medicilor, în general, şi al celor de familie, în special, va scădea dramatic, conducând la o scădere a accesului la îngrijiri şi creşterea cheltuielilor de funcționare a sistemului.

Analiştii economici şi financiari au elaborat prognoze conform cărora dacă se păstrează acelaşi ritm al intrărilor şi ieşirilor din sistem din ultimii doi ani, în anul 2021 nu vor mai fi medici care să lucreaze în România (13).

În prezent, media anuală a ieşirilor medicilor din sistem se ridică la 7.615 (3.333 prin migrație şi 4.282 ieşiri naturale).

La o medie anuală a intrărilor în sistem de 2.916, există în contrapondere o medie anuală a ieşirilor medicilor din sistem (prin migrație şi pensionare) de 7.615. Astfel, anual în România 4.699 de medici nu mai sunt înlocuiți, ceea ce înseamnă că raportarea la numărul total de medici din sistem înregistrat în 2009 (50.386), sugerează că, în aproximativ 11 ani, nu vor mai fi medici autohtoni care să asigure asistența pentru sănătate a populației.

CONCLUZII

Migrația forței de muncă din sănătate este un fenomen care in România a luat proporții îngrijorătoare, urmare a unui sistem de motivare insuficientă a personalului medical prin veniturile obținute, condițiile nesatisfăcătoare de muncă şi posibilitatea redusă de promovare profesională pe baza unor criterii obiective.

Principalele direcții de acțiune în vederea optimizării situației actuale a resurselor umane din sănătate sunt prezentate în Raportul Comisiei Prezidențiale privind politicile de sănătate publică (12).

1. *Elaborarea unor politici sectoriale coerente de formare, dezvoltare şi alocare a resurselor umane în sănătate.* În acest scop, Ministerul Sănătății trebuie să îşi îmbunătățească propria capacitate de analiză politică, planificare şi management de resurse umane la nivel central, concomitent

cu îmbunătățirea capacității de evaluare şi prognoză a numărului şi categoriilor/specialităților de personal adecvat, astfel încât să răspundă nevoilor de sănătate ale populației pe termen mediu şi lung.

2. *Creşterea disponibilității resurselor umane în sectorul de sănătate din România*. Este vorba de creşterea numărului de locuri în instituțiile de învățământ medical, concomitent cu limitarea pierderilor prin ieşirile din sistem. Pentru menținerea personalului factorii motivaționali trebuie corect identificați şi aplicați adecvat. Creşterea veniturilor până la atingerea unui nivel decent, ar însemna recunoaşterea imensei responsabilități a unei profesii făcute cu seriozitate şi dăruire.

3. *Stimularea dezvoltării carierei profesionale în domeniul medical.* Introducerea unui mecanism de recunoaştere şi încurajare a performanțelor profesionale şi a calității serviciilor la toate nivelurile sistemului de sănătate ar permite bun un sistem de motivare. La acesta ar contribui şi organizarea de cursuri de formare continuă pentru medici şi asistenți, în concordanță cu politicile de dezvoltare a sistemului de sănătate, neinfluențate de interesele comerciale ale distribuitorilor de medicamente sau aparatură medicală.

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NECESARUL DE TRATAMENT ORTODONTIC LA STUDENŢII FACULTĂŢII DE MEDICINĂ DENTARĂ DIN IAŞI

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ORTHODONTIC TREATMENT NEED OF THE STUDENTS OF THE FACULTY OF DENTAL MEDICINE FROM IASI (Abstract): Nowadays, people are becoming more aware of the importance ofaesthetics in the integration of the individual in society. The purpose of this study was to determine the need for orthodontic treatment and their aesthetic perception in agroup of medical students in Iasi. **Material and methods**. The clinical study was conducted on a sample of 254 subjects. Data were collected after completion of the clinical examination.The clinical evaluation of the status we used orthodontic index IOTN (Index Orthodontic Treatment Needs) and a questionnaire on aesthetic appreciation by the student. All subjects included in the study were informed verbally about the purpose of the study noting the consent form. Statistical data processing was done with SPSS14.00 program for Windows. **Results**: 20% of the subjects were dental IOTN 0, 33% of the value of a IOTN subjects, 28% of subjects IOTN value 2, 3 and 9% IOTN value IOTN 4and 5 values of 4% and 6% respectively. The need for orthodontic treatment is greater in subjects with IOTN 4 and 5 and 10% of the subjects presented severe dento-maxillary. IOTN aesthetic component of the index is divided into three groups: Group 1 - 4 with 91% of subjects, group 5-7 with 5% of subjects and group 8-10 with4% of subjects. **Conclusions**: The need for orthodontic treatment for subjects with IOTN4 and 5 is mare. There is an increasing number of orthodontic treatment in young people, the primary motivation being to improve the aesthetic appearance. **Key words**: IOTN, NECESSARY ORTHODONTIC TREATMENT, STUDENTS

Percepţia individului în ceea ce priveşte aspectul estetic şi integrarea socio-comportamentală a acestuia sunt factori care contribuie la motivarea pacientului în realizarea de tratamente ortodontice.

In trecut determinarea necesarului de tratament ortodontic se realiza strict din punct de vedere medical urmărindu-se prezenţa anomaliilor dentare, în prezent studiile subliniind rolul important al modului de percepţie a anomaliilor din punct de vedere estetic de către pacient, fapt ce motivează începerii acestor tratamente ortodontice. În acest scop indici precum Index of Orthodontic Treatment Need (IOTN), Dental Aesthetic Index (DAI) şi Index of Complexity Outcome and Need au fost dezvoltaţi pentru a ţine cont şi de percepŢia pacientului în ceea ce priveste anomalia dentara şi evaluarea rezultatelor tratamentului ortodontic (1,2).

IOTN este un sistem de notare al anomaliile dento-maxilare dezvoltat de Brook & Shaw (1989). Acest indice se compune din două componente independente: component dentară, care are un sistem de notare format din cinci grade de evaluare ce încadrează subiectul într-o grupă de risc în funcţie de anomaliile dentare pe care le prezintă (tab. I) şi componenta estetică care inregistrează perceptia estetica a anomaliilor dentomaxilare pe o scară de zece grade (fig.1)

Scopul acestui studiu a fost evaluarea statusului ortodontic la studenţilor medicinişti,

TABELUL 1
Componenta dentara a IOTN

IOTN 1	Ocluzie aproape perfecta
IOTN 2	Usoare anomalii dentare
IOTN 3	Anomalii dento-maxilare moderate dar care nu necesita tratamente ortodontice
IOTN 4	Anomalii dento-maxilare grave dar care necesita tratamente ortodontice
IOTN 5	Anomalii dento-maxilare foarte grave

stabilirea necesarului de tratament ortodontic dar şi percepţia acestora privind aspectul estetic al anomaliilor dento-maxilare.

MATERIAL ŞI METODĂ

Studiul a fost realizat pe 254 de subiecţi, studenti în anul II şi III la Facultăţii de Medicină Dentară, UMF Iaşi, în anul universitar 2009-2010. Lotul cuprinde 170 subiecţi de sex femenin(66.9%) şi 84 subiecţi de sex masculin(33.1%), cu o medie de vârstă de 22.7 ani(minim 21 ani, maxim 30 ani).

Datele s-au obţinut prin examenul clinic şi completarea fişei de observaţie la sediul Disciplinei de Prevenţie Oro-Dentara de către examinatori care au fost calibrati anterior.

Analiza statistica a datelor a fost realizata cu ajutorul programului informatic SPSS 14 pentru Windows.

REZULTATE

IOTN (Index Orthodontic Tretament Needs) prin cele două component IOTN dentar şi IOTN estetic subliniază necesarul de tratament ortodontic. Astfel, 20% dintre subiecţi au IOTN dentar valoarea 0, 33% dintre subiecţi IOTN valoarea 1, 28% dintre subiecţi IOTN valoarea 2, 9% IOTN valoarea 3 şi IOTN 4 şi 5 valorile de 4% şi respectiv 6%. Necesitatea realizării de tratamente ortodontice este mare în cazul subiectilor cu IOTN 4 şi 5, respective 10% dintre subiecţi prezintă anomalii dento-maxilare grave (fig.2).

Componenta estetică a indicelui IOTN se împarte în 3 grupe : grupa 1- 4 cu 91% dintre subiecţti, grupa 5-7 cu 5% dintre subiecţi şi grupa 8-10 cu 4% dintre subiecti.

Componenta estetică a indicelui IOTN se împarte în 3 grupe : grupa 1- 4 cu 91% dintre subiecţi care nu necesită tratamente ortodontice , grupa 5-7 cu 5% dintre subiecţi la care necesarul de tratament este “la graniţă” şi grupa 8-10 cu 4% dintre subiecţi care au nevoie de tratamente ortodontice complexe(fig.3).

In ceea ce priveşte tipul de anomalie dento-maxilară prezentată : 95% dintre subiecţi au prezentat anomalii dento-maxilare clasa I Angle cu uşoare anomalii dentare care nu modifică vizibil funcţional şi estetic starea pacientului şi 5% dintre subiecţi clasa aIIa Angle . Nu au existat subiecţi cu anomalie de clasa a III a Angle (fig.4).

Cele mai frecevente anomalii ortodontice semnalate au fost : anomaliile dentare izolate (89,1%), urmată de crossbite (44,5%), overbite marit (33,6%), a overjet mărit (33.6).

Situaţia tratamentelor ortodontice realizate pâna în prezent a fost următoarea : 4.3% dintre subiecţi sunt purtatori de aparate ortodontice fixe şi 2.8% de aparate mobile (aparate de contentie). 10, 6% dintre subiecţi au purtat aparate ortodontice mobile şi 7.5 % aparate fixe .

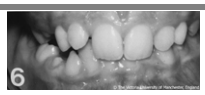
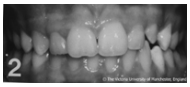
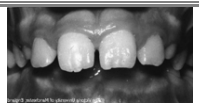
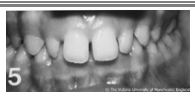
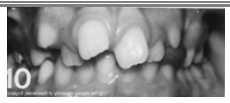
1		6	
2		7	
3		8	
4		9	
5		10	

Fig. 1. Componenta estetică a IOTN

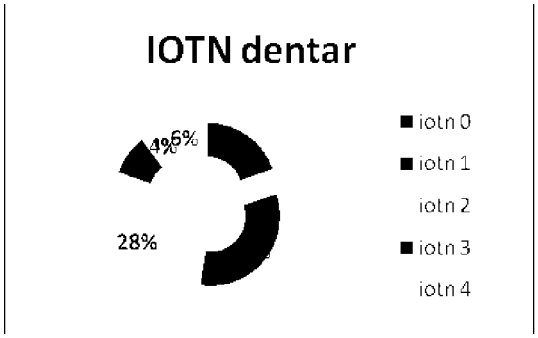


Fig. 2. Componenta dentara a IOTN-ului

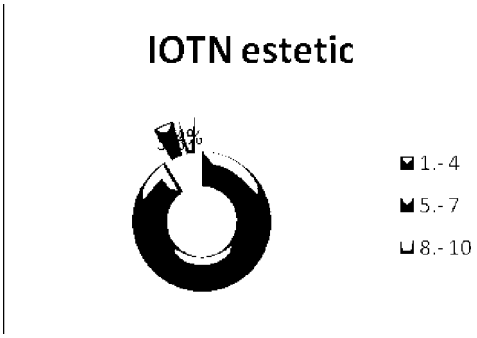


Fig. 3. Componenta estetica a IOTN-ului

La intrebarea "dacă există efecte secundare" 6% dintre subiecţi au raspuns "da", numai 80.7% declarându-se multumiţi de rezultatele tratamentelor efectuate. Subiecţii de sex femenin au fost mai nemulţumiţi de aspectul estetic în comparatie cu cei de sex masculine (p=o.o5).

Rezultatele obţinute subliniază necesarul mare de tratament ortodontic la populaţia adultă tânără din Romania, rezultate similare fiind întâlnite la populaţia tânără din Peru (3). Majoritatea studiilor epidemiologice publicate sunt realizate în populatia adultă tînără din Europa, unde tratamentul ortodontic este sustinut de către sistemul public de sănătate. In Finlanda 38.5% dintre studenţi au avut tratamente ortodontice,în Suedia 40%, Norvegia 61.5%(2), valori mari în comparatie cu cele obţinute în Romania unde aproximativ 20% dintre studenti au beneficiat de tratamente ortodontice .

CONCLUZII

Desi necesarul de tratament ortodontic

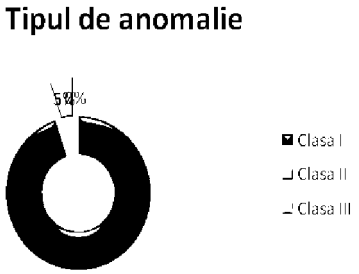


Fig. 4. Tipurile de anomalii dento maxilare

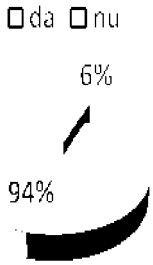


Fig. 5. Prezenţa efectelor secundare în urma tratamentului ortodontic

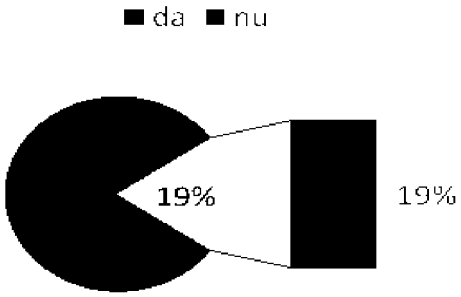


Fig. 6. Distribuţia răspunsurilor la întrebarea „Sunteţi multumiţi de rezultatele tratamentului ortodontic”

este mare la populaţia tânără se constată totuşi o creştere a numărului de tratamente ortodontice, majoritatea fiind motivate de necesitatea îmbunătăţirii aspectului estetic, componentă importantă în intergrarea socială a acestor tineri.

Procentul scăzut de tratamente ortodontice realizate la populaţia tânără este limitat din două motive : primul motiv ar fi numărul redus de medici specialişti din Romania care sunt concentraţi în oraşele mari făcând dificilă accesarea acestor servicii, iar cel de-al doilea motiv este reprezentat de susţinerea slabă a acestor tratamente de către sistemul de sănătate naţional, aceste tratamente fiind susţinute financiar în cea mai mare parte de către pacienti.

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EATING HABITS IN AN ADOLESCENT POPULATION FROM TIMIŞ COUNTY

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EATING HABITS IN AN ADOLESCENT POPULATION FROM TIMIŞ COUNTY (Abstract): Dietary patterns during adolescence may contribute to eating disorders and may increase the risk for several important chronic diseases later in life. **Objectives**: The aim of this study is to analyze eating behaviours among high school students residing in Timis county area. **Material and method**: We conducted a cross sectional study using a stratified cluster sample design to produce a representative sample of students for the Timis county. From 1083 primary sampling units (classes) 149 were randomly selected, stratified proportionally according to grades 9–12, high school profile (theoretical, industrial, vocational, and confessional), urban and rural environment. The study was carried out in 2004. **Results**: The overall response rate of 74.6%. A total of 2908 students were included in the survey. The proportion of students consuming less than daily milk & diary products, green salad and fruits was: 58.3% for milk & diary products, 71.2% for green salad and 59.1% for fruits. Based on multivariate analysis three common predictors for consuming fresh fruits, green salad and milk and diary products were identified: perceived weight, school performance and school grades. **Conclusions**: Through examination of prevalence rates of nutrition-related concerns we have identified priority areas to be targeted with future interventions. **Key words**: DIETARY PATTERNS, ADOLESCENTS, FOOD CONSUMPTION

Adolescence is considered an especially nutritionally vulnerable period of life for several reasons. First is the greater demand for nutrients because of the dramatic increase in physical growth and development. Second is the change of lifestyle and food habits of adolescents that affect both nutrient intake and needs. Third are those adolescents with special nutrient needs such as those who participate in sports, have a chronic illness, diet excessively, or those who use alcohol and drugs (1).

Dietary patterns during adolescence may contribute to eating disorders and may increase the risk for several important chronic diseases later in life (2,3). The main inadequate food consumption patterns are low consumption of fruits and vegetables (4,5). Dairy foods are the major source of calcium in youth diet and calcium intake is critical to achieving optimal peak bone mass and may play an important role in the prevention of degenerative bone disease in later years (6). Despite the importance of nutrition during adolescence, little is known about the eating behaviours of young people.

The aim of this study is to analyze eating behaviours among high school students residing in Timiş county area.

MATERIAL AND METHOD

We conducted a cross sectional study using a stratified cluster sample design to produce a representative sample of students for the Timis county, Romania. From 1083 primary sampling units (classes) 149 were randomly selected, stratified proportionally according to grades 9–12, high school profile (theoretical, industrial, vocational, and confessional), urban and rural environment. A 126-item questionnaire was developed to investigate health-risk behaviours of adolescents. Some of the questionnaire items were modified from other instruments, including the 1999 Youth Risk Behaviour Survey (YRBS) and the European School Survey Project on Alcohol and Other Drugs (ESPAD). The survey focused on health risk behaviours established during adolescence that result in the most significant mortality, morbidity, disability, and social problems for youths and, later, adults. The following issues were examined: family milieu, out-of-family entourage, tobacco use, alcohol use, illicit drug use, violence-related behaviours and school-related violent behaviours, suicide ideation, attempted suicides, and the seriousness of those attempts, preventive conduct in road traffic, sexual behaviours, dietary behaviours, overweight and weight control, physical activity, medical attendance. In total, 23 items were dedicated to dietary behaviours.

A pilot test was conducted to establish the validity and reliability of the instrument. The study was carried out between May–June 2004. The Timiş County Scholastic Supervision Board approved this study. The questionnaire was administered in a regular classroom setting and took students about 60 minutes to complete. No filter questions were used. By doing this, comparable amounts of time are required to complete the questionnaire, regardless of risk behaviour status and students cannot detect the risk behaviours of their colleagues simply by looking at the pattern of responses. Only students present the day of the survey were eligible for participation. Students were identified only by their classroom identification number. Trained public health residents and undergraduate students conducted the survey. The data collectors read aloud scripts that explained the survey procedures. Students were told that they do not have to put their names on answer sheets, and that obtained data would be used only for general assessment of the situation in the county and would not be ever associated with their schools or grades. A weighting factor was applied to each student record to adjust for non-response and for the varying probabilities of selection. The weights were scaled so that the weighted count of stu-

TABLE I

Demographic Characteristics of the Sample

Characteristics (n=2908)		95%CI
Age, y		
Mean	16.79	
SD	1.19	
Range	14-25	
Sex, %		
Male	48.5	46.7-50.3
Female	51.5	49.7-53.3
Residence area, %		
Cities	81.6	80.1-82.9
Towns	13.9	12.7-15.2
Villages	4.5	3.8-5.4
Ethnicity, %		
Romanian	92.3	91.2-93.3
Hungarian	3.4	2.7-4.1
Serbian	1.9	1.4-2.5
German	0.8	0.5-1.2
Ukrainian	0.5	0.3-0.9
Bulgarian	0.3	0.2-0.6
Croatian	0.3	0.1-0.6
Other	0.5	0.3-0.8

dents was equal to the total sample size.

Chi square test was used to compare categorical variables. Logistic regression modelling was used to detect predictors for certain behaviours. Using backward elimination procedures, the most parsimonious multivariate logistic regression model was produced. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated. All *P* values were calculated based on two--sided statistical tests. Statistical tests were performed using Epi Info v. 3.3.2 or SPSS v.10 software.

RESULTS AND DISCUSSION

The class response rate was 97.9%, and the student response rate was 76.2%, resulting in an overall response rate of 74.6%. A total of 2908 students were included in the survey. Demographic characteristics of the sample are shown in table I.

Based on self-reported height and weight, 6.1% of students were overweight and 0.8% were obese. The overall mean (SD) body mass index (BMI) was 20.7 (2.9) kg/m^2. Male students were more likely than female students to be overweight or obese (OR= 2.0; 95%CI: 1.5-2.8).

A fundamental component of a healthy diet is to eat a balanced breakfast, which is important for nutritional adequacy. A good breakfast can contribute to a healthy diet rich in carbohydrates and micronutrients (7,8,9). We found that 9.8% of the students never eat breakfast and 34.3% rarely do. Girls were more likely than boys to skip

TABLE II
Predictors for eating fresh fruits less than daily

Variables	*P* value	OR	95% C.I. for OR	
Gender (male)	<0,001	1.39	1.17	1.64
School performance (marks)	0.002			
9-10		1(ref)		
8-8.99	0.781	1.03	0.84	1.27
6-7.99	0.020	0.77	0.62	0.96
5-5.99	0.174	1.39	0.87	2.22
Grades	<0.001			
Grade 9		1(ref)		
Grade 10	0.073	1.20	0.98	1.47
Grade 11	0.016	1.29	1.05	1.59
Grade 12	<0.001	2.07	1.62	2.64
Perceived weight	0.006			
Underweight		1(ref)		
About the right weight	0.013	1.28	1.05	1.55
Slightly overweight	0.003	1.48	1.15	1.90
Very overweight	0.010	1.81	1.15	2.82
Exercising to lose weight or to keep from gaining weight during the past 30 days (yes)	0.018	1.22	1.04	1.44
Taking any diet pills, powders, or tea without a doctor's advice to lose weight or to keep from gaining weight during the past 30 days (yes)	0.013	0.66	0.48	0.92
Financial situation	<0.001			
Very satisfied		1(ref)		
Satisfied	0.082	1.22	0.98	1.52
Neither satisfied or not satisfied	<0.001	1.77	1.37	2.29
Not so satisfied	<0.001	1.80	1.35	2.41
Not at all satisfied	<0.001	2.51	1.54	4.11

Model chi-square test was 126.58 with 16 degrees of freedom ($P<0.001$)

TABLE III
Predictors for eating green salad less than daily

Variables	*P* value	OR	95% C.I. for OR	
School performance (marks)	0.041			
9-10		1(ref)		
8-8.99	0.584	0.94	0.75	1.18
6-7.99	0.013	0.75	0.59	0.94
5-5.99	0.542	0.86	0.54	1.39
Grades	0.013			
Grade 9		1(ref)		
Grade 10	0.033	1.27	1.02	1.57
Grade 11	0.019	1.31	1.05	1.64
Grade 12	0.003	1.48	1.14	1.92
Perceived weight	0.004			
Underweight		1(ref)		
About the right weight	0.001	1.42	1.16	1.74
Slightly overweight	0.019	1.36	1.05	1.76
Very overweight	0.026	1.73	1.07	2.80
Financial situation	0.001			
Very satisfied		1(ref)		
Satisfied	0.031	1.29	1.02	1.63
Neither satisfied or not satisfied	<0.001	1.66	1.26	2.18
Not so satisfied	0.006	1.53	1.13	2.08
Not at all satisfied	0.004	2.21	1.28	3.80
Taking any diet pills, powders, or tea without a doctor's advice to lose weight or to keep from gaining weight during the past 30 days (yes)	0.002	0.59	0.43	0.83

Model chi-square test was 68.51 with 14 degrees of freedom ($P<0.001$).

breakfast (OR=1.98; 95%CI: 1.53-2.57). Other studies also found more breakfast skippers among females than among males (5,10,11). In general, female and older adolescents tend to omit the morning meal. Various reasons for skipping breakfast such as lack of time or not being hungry in the morning have been observed (8,12). When it comes to frequency of eating, the highest prevalence (46.5%) is among those eating three meals each day. When asked about fresh juice intake, 34.2 % of the students responded they never drink such beverage. One third (33.0%) of the students reported eating fresh fruits between one and three times a week. Only 3.4% of the students reported never eating fruits during a week period. Green salad was consumed by 37.2% of the students between one and three times a week and never by 18.3% of the students.

The proportion of students consuming less than daily milk & diary products, green salad and fruits was: 58.3% for milk & diary products, 71.2% for green salad and 59.1% for fruits. Because of these high figures we decided to build multivariate logistic regression models to find out the most important predictors (tables II, III, IV).

Perceived weight was directly associated with inadequate consumption of fruit, green salad, milk and diary products among those who considered themselves as overweight or obese. Surprisingly, we did not find any association with body mass index.

Fruit, green salad, milk and diary products intake decreased among older adolescents in grades 11 and 12.

Students who were not satisfied with family financial situation were more likely not to eat fruit and green salad on a daily basis.

TABLE IV
Predictors for eating milk and diary products less than daily

Variables	*P* value	OR	95.0% C.I. for OR	
Gender (male)	0.007	0.80	0.67	0.94
School performance (marks)	0.001			
9-10		1(ref)		
8-8.99	0.184	0.87	0.70	1.07
6-7.99	0.000	0.65	0.52	0.81
5-5.99	0.693	0.91	0.58	1.43
Environment	0.019			
Towns		1(ref)		
Villages	0.756	0.93	0.59	1.47
Cities	0.016	1.32	1.05	1.65
Grades	0.001			
Grade 9		1(ref)		
Grade10	0.781	1.03	0.84	1.26
Grade 11	0.231	1.14	0.92	1.40
Grade 12	<0.001	1.62	1.27	2.06
Perceived weight	0.008			
Underweight		1(ref)		
About the right weight	0.494	1.07	0.88	1.30
Slightly overweight	0.012	1.38	1.07	1.76
Very overweight	0.012	1.80	1.14	2.86
Mother's highest level of education	0.016			
University		1(ref)		
College	0.865	1.03	0.76	1.39
High school	0.009	1.34	1.08	1.66
Vocational school	0.005	1.48	1.12	1.95
Primary school or less	0.108	1.30	0.94	1.80

Model chi-square test was 97.88 with 16 degrees of freedom ($P<0.001$).

We did not find any association between watching TV and playing computer games and low fruit and vegetables consumption as did other studies.21

No association between poor school achievement and inadequate consumption of fruits, green salad, milk and diary products was identified. Instead, we found that adolescents reporting average school marks (6-7.99) were protected against this kind of behaviour.

Students exercising to lose weight or to keep from gaining weight during the 30 days preceding the survey were more likely to eat fresh fruits less than daily (OR= 1.22; 95%CI: 1.04-1.44).

Parental educational level was related only to milk and diary products consumption, adolescents whose mothers finished vocational schools being at highest risk for inadequate consumption (OR=1.48; 95% CI: 1.12-1.95).

Assessment of dietary intake faces many problems, both related to the true nature of variation in an individual's diet and to a number of potential sources of error linked to the measurement instrument and to the reporting subject.9

Strengths of this study include the large and population-based sample of adolescents and a reasonably high response rate.

However, the study also has some limitations that should be taken into account in interpreting the findings. Although the study sample was large and diverse, it is not a nationally representative sample. Therefore, implications for other adolescent population should be made with caution. On the other hand, adolescents who gave up school were

not included in the study. Finally, body mass index was based on self-reported height and weight measures. Boys tend to overestimate their height, while girls have a tendency to underestimate their weight (13,14,15).

CONCLUSIONS

The area of nutrition may be neglected for several reasons : low priorities in comparison with other behaviours, difficulties in adequately assessing nutrition intake with a limited number of items and concerns about self-reported heights and weight (6).

Misreporting is a major problem in dietary surveys of adolescents and so all the dietary data collected should be interpreted with caution.

Through examination of prevalence rates of nutrition-related concerns we have identified priority areas to be targeted with future interventions.

We also identified three common predictors for consuming fresh fruits, green salad and milk and diary products : perceived weight, school performance and school grades.

The development of prevention programmes in school is vital as such programmes interfere with the trend of bad dietary habits among adolescent students. Associations between nutrition-related behaviours and other health behaviours indicate that eating behaviour do not occur in isolation (16,17). Interventions aimed at changes in eating behaviours should be integrated with other interventions aimed at promotion of healthy lifestyle (6). Counselling adolescents about nutrition is a true challenge. It can be very difficult to convince teenagers to modify their habits, unless they have a particular objective, are highly motivated and are capable of delaying immediate gratification for a larger future goal (2).

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NOUTĂŢI

Cardioncologia : o nouă disciplină

Tratamentul chimioterapic este grevat de o serie de efecte adverse importante, în ultima perioadă de timp interesul îndreptându-se asupra efectelor adverse cardiace, datorită creşterii ratei de supravieţuire la pacienţii oncologici. Această creştere a supravieţuirii a adus cu sine demascarea efectelor cardiotoxice ale chimioterapiei. Se cunoaşte de multă vreme că antraciclinele au o cardiotoxicitate crecută, însă în prezent s-a demonstrat că şi noile clase de chimioterapice determină complicaţii cardiovasculare ameninţătoare de viaţă. Astfel, trastuzumabul (anticorp anti-HER2), imatinibul şi mai nou sunitinibul (inhibitor de tirozină) determină apariţia insuficienţei cardiace. Creşterea supravieţuirii pacienţilor oncologici este datorată apariţiei unei medicaţii chimioterapice eficiente, însă cu efecte adverse pe termen lung, care în mod ironic pot duce la decesul pacientului, care sunt dificil de tratat dar şi mai dificil de prevenit. Acesta este motivul pentru care s-a dezvoltat o nouă ramură medicală, cea a cardioncologiei, care este abia la început, existând puţine studii care să poată stabili care este tratamentul cardiovascular cel mai eficient în cazul pacienţilor oncologici ; aceasta deoarece marile firme de medicamente au exclus din studii pacienţii oncologici, care puteau influenţa în mod negativ supravieţuirea. Abia de curând s-au introdus în ghidurile de hipertensiune recomandări privind managementul hipertensiunii arteriale secundare tratamentului cu inhibitori de factor de creştere endotelial, precum bevacizumab (Avastin) şi sorafenib (Nexavar). În acest moment se deschide o nouă eră în tratamentul pacienţilor oncologici, în care se va putea vorbi în curând de prevenţie cardiovasculară, la un pacient aflat în remisiune sau vindecat de cancer (Mann DL, Krone RJ. Cardiac disease in cancer patients. *Prog Cardiovasc Dis* 2010 ; 53 (2) : 80-87).

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EFECTELE SUPLIMENTĂRII DIETEI CU ACIZI GRAŞI POLINESATURAŢI OMEGA 3 ŞI ACETILCISTEINĂ ASUPRA PERFORMANŢEI ÎN ÎNVĂŢARE

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OMEGA-3 FATTY ACIDS AND ACETYLCISTEINE DIET SUPPLEMENTATION EFFECTS ON LEARNING PERFORMANCE (Abstract): Omega-3 fatty acids are essential for human health, are crucial for the brain, body development and also in prevention of cardiovascular diseases. **Material and method**: We used 60 male Wistar rats, weight 180 ± 20 grams, divided into two study groups: normal control animals (M) and test animals (T) divided in groups I (N3-PUFA), II (ACC) and III (N3-PUFA and ACC). Administration of the substances was made for 36 weeks (nine months), after which the animals were evaluated and subsequently sacrificied for further biochemical analysis. As an evaluation method has been used multiple T maze labyrinth. **Results**: Group I recorded a time maze percentage decrease from -4.26% to -33.88%. In group II we recorded a decrease time maze percentage from -5.37% to -34.28%. During the experiment, group III recorded a decrease time maze percentage from -5.1% to -32.33% at the end of the experiment. The control group evolution for the maze recorded a decrease in time maze percentage, reaching 17-18% at the end of the experiment. **Conclusion**: By improving the diet with polyunsaturated fatty acids increased exponentially cognitive performance compared with normal diet, low in these acids. **Key words**: POLYUNSATURATED FATTY ACIDS, T MAZE, WISTAR RATS

Omega 3 sunt acizi graşi esenţiali pentru sănătatea umană. Omega 3 nu pot fi fabricaţi de către organismul nostru. Din acest motiv, acizii graşi omega 3 trebuie să fie obţinuti din alimentaţie sau suplimente alimentare. Acizii graşi omega-3 pot fi găsiţi în peşte (cum ar fi somon, ton sau halibut), în anumite alge şi în nuci. Acizii omega 3 mai sunt cunoscuţi şi cu denumirea de acizi graşi polinesaturaţi (PUFA) (1,2). Aceştia joacă un rol crucial pentru creier, precum şi pentru creşterea şi dezvoltarea normală a întregului organism. Există trei mari tipuri de acizi graşi omega 3, care sunt ingeraţi din alimentaţie şi utilizaţi de către organism: acid alpha-linolenic (ALA), acidul eicosapentaenoic (EPA) şi acidul docosahexaenoic (DHA). După ce mâncăm, organismul transformă ALA în EPA şi DHA, cele două tipuri de omega-3 acizi graşi mai uşor de utilizat de către corpul nostru.

Cercetările în domeniu indică faptul că, acizii graşi omega 3 reduc inflamaţiile şi ajută la prevenirea unor factori de risc asociaţi cu boli cronice cum ar fi bolile cardiace, cancerul şi de artrita. Aceşti acizi graşi esenţiali sunt foarte concentraţi în creier şi par a fi deosebit de importanţi pentru funcţia cognitivă (partea de memorie şi de performanţă intelectuală) şi funcţia de comportament (2). Copiii care nu primesc suficient omega-3 de la mamele lor în timpul sarcinii sunt expuşi riscului de a dezvolta patologii ale sistemului nervos,

deficite de vedere şi auz.

În ultimele decenii, odată cu creşterea galopantă a incidenţei bolilor cardiovasculare cu etiopatogenie aterosclerotică ce influenţează sistemul nervos central precum şi a patologiei neurodegenerative, a apărut preocuparea crescândă atât pentru cunoaşterea factorilor de risc aterogeni şi pentru îmbunătăţirea metodelor de evaluare a disfuncţiei endoteliale (element cheie în ambele stadii de evoluţie ale aterosclerozei), cât şi pentru încetinirea proceselor degenerative dar şi pentru găsirea unor noi modalităţi de prevenţie şi terapie.

Identificarea factorilor de risc favorizanţi, cât mai ales modularea factorilor cu rol protector, prin suplimente nutritive, poate avea o importanţă de prim ordin în scopul găsirii unei atitudini terapeutice pentru întârzierea sau ameliorarea bolilor sistemului nervos central şi eventual a complicaţiilor acestora (3,4,5).

MATERIAL ŞI METODĂ

În experiment au fost utilizaţi 60 de şobolani masculi, rasa Wistar, cu greutatea de 180±20 grame. Şobolanii masculi Wistar au fost împărţiţi în două grupe de studiu : animale normale martor (M) şi animale test (T).

Grupul test a fost ulterior divizat în câte trei loturi – fiecare lot fiind format din 15 animale, în raport cu tipul de substanţă administrată după cum urmează :

- Lot 1 – şobolani albi, masculi, rasa Wistar, administrare intraperitoneală de acetilcisteină (ACC) 35 mg/100 g animal ;
- Lot 2 – şobolani albi, masculi, rasa Wistar, administrare intraperitoneală de PUFA 50 mg/100 g animal ;
- Lot 3 – şobolani albi, masculi, rasa Wistar, administrare intraperitoneală de ACC 35 mg/100 g animal şi PUFA 50 mg/100 g animal ;

Grupul martor a curpins un singur lot de 15 animale care au primit doar dietă normală şi fără alte intervenţii

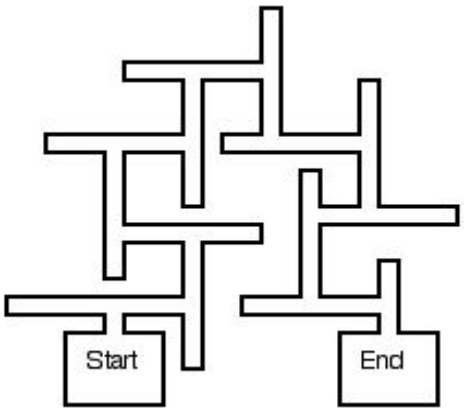

Fig. 1. Prezentare schematică a labirintului multiplu T

- Lot 4 – şobolani albi, masculi, rasa Wistar, cu dietă normală ;

Administrarea substanţelor s-a făcut timp de 36 săptămâni (9 luni), după care animalele au fost evaluate şi ulterior sacrificate.

Ca metoda de evaluare a fost folosit labirintul multiplu T. Labirintul multiplu T-este un labirint complex format din mai multe piese în formă de T. Performanţa în cadrul labirintului multiplu T este uşor de evaluat deoarece fiecare intersecţie este identică şi are un răspuns clar corect sau greşit (2,5,6). Labirintul multiplu T a fost folosit pentru a evalua răspunsul la învăţare şi elaborarea hărţilor cognitive la animalul de experienţă.

REZULTATE

Monitorizarea comportamentului în labirint a şobolanilor din Lotul I concretizat prin numărul de minute necesar traversării acestuia, pe parcursul celor 10 evaluări anterioare sacrificării, prezintă serii de valori cu varianţe ample, variind de la 10,40 CV% la a 2-a monitorizare până la 24,42 CV% la cea de a 5-a monitorizare (7,8).

La finalul experimentului, timpul necesar şobolanilor de a traversa labirintul a fost de 6,0 ori mai mic ($M_{iniţial}$ / M_{final} = 6,0), fiind semnificativ mai redus decât la

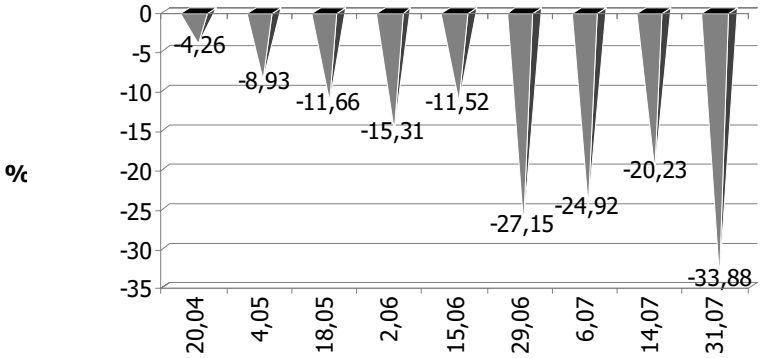

Fig. 2. Evoluţia procentuală a timpilor de labirint la lotul I pe parcursul monitorizării

debutul studiului (t= 25,24; GL= 28; p<0,001).

Pe cazuistica lotului I s-a evidenţiat o descreştere procentuală a timpului de labirint de la 4,26% până la -33,88%.

La lotul II de studiu, monitorizarea comportamentului în labirint a şobolanilor, pe parcursul celor 10 evaluări, prezintă serii de valori cu varianţe ample, variind de la 11,18 până la 23,08 CV%, cele mai omogene înregistrându-se la începutul experimentului, la a 2-a monitorizare (6).

La finalul experimentului, timpul necesar şobolanilor de a traversa labirintul a fost de 6,4 ori mai mic ($M_{iniţial}$ / M_{final} = 6,4), fiind semnificativ mai redus decât la debutul studiului (t= 19,97; GL= 28; p<0,001).

La lotul II s-a evidenţiat o descreştere procentuală a timpului de labirint de la 5,37% până la -34,28%.

La lotul III de studiu, monitorizarea comportamentului în labirint a şobolanilor, pe parcursul celor 10 evaluări anterioare sacrificării, prezintă serii de valori cu varianţe ample, variind de la 4,0 până la 21 57CV%, cele mai omogene înregistrându-se la finalul experimentului (1,7).

La finalul experimentului, timpul necesar şobolanilor de a traversa labirintul a fost de 6,2 ori mai mic ($M_{iniţial}$ / M_{final} = 6,2), fiind semnificativ mai redus decât la debutul studiului (t= 27,63; GL= 28; p<0,001).

Pe parcursul experimentului, la şobolanii din lotul III s-a observat descreşterea procentuală de la o etapă la alta a timpului de labirint până la 32,33% la sfârşitul experimentului (8,9).

La lotul martor, evaluarea comportamen-

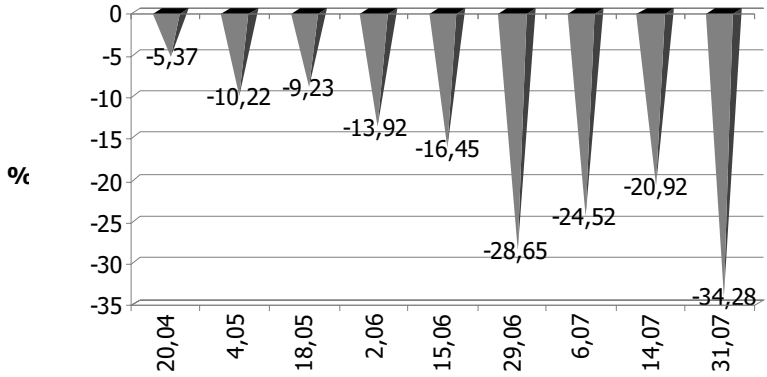

Fig. 3. Evoluţia procentuală a timpilor de labirint la lotul II pe parcursul monitorizării

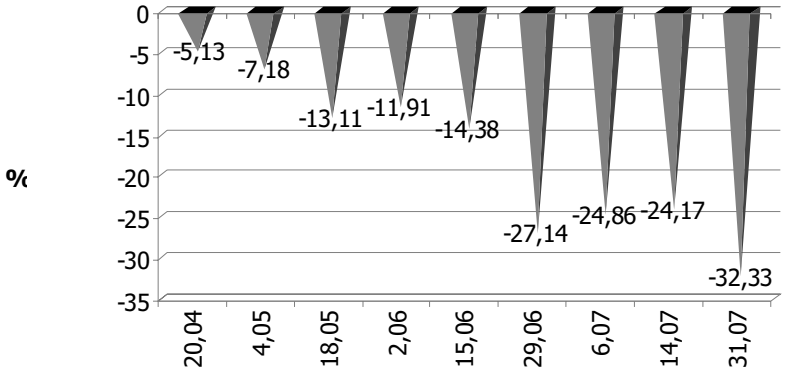

Fig. 4. Evoluţia procentuală a timpilor de labirint la lotul III pe parcursul monitorizării

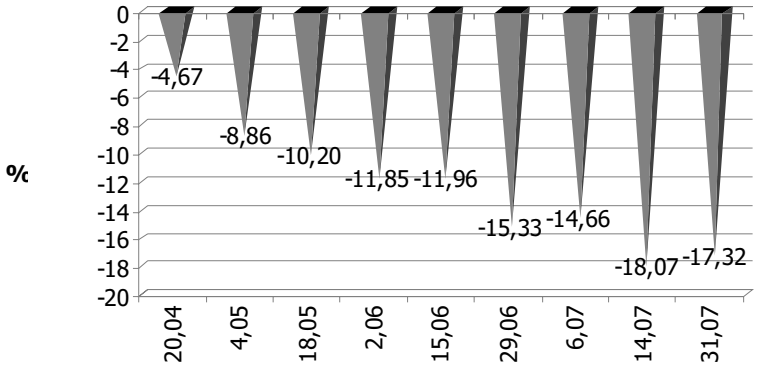

Fig. 5. Evoluţia procentuală a timpilor de labirint la lotul martor pe parcursul monitorizării

tului şobolanilor în labirint, pe parcursul celor 10 monitorizări, prezintă serii de valori cu varianţe ample, variind de la 6,73 până la 25,13 CV%, cele mai omogene înregistrându-se la a 3-a monitorizare de la debutul experimentului (7,9).

La finalul experimentului, timpul necesar şobolanilor de a traversa labirintul a fost de 3,4 ori mai mic ($M_{iniţial}$ / M_{final} = 2,8), fiind semnificativ mai redus decât la debutul studiului ($t = 27,92$; $GL = 28$; $p < 0,001$).

La lotul martor evoluţia timpilor de labirint a fost descrescătoare de la o etapă la alta, ajungând la 17-18% la finalul experimentului (10,11).

DISCUŢII

Comparând între loturi timpul de labirint se observă următoarele aspecte:

- la debutul studiului:

§ timpul mediu la labirint la lotul II, nu a prezentat diferenţe semnificative comparativ cu timpul mediu de labirint la lotul martor ($p > 0,05$);

§ animalele din loturile I ($p < 0,05$) şi III ($p < 0,001$) au prezentat diferenţe semnificative faţă de cele din lotul martor;

- la finalul experimentului:

§ timpul mediu de labirint la loturile de studiu a fost semnificativ mai mic la finalul experimentului comparativ cu cel obţinut la lotul martor ($p < 0,001$);

§ timpul mediu din lotul I nu a prezentat diferenţe semnificative comparativ cu cel obţinut la lotul II ($p > 0,05$), dar a fost semnificativ mai mare comparativ cu timpul mediu înregistrat la lotul III

($p < 0,05$) (10,11).

CONCLUZII

Cercetarea de faţă aduce un aport important în studiul acizilor graşi polinesaturaţi şi importanţa introducerii lor în alimentaţia zilnică. Astfel, prin îmbunătăţirea dietei cu acizi graşi polinesaturaţi performanţele cognitive au crescut exponenţial comparativ cu dieta normala, săracă în aceşti acizi.

Necesitatea utilizării pe scară largă a acizilor graşi polinesaturaţi este dovedită de multiplele experimente ştiinţifice, precum şi de rezultatele obţinute pe animalele de experienţă.

Studiul de faţă aduce o nouă lumină asupra importanţei existenţei unei balanţe între aportul de PUFA şi dieta zilnică.

Progresul semnificativ mai mare în cazul utilizării acestor acizi dovedeşte încă o dată necesitatea adopatării unei diete echilibrate şi bogate în nutrienţi esenţiali pentru funcţionarea organismului în condiţii optime.

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VIRUSUL POLYOMA BK ŞI IMPLICAŢIILE ACESTUIA ÎN PATOLOGIA ASOCIATĂ TRANSPLANTULUI RENAL

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POLYOMAVIRUS BK AND ITS ROLE IN ASSOCIATED KIDNEY TRANSPLANTATION PATHOLOGY (Abstract): In the last three decades kidney transplantation became a therapeutic approach in Romania but, unfortunately, the increasing of kidney transplantation procedures is related to an increasing rate of associated pathology such as Polyomavirus BK Associated Nephropathy (BKVAN). Studies published recently emphasize that BKVAN is a consequence of different risks factors, particular viral pathogenesis, humoral and cellular immunity and high doses of immunosuppressive drugs used for a long time are the main conditions that influence the BKVAN's evolution. Since there is no specific and effective treatment, optimal medical management is to diagnose nephropathy in early stages, to stop viral replication, limiting tubular lesions and prevent progress toward destruction by decreasing doses of immunosuppressive agents. **Key words**: POLYOMAVIRUS BK, NEPHROPATHY, IMMUNOSUPRESSION

EXPERIMENTAL RESEARCH FOR THE DETERMINATION OF BISOPROLOL FUMARATE IN BULK AND DOSAGE FORMS USING LIQUID CHROMATOGRAPHY

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EXPERIMENTAL RESEARCH FOR THE DETERMINATION OF BISOPROLOL FUMARATE IN BULK AND DOSAGE FORMS USING LIQUID CHROMATOGRAPHY (Abstract) **Aim:** To develop a simple, economic, feasible, and effective RP-HPLC method for the quantitative determination of bisoprolol in bulk and pharmaceutical formulations. **Material and methods:** Chromatographic separation was achieved on a Zorbax SB-C18 chromatographic column. The mobile phase was a mixture of methanol and 0.02M potassium acetate (40:60) with a 1 mL/min flow rate. The UV detection was done at 225 nm. The method was validated in accordance with the current ICH guidelines in terms of linearity, limit of detection, limit of quantification, precision, accuracy, recovery, and system suitability. **Results:** The retention time for bisoprolol was 2.38 minutes. A linear response was observed over the concentration range 5-80 µg/mL of bisoprolol fumarate. The limit of detection and the limit of quantitation for bisoprolol fumarate were 1.15 and 3.81µg/mL, respectively. The precision of the method was satisfactory and the relative standard deviations did not exceed 1%. The accuracy of the method was proved and the mean recovery of drug was in the range 99.74-100.02%. **Conclusions:** The proposed method proved to be selective, simple, precise, accurate, and it was successfully applied for the determination of bisoprolol in tablets. **Keywords:** ANTIHYPERTENSIVE, BISOPROLOL, CHROMATOGRAPHIC ANALYSIS.

Bisoprolol fumarate (BF), chemically known as (±)-1-[4-[[2-(1-methylethoxy) ethoxy]methyl]phenoxy]-3-[(1-methylethyl) amino]-2-propanol(*E*)-2-butenedioate, is a synthetic, beta1-selective adrenoceptor blocking agent used in the treatment of hypertension. The two substituents present in the para position of the benzene ring might be the reason for its β1-adrenergic receptor selectivity. BF is used alone on in combination with other drugs to treat hypertension. BF is well absorbed following oral administration. The absolute bioavailability for a 10 mg dose is greater than 80%. Absorption is not influenced by meals. The first pass metabolism *of* bisoprolol fumarate is less than 20% and it is eliminated through renal and non-renal pathways, as about 50% of the dose is found unchanged in the urine and the remainder is eliminated as inactive metabolites (1-3).

Chemical analysis methods are widely used in pharmaceutical practice to ensure

the quality and safety of drugs. Quality control should be achieved through faster, more reliable and easily achievable processes. Several analytical methods for the determination of BF in bulk, dosage forms, and human plasma have been proposed, but they are devaluated by long retention times and the use of expensive chemicals (4-9). Hence, the aim of the present study was to develop a simple, economic, feasible, and effective RP-HPLC method for quantitative determination of BF in bulk and pharmaceutical formulations.

MATERIAL AND METHODS

Instrumentation and chromatographic conditions: The analysis of BF was carried out using an Agilent 1100 system equipped with a photodiode array detector. A reversed-phase HPLC column Zorbax SB-C18, 3 x 100 mm, 3.5 µm particle size was used. The column and autosampler temperature were maintained at 25°C. The chromatographic separation of BF was achieved by isocratic elution. The mobile phase used for the analysis was a mixture of methanol and 0.02M potassium acetate (40:60 v/v). A 0.45 µm membrane filter was used to filter and degas the mobile phase. The injection volume was 25 µL. The output signal was monitored and integrated at 225 nm.

Chemicals and Reagents: All solvents used were analytical grade. Milli-Q water, methanol, potassium acetate were obtained from Merck (Darmstadt, Germany). Pharmaceutical grade BF was produced by Unichem Laboratories LTD, India. Two tablet formulation of 5 mg BF (A) and 10 mg (B) were procured from the local pharmacies. Only class A standard volumetric glassware was used.

Standard solution preparation: 100 µg/mL BF standard stock solution was prepared by transferring 10 mg of BF reference standard into a 100-mL volumetric flask using 25 mL methanol. The solution was diluted up to the volume with mobile phase.

Extraction procedure for dosage forms: Twenty tablets labeled to contain 5 and 10 mg of BF were individually weighed, mixed and finely powdered. The amount of powder equivalent to one tablet was accurately weighed, transferred into 100 mL volumetric flask using the mobile phase and then sonicated for 15 minutes. The sample solution was filtered using 0.45 µm filter.

RESULTS AND DISCUSSION

To optimize the analysis method, a number of parameters (organic modifier, flow rate) have been investigated. Different mobile phases were used to achieve the best separation and resolution. BF was eluted with the optimum peak shape (fig. 1) achieved when using a mixture of methanol and 0.02M potassium acetate (40:60, v/v). The UV detection was set at 225 nm. System suitability tests, evaluating column efficiency (N), tailing factor, resolution, were done to prove that the chromatographic system was adequate for the analysis (tab. I). These tests were carried out on freshly prepared BF standard stock solutions.

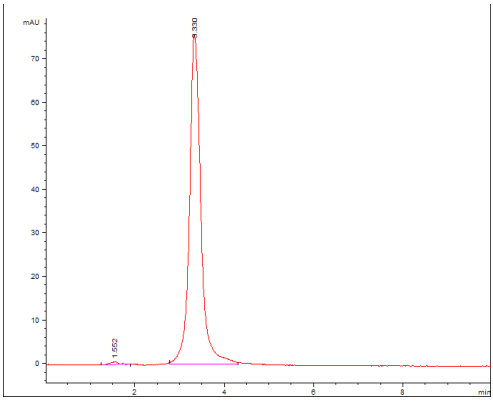

Fig. 1. Retention time of BF

TABLE I
System suitability parameters

Retention time	3.33
Area	2678
Tailing factor	1.20
Symmetry	0.74
Theoretical plates (N)	4210
Resolution	1.11

Linearity study: The standard stock solution was diluted to prepare three sets of BF standard solutions in the 5-80 µg/mL concentration range. Each set was analyzed to plot a calibration curve (fig. 2). To evaluate the linearity of the method (tab. II), standard deviation (SD), slope, intercept, regression coefficient (r2), limit of detection and quantification were calculated (10-12).

The precision of the method was studied for a 45 µg/mL BF solution through six replicate determinations (9). The percentage relative standard deviation (RSD%) was lower than 1.0% proving the high repeatability of the new method (tab. III).

The accuracy of the proposed method was established by means of the standard addition technique, by adding known amounts of standard substance to the 5µg/mL solution to reach three different concentration levels (25%, 100% and 125%) (10). The accuracy of the developed method for BF determination ranged from 99.74% to 100.07% (tab. IV).

As to the robustness studies, deliberate changes in the flow rate of mobile phase (± 0.2 mL/min) and column temperature (± 2°C) were made to evaluate the impact on the method. These small and deliberate variations had no significant effect on the measured system suitability parameters (tab. V).

The proposed method was successfully applied for the simultaneous determination of BF in tablets without interference from the excipients and without prior separation (tab. VI).

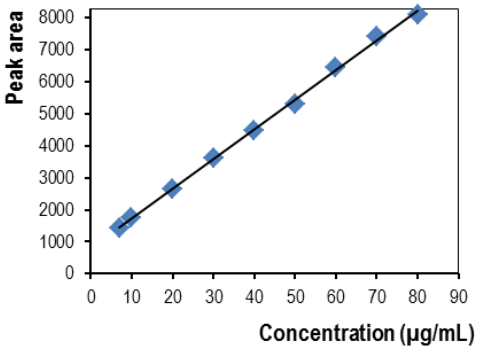

Fig. 2. Calibration curve

TABLE II
Linearity studies of the method

Regression coefficient (r^2)	0.9993
Correlation coefficient (r)	0.9996
Standard Error (SE)	54.14
Intercept	740.00
Slope	94.00
LOD (µg/mL)	1.15
LOQ (µg/mL)	3.81

TABLE III
Precision of the method

Theoretical concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	RSD %
45	4971	45.01	0.67
	4932	44.59	
	4899	44.24	
	4958	44.87	
	4893	44.82	
	4975	45.05	

TABLE IV
Accuracy of the method

Spiked level %	Concentration of BF (µg/mL)		Recovery %	Mean
	Added	Found		
25	5	9.98	99.80	100.07
	5	9.92	99.20	
	5	10.12	101.20	
100	35	39.92	99.85	99.86
	35	40.03	100.08	
	35	39.87	99.72	
125	45	49.86	99.72	99.74
	45	50.20	100.40	
	45	49.56	99.12	

TABLE V
Robustness

Parameter	Value	Area	Theoretical Plates	Tailing factor
Temperature	25 - 2°C	2679	4220	1.23
	25 + 2°C	2709	4235	1.35
Flow rate	1 - 0.2 mL/min	2694	4210	1.29
	1 + 0.2 mL/min	2689	4215	1.30

TABLE VI
Experimental results of BF assay in tablets

Label claim (mg/tablet)	Concentration founds n=3 (mg/tablet)	Recovery (%)	RSD (%)	Obliquity
A (5)	4.98	99.60	0.77	0.4
B (10)	9.95	99.5	0.62	0.5

CONCLUSIONS

The RP-HPLC method we have developed offers various advantages, including minimal sample prepping before chromatographic separation, enough sensitivity for quantitative analysis of bisoprolol fumarate in pharmaceutical products, good precision and accuracy.

The method involves the use of easily available, inexpensive laboratory reagents and it can thus be used for routine analysis and quality control.

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NEWS

STRUCTURE-BASED DESIGN, SYNTHESIS AND ANTITUMORAL EVALUATION OF ENULOSIDES

The purpose of this research is to designe and obtaine in high yields a new class of antitumoral agents with lower structural complexity. The general strategy for the synthesis of enulosides is based on the use of glycals as starting materials. From the pharmacological point of view, some synthetic enulosides exhibited activity against Mycobacterium tuberculosis H37Rv and they can also act as glycosidase inhibitors. All compounds exhibited high antitumoral activity in micromolar range against the tested tumor cell lines. (Santos JAM, Santos CS, Almeida CLA, et al. Structure-based design, synthesis and antitumoral evaluation of enulosides. *European Journal of Medicinal Chemistry* 2017, 128: 192-201).

Oana Dragostin

SURVEILLANCE SEASONAL PROGRAM. ANTIBIOTIC RESISTANCE OF PATHOGENIC AGENTS ISOLATED FROM CHILDREN WITH ACUTE GASTROENTERITIS, IN A REGION OF NORTH-EAST ROMANIA

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SURVEILLANCE SEASONAL PROGRAM. ANTIBIOTIC RESISTANCE OF PATHOGENIC AGENTS ISOLATED FROM CHILDREN WITH ACUTE GASTROENTERITIS, IN A REGION OF NORTH-EAST ROMANIA (ABSTRACT): The acute gastroenteritis is a population health problem, frequently met everywhere in the world. In Romania, for certain etiologies the reporting is compulsory. Also, these infections are reported in the TESSy system, too *(The European Surveillance System)*. In this context, antibiotics therapy and antibiotics resistance have a particular importance. The study **aim** was to assess the prevalence of bacterial pathogens involved in the etiology of acute gastroenteritis (AGE) on the Iaşi County, Romania and the surrounding areas and the determination of the profile of resistance to the antibiotics for the identified agents. **Material and methods**: 72 strains of isolated and identified bacterial agents involved in the acute gastroenteritis (AGE) in the case of children (0-14 years), with the exception of the *Campylobacter spp*., in the municipality of Iasi and its surrounding areas were studied in 2012, during the surveillance period of AGE (May to October). **Results**: The most frequent has been the *Salmonella spp.* (63.9%) from which the *S. enterica Thyphimurium* in 21 cases (29.2%) and *S. enteritidis* in 7 cases (9.7%), followed by *E. coli* (20.8%) from which *E. coli* O:127 in 3 cases (4.2%), *Shigella sonnei S* (13.9%) and *Yersinia enterocolitica* (1.4%). In the case of the children from the group of 0-1 year, the *Salmonella enterica Thyphimurium* has been the pathogenic agent most frequently encountered (6 cases – 29.2%), followed by *Salmonella* Colindale, *S. enteritidis*, *Shigella sonnei S*, *E. coli* O:127 registered with a frequency of 5.6%. The antibiotic resistance rates are high in the case of ampicillin (43.1%), tetracycline (31.9%), amoxicillin – clavulanic acid (26.4%), trimethoprim/ sulfametoxazole (22.2%), sulfonamides (11.1%). **Conclusions**: The antibiotic resistance rates are high in the case of ampicillin, tetracycline, amoxicillin – clavulanic acid, trimethoprim/ sulfametoxazole, sulfonamides. **Keywords**: ANTIBIOTIC RESISTANCE, SURVEILLANCE, ACUTE GASTROENTERITIS.

Acute gastroenteritis (acute diarrheal disease - AGE) is one of the most common causes of morbidity and mortality worldwide. It is estimated that AGE is among the top 5 causes of death around the world, in particular towards younger age groups in developing countries. Worldwide, data show that $1x10^9$ episodes of AGE are reported yearly, resulted in nearly 5 million deaths, mostly recorded in children under 5 years old (1). In USA, it is estimated a number of more than 170 million episodes of AGE is estimated yearly (1, 2). In Europe, AGE is a major health problem nowadays, especially in the pediatric age and, most importantly, throughout the continent. In the European region, AGE is a disease responsible for a high number of days of hospitalization, high costs and average figures of mortality, but still cannot be neglected. In 2010, the WHO database shows that the number of deaths from intestinal infectious diseases in the age group of 0-14 years range from 28 (Ukraine), 24 in France, 31 in Romania, Germany - 12, Bulgaria - 6, Poland - 4, Czech Republic – 3, but 1 death in Switzerland, Sweden or 0 in Finland (2).

Despite the real progress, evidence-based, regarding surveillance, diagnostic techniques, prevention and therapeutic methods, AGE remains a global health population problem (3).

The aim of the present study is that to assess the prevalence of bacterial pathogens involved in the etiology of acute gastroenteritis (AGE) on the territory of the municipality of Iaşi, Romania and the surrounding areas and the determination of the profile of resistance to the antibiotics for the identified agents.

MATERIAL AND METHODS

We have studied 72 strains of isolated and identified bacterial agents involved in the acute gastroenteritis in the case of children (0-14 years), with the exception of the *Campylobacter spp*., in the municipality of Iasi and its surrounding areas, during the surveillance period of AGE (May to October 2012). The isolated strains have been transported to the "I. C. Cantacuzino" Institute, Bucharest, The Reference Laboratory of enteric bacterial infections, in order to confirm the enteric agents. In the case of all the strains identified the antibiotic resistance has been tested. 15 strains of *Campylobacter* from the feces samples collected from the Pediatric sections have been isolated, identified, and tested for the antibiotic resistance in the Regional Public Health Laboratory Centre of Iasi, at the same time. The isolation of the *Campylobacter spp*. by culture is not a current practice, and these 15 strains will be the subject of a separate study.

The database was realized in the MS EXCEL program. The statistical analysis has been performed with the SPSS 20.0 software. For the comparison, the $\chi 2$ (chi-square) test has been applied. The value of p (the materiality) that reflects the probability that the difference between the analyzed variables to be random, has been considered to be higher than 0.05.

RESULTS

From the 72 strains studied, 39 (54.2%) have been isolated from male subjects and 33 (45.8%) from female subjects and the distribution on age groups has been the following: 26 cases (36.1%) for 0-1 year, 25 for 1-4 years (34.7%), 14 from 4-10 years (19.4%) and 7 children aged 10-14 years (9.7%). During the active period of surveillance (May – October), it has been observed an incidence peak in the month of July (25 cases), followed by the same value

in the months of June and August (with 21 cases), and afterwards the rate of incidence decreases in September at 2 cases, and in October no other case of acute diarrheal disease has been registered (tab. I).

Referring to the isolated strains, the most frequent has been the *Salmonella spp.* (63.9%) from which the *S. enterica Typhimurium* in 21 cases (29.2%) and *S. enteritidis* in 7 cases (9.7%), followed by *E. coli* (20.8%) from which *E. coli* O:127 in 3 cases (4.2%), *Shigella sonnei S* (13.9%) and *Yersinia enterocolitica* (1.4%).

In the case of the children from the group of 0-1 year, the *Salmonella enterica Typhimurium* has been the pathogenic agent most frequently encountered (6 cases – 29.2%), followed by *Salmonella Colindale, S. enteritidis, Shigella sonnei S, E. coli* O:127 registered with a frequency of 5.6% (tab. II).

The antibiotic resistance rates are high in the case of ampicillin (43.1%), tetracycline (31.9%), amoxicillin – clavulanic acid (26.4%), trimethoprim / sulfametoxazole (22.2%), sulfonamides (11.1%) (fig. 1).

The *Salmonella spp.* (19 strains) and *E. coli* (10 strains) present resistance to the ampicillin and just one strain of *Shigella* from among the 10 studied has been resistant to this antibiotic. The *Salmonella* (13 strains) and *E. coli* (5 strains) present resistance also to the amoxicillin – clavulanic acid.

The most of the strains are sensitive to the most of the tested antibiotics; for example, a single strain of *Salmonella* has been resistant to the gentamicin (tab. III).

TABLE I

The distribution taking into account the month of isolation of the strains

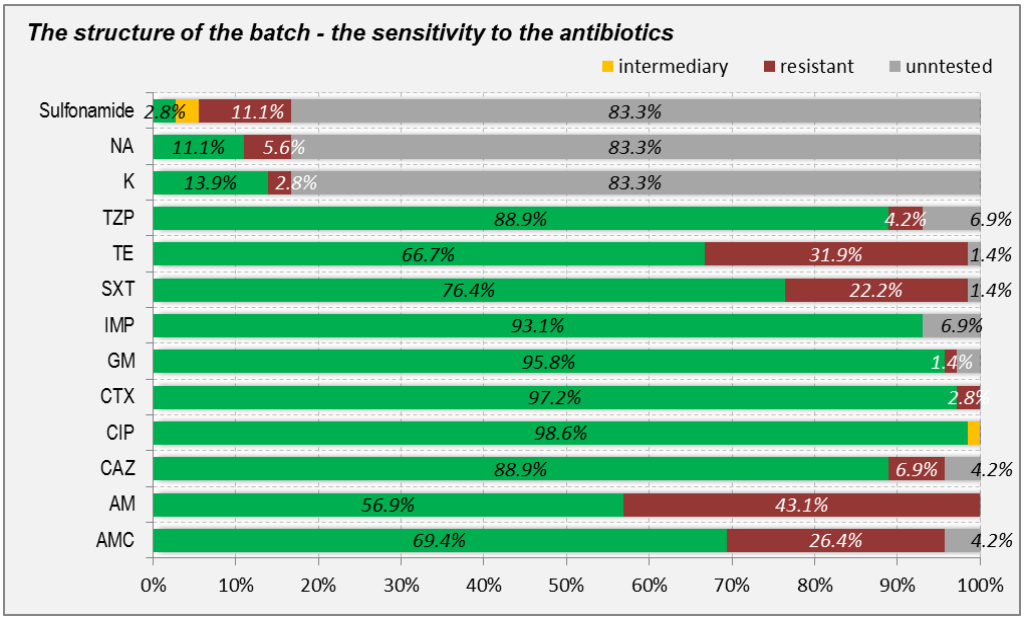
Month of isolation	No. of isolated strains
May	3
June	21
July	25
August	21
September	2
October	0

TABLE II

The type and frequency of the pathogenic agents isolated in the case of the AGE

The pathogenic agent	The absolute frequency	The percentage frequency
E. coli non-agglutinating	1	1.4
E. coli O:127	1	1.4
E. coli O:26	2	2.8
E. coli O:55	2	2.8
E. coli O:111	1	1.4
E. coli O126	2	2.8
E. coli O:127	3	4.2
E. coli O:128	1	1.4

The pathogenic agent	The absolute frequency	The percentage frequency
E. coli EPEC+ O:26B6	1	1.4
E. coli EPEC+ O:55B5	1	1.4
Salmonella Brandenburg	1	1.4
Salmonella Colindale	2	2.8
Salmonella Derby	2	2.8
Salmonella Enteritidis	7	9.7
Salmonella Heidelberg	1	1.4
Salmonella Infantis	4	5.6
Salmonella Kottbus	4	5.6
Salmonella Newport	1	1.4
Salmonella Stanley	1	1.4
Salmonella Typhimurium	21	29.2
Salmonella Wien	2	2.8
Shigella sonnei S	10	13.9
Y. enterocolitica O:3	1	1.4
Total	**72**	**100.0**



green – sensitive red - resistant gray – untested yellow - intermediary

Fig. 1. The strains sensitivity to various antimicrobials

Legend: AMC – amoxicillin – clavulanic acid; AM – ampicillin; C – chloramphenicol; CIP - ciprofloxacin; CTX – cefotaxime; CF- cephalotin; E – erytromycine; GM – gentamicin; K- kanamycin; NA – nalidixic acid; SXT – trimethoprim/ sulfamethoxazole; TE – tetracycline; TM – tobramycin; CAZ – ceftazidime; IMP – imipenem; TZP – piperacillin /tazobactam

TABLE III

The sensitivity to the antimicrobials ranged on types of pathogenic agents

		sensitive		intermediary		resistant		untested		chi-square	p
		N	%	N	%	N	%	N	%		
AMC	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	8.890	0.180, *NS
	E. coli	10	66.7			5	33.3	0	0.0		
	Salmonella	32	69.6			13	28.3	1	2.2		
	Shigella SS	7	70.0			1	10.0	2	20.0		
AM	*Y. enterocolitica*	0	0.0			1	100.0			9.247	0.026, **SS
	E. coli	5	33.3			10	66.7				
	Salmonella	27	58.7			19	41.3				
	Shigella SS	9	90.0			1	10.0				
CAZ	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	9.194	0.163, NS
	E. coli	15	100.0			0	0.0	0	0.0		
	Salmonella	41	89.1			4	8.7	1	2.2		
	Shigella SS	7	70.0			1	10.0	2	20.0		
CIP	*Y. enterocolitica*	1	100.0	0	0.0					0.573	0.903, NS
	E. coli	15	100.0	0	0.0						
	Salmonella	45	97.8	1	2.2						
	Shigella SS	10	100.0	0	0.0						
CTX	*Y. enterocolitica*	1	100.0			0	0.0			4.114	0.249, NS
	E. coli	14	93.3			1	6.7				
	Salmonella	46	100.0			0	0.0				
	Shigella SS	9	90.0			1	10.0				
GM	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	13.275	0.039, SS
	E. coli	15	100.0			0	0.0	0	0.0		
	Salmonella	45	97.8			1	2.2	0	0.0		
	Shigella SS	8	80.0			0	0.0	2	20.0		
IMP	*Y. enterocolitica*	1	100.0					0	0.0	5.280	0.152, NS
	E. coli	13	86.7					2	13.3		
	Salmonella	45	97.8					1	2.2		
	Shigella SS	8	80.0					2	20.0		
SXT	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	1.057	0.983, NS
	E. coli	11	73.3			4	26.7	0	0.0		
	Salmonella	35	76.1			10	21.7	1	2.2		
	Shigella SS	8	80.0			2	20.0	0	0.0		
TE	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	8.981	0.175, NS
	E. coli	7	46.7			8	53.3	0	0.0		
	Salmonella	30	65.2			15	32.6	1	2.2		
	Shigella SS	10	100.0			0	0.0	0	0.0		
TZP	*Y. enterocolitica*	1	100.0			0	0.0	0	0.0	5.945	0.429, NS
	E. coli	12	80.0			1	6.7	2	13.3		
	Salmonella	43	93.5			2	4.3	1	2.2		
	Shigella SS	8	80.0			0	0.0	2	20.0		

		sensitive		intermediary		resistant		untested		chi-square	p
		N	%	N	%	N	%	N	%		
K	*Y. enterocolitica*	0	0.0			0	0.0	1	100.0	10.087	0.121, NS
	E. coli	2	13.3			2	13.3	11	73.3		
	Salmonella	8	17.4			0	0.0	38	82.6		
	Shigella SS	0	0.0			0	0.0	10	100.0		
NA	*Y. enterocolitica*	0	0.0			0	0.0	1	100.0	4.985	0.546, NS
	E. coli	3	20.0			0	0.0	12	80.0		
	Salmonella	5	10.9			4	8.7	37	80.4		
	Shigella SS	0	0.0			0	0.0	10	100.0		
Sulfonamide	*Y. enterocolitica*	0	0.0	0	0.0	0	0.0	1	100.0	15.900	0.069, NS
	E. coli	0	0.0	0	0.0	3	20.0	12	80.0		
	Salmonella	2	4.3	0	0.0	5	10.9	39	84.8		
	Shigella SS	0	0.0	2	20.0	0	0.0	8	80.0		

Legend: AMC – amoxicillin – clavulanic acid; AM – ampicillin; C – chloramphenicol; CIP - ciprofloxacin; CTX – cefotaxime; CF- cephalotin; E – erytromycine; GM – gentamicin; K- kanamycin; NA – nalidixic acid; SXT – trimethoprim/ sulfamethoxazole; TE – tetracycline; TM – tobramycin; CAZ – ceftazidime; IMP – imipenem; TZP – piperacillin /tazobactam; *NS – statistically not significant; ** SS – statistically significant

The tests of statistical significance confirm the resistance of the *Salmonella* to ampicillin (p=0.026). For the gentamicin (p=0.039) it can be spoken about the maintenance of the sensitivity of the *Salmonella* (a single resistant strain), compared to the resistance to other antibiotics: ceftazidime – 4 resistant strains, tetracycline – 15, trimethoprim/ sulfamethoxazole – 10, piperacillin/ tazobactam – 2, nalidixic acid - 4, sulfonamides – 5. In the case of ciprofloxacin and imipenem all the strains studied have been sensitive.

DISCUSSION

Acute gastroenteritis is a population health problem frequently met worldwide, responsible for higher levels of morbidity and even for mortality especially in the cases of the extremes of age and of the persons with a compromised immunity and especially in the developing countries (4).

In Romania, the epidemiological and microbiological surveillance of AGE is passive and it is developed at the level of all the county public health departments and at those of the Bucharest municipality. For certain etiologies *(Salmonella, Shigella, Campylobacter, Yersinia* and *E. coli* - VTEC/ STEC) the reporting is compulsory. Also, these infections are reported in the TESSy system, too *(The European Surveillance System)*. The microbiological diagnosis in the case of AGE in the current context, when there is a large number of known and identifiable pathogenic agents, implies the existence of a clear working methodology and especially accessible in the current medical practice and not only in the centers of reference and in the specialized laboratories (the case *E. coli* – the diarrhea genic pathotypes or *Campylobacter* – difficult to cultivate) (5, 6, 7).

Traditionally, *E. coli* is one of the most wide-spread microorganisms resistant to the antibiotics. The antibiotic resistance of *E. coli* is worrisome, because it may determine the most frequent infections with

gram negative bacteria, and the emergence of the phenotype producer of β-lactamase on a wide spectrum, which confers resistance to the third-generation of cephalosporin is in a continuous growth in Europe. Such strains isolated from the bacteremia have also shown an increased cross resistance to the fluoroquinolones (> 80%) and to gentamicin (> 40%) (8, 9).

The resistance to the fluoroquinolones is, also, frequent among the *E. coli* strains, which overexpresses plasmid genes *FomA* and *FomB* capable to inactivate the fosfomycin by means of the phosphorylation. The *E. coli* are, also, the most frequent pathogenic agents with transmission from animals to humans. Another enterohaemorrhagic strain, *E. coli* O104: H4, has infected more than 3.800 persons in Germany, in 2011, causing 54 deaths (8, 9).

According to the ECDC report from 2012, in Romania, Bulgaria, Slovenia and Italy, among 25-50% from the isolated strains of *E. coli* are resistant to the third-generation of cephalosporin, the same resistance being acknowledged also for the fluoroquinolones, only that this one is much more extended in Europe (10).

The results of a 5-year prospective surveillance study of the resistance to the antibiotics among *Salmonella enterica* isolates and the ceftriaxone therapy among children hospitalized for acute diarrhea ranks the tetracycline (60.6%) with the highest rate of resistance, then ampicillin (46.8%) and chloramphenicol (21.6%). The ceftriaxone resistance occurs rarely (1.9%). The resistance to the ciprofloxacin (8.7%) is more important than that to ceftriaxone. In all the 56 cases studied by authors from Italy, the ceftriaxone was indicated and effective (11).

In a study performed in Turkey, the first choice option drug was changed from TMP-SMX to cefixime because of the observed resistance to the TMP-SMX reported in proportions ranging from 47.4% to 96.5% in several regions of the country. The second choice drug, ampicillin, specific in the 1987-1994 interval, it was rarely indicated during the period 1995-2002 and for the period 2003-2009 only one patient in the study received a prescription with ampicillin. The resistance to the ampicillin in this study had high resistance rates of 58.4% and even 86% but with important differences between the strains of *Shigella spp., S. flexneri* being constantly resistant. For the *S. sonnei* a different evolution was reported. After a decline in resistance to 5.7% between 1995 and 2002, the resistance was observed in approximately 31.4% of isolates from 2003 to 2009. Most *Shigella* strains are sensitive to ampicillin in Turkey. The authors concluded that a better clinical management may help to reduce the transmission of these pathogens in the general population in the future (12).

CONCLUSIONS

Our study on surveillance of the resistance to the antibiotics in a territory may provide useful data for the elaboration of hospital guides with a regional specific, so that the emergence of new resistant phenotypes should be limited and the eventual self-medication with antibiotics should be discouraged, frequently encountered in certain areas.

In the context of global travelling, the close collaboration among the epidemiologist, microbiologist and the pediatrician is essential in the surveillance of AGE, and the laboratory diagnosis has a paramount importance in this system, at the national as well as at the international level.

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STRESS IN PEDIATRIC PATIENTS – THE EFFECT OF PROLONGED HOSPITALIZATION

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STRESS IN PEDIATRIC PATIENTS–THE EFFECT OF PROLONGED HOSPITALIZATION (Abstract): Long-term hospitalization emotionally impacts any patient, especially children, and is defined as a long period of time during which the patient is hospitalized and experiences isolation from his or her family, friends and home. Stressful situations trigger a nonspecific response that involves multiple physiological mechanisms. Currently, because of the complexity of these mechanisms, there are no laboratory markers that allow the quantification of the stress intensity felt by the patient. Laboratory determinations currently used in evaluating the response to stress are neuroendocrine, immunological and metabolic. The neuroendocrine system is the first to respond to stressful events. Stress stimulates the hypothalamus, leading to the release of CRH, which stimulates the pituitary gland to produce ACTH. Chronic stress directs the synthesis towards cortisol, which may lead to hypo secretion of the other adrenal steroid hormones. The hospital and the disease are stressors for children and caregivers, since stress can interfere with the normal development of young patients, affecting them in the long term. Admitting a child to hospital means interrupting his or her normal daily life and changing the environment that is familiar to him or her. Therefore, the involvement of the family doctor is very important, as many conditions can be solved by visiting his or her office and thus eliminating the need for hospitalization in a pediatric hospital. If, however, the nature of the condition requires that the child should be seen by a pediatrician, the period of hospitalization should not be much extended so as to prevent the appearance of other possible problems that might influence the child's state. **Keywords**: LONG-TERM HOSPITALIZATION, STRESS IN PEDIATRIC PATIENTS, CHROMOGRANIN A, SALIVARY ALPHA AMYLASE.

Long-term hospitalization has a significant emotional impact on any patient, and especially on pediatric patients. The concept of long-term hospitalization is defined as a period of time during which the patient is hospitalized and he or she feels and / or becomes aware of his or her isolation from family, friends and home. Long-term hospi-

talization involves not only a particular period of time, but also the moment when a person loses emotional balance. Pediatric patients are in the process of determining their own identity, and they are psychologically, emotionally and physically dependent on their family and friends. Fortunately, medical advances have increased survival rates, while decreasing mortality rates. Children go through "normal developmental stress", doubled by the negative impact that the long-term hospitalization can have on their neuropsychological, emotional and physical development (1).

Compared to adults, infants and young children are significantly influenced by the hospital environment and its adjacent factors. Children's lack of proper and full understanding of what is happening, their communication skills that are insufficiently developed to transmit their needs, as well as their emotions and experiences may often go unnoticed by caregivers. At the same time, young patients' state of anxiety increases (2).

For all patients, temporarily giving up their personal belongings, intimacy, entourage and known environment, trigger anxiety. Wearing a certain outfit during their hospital stay, sleeping in a different bed, being imposed a certain program, diet, medication, as well as going through all the tests increases stress. On top of all this, patients come into contact with many strangers; this causes them to feel deprived of almost all aspects related to their personal space. Patients find themselves in an unfamiliar environment, which is worrying for most adults, and especially for children. In addition, hospitalization has different effects on newborn patients' long-term development, compared to adolescent patients (2, 3). Infants often get scared by unexpected noises, sudden maneuvers and movements. Starting with the age of 6 months, children already have a strong attachment towards the people who take care of them (and especially towards their mothers). Separation from these people can generate a significant discomfort in young patients, leading to the fear of separation. Moreover, children begin to be afraid of strangers and of people with atypical appearance or traits they are not used to (e.g., people wearing protective masks on their face). Up to 1 year, children acquire a lot of new skills; if they are admitted to hospital for a long period of time, developmental delays may occur. They may lack the sensory stimulation that would help them develop (music, light, reading stories, playing with the people who take care of them, toys, etc.). If parents do not spend enough time with their children in hospital, the relationships between them can be affected. The development of children aged between 1-2 years may be delayed due to the fact that the time spent in hospital interferes with the activities that could help stimulate new skills. Sleep problems may occur (2, 3, 5). Dietary imbalances are a result of home routine change and stress. At this age, children are very afraid of strangers, thus the presence of nurses and doctors increases their level of anxiety. Children are irritated, frustrated and frightened because, at this age, it is hard for them to understand why they are hospitalized. For children between 2-5 years, the main negative impact is the stress of being away from everything that is familiar to them. They may exhibit phobia towards medical procedures, which are perceived like something that can hurt them; they also feel threatened, restricted, punished and tortured (1, 3). They may feel that they have done something wrong and that this is why they are in hospital. They roughly know their bodies, but their under-

standing on how the body works is far from clear to them, and this can create frustration. Language is largely developed by the age of 5 years, but children may misinterpret what they hear. Starting with the age of 2-3 years, children begin to have the conscience of the self and their fears start to become somewhat specific. In general, at this age stage, children are afraid of things or phenomena they do not understand or cannot control (3, 6). Young children have a limited understanding of the dimensions of the things surrounding them and of the relation between cause and effect, which triggers irrational fears. The fear of separation from the people who take care of them is still present at this age. Other fears that appear are the fear of large animals, of water, of the unknown and of pain. Children's active imagination and their limited ability to distinguish between real and imaginary things cause them to be afraid of real characters. Between 3 and 6 years old, children are often afraid of being left alone or getting lost from parents. They are afraid of things such as insects, darkness, noise and actions they cannot explain or they have not come across with, imaginary creatures, physical injuries, going to the doctor, thunder and lightning (3, 7). Children aged 5-12 years may experience any of the states listed above, plus the fact that, during this period, they show normal fear of doctors, needles and pain. As children learn more about the complexity of the world that surrounds them, many childhood fears disappear; yet, they are replaced by other fears. As children's social life becomes increasingly important, many fears appear which are related to their way of interacting with other children and even with adults. In addition, abstract thinking gradually begins to develop, and children's fears also "shift" from concrete to abstract things. Fears gradually shift from the present to the future and from the certain to the hypothetical or the potential (2, 6, 7). Children begin to fear that someone in the family could be hurt or die. Other fears appear, i.e. the fear of being rejected by other children or not having friends, being ridiculed, humiliated, as well as the fear of not meeting the expectations their parents or other adults (e.g. teachers) might have related to their performance (at school or in other activities) (3, 4). Other fears continue to be normal, such as the fear of doctors and of medical procedures, the fear of natural disasters and, in general, of any potentially destructive phenomenon over which the child has no control. In addition, what is new is the appearance of a certain discomfort caused by the invasion of own privacy. As children grow, they begin to understand that they are suffering from a serious illness or that something bad is happening, with major physiological consequences. Chemotherapy, medical tests, surgery, invasive maneuvers cause a constant fluctuation of the stress level in children. Some children tend to associate the restriction of movement with death, while for older children the loss of interaction represents the isolation from the processes of everyday life (5.7).

The combination between these states and the boredom caused by the interruption of the activities that children were used to doing can cause extreme rises in the levels of anxiety and stress. Some children, after being hospitalized for a long period of time, may exhibit low self-esteem and a distorted image of own body. They may feel harassed and ignored to such an extent that they end up feeling unprepared for the transition back to normality. Because of a severe diagnosis and of prolonged hospitalization, some children and adolescents may

feel incapable of keeping up with school activities; they may feel marginalized and "left behind" (3, 4, 5). Isolation and emotional regression are also serious consequences of an illness occurring in childhood. After setting the diagnosis and initiating the treatment of a serious illness, such children often tend to become more mature than their friends. At school, it is difficult for them to find friends with whom they can socialize. Their health status often limits their participation in sport activities and causes them to end certain relationships, feel lonely and emotionally isolated. In addition, their school performance declines (2, 7). Given the factors causing isolation and a bad self-image, these patients may show depressive disorders from an early age. The complexity of fears in adolescence reflects the cognitive level and the physical and mental transformations specific to this stage. Adolescents are afraid of academic failure, parents' disappointment and rejection from friends. They are also afraid they might be put in an unfavorable light or ridiculed in front of others. In addition, they are worried about physical imperfections (e.g. acne, too high or too low body weight), death, physical and emotional harassment. During this period, great emphasis is placed on physical appearance, self-image, other people's opinion about them, the feeling of belonging to a group and peers' acceptance, as well as individual performance (3, 5).

Stressful situations trigger a nonspecific response that involves multiple physiological mechanisms. Due to the complexity of these mechanisms, there are still no specific laboratory markers that allow the quantification of stress intensity experienced by the patient. Laboratory determinations currently used in evaluating the response to stress are neuroendocrine, immunological and metabolic. The neuroendocrine system is the first to respond to stressful events. Stress stimulates the hypothalamus, leading to the release of CRH (corticotropin releasing hormone), which stimulates the pituitary gland to produce ACTH (adrenocorticotropic hormone). Under the action of ACTH on the adrenal gland, adrenaline and norepinephrine are released (from medulla) along with cortisol (from cortical area) (8). Chronic stress directs the synthesis towards cortisol, which may lead to hyposecretion of the other adrenal steroid hormones.

Cortisol exerts immunosuppressive and anti-inflammatory effects. Secretion of cortisol has a circadian rhythm, with elevated levels in the morning. Cortisol levels peak 20-40 minutes after acute stressful events. Cortisol blood levels are influenced by other factors (genetic factors, obesity). Cortisol may be measured in urine, saliva and serum, but salivary cortisol is often considered a better marker. In saliva, cortisol appears mainly in free form, not bound to proteins (such as blood); it is relatively resistant to the enzymatic degradation and it is unaffected by the salivary flow concentration. In addition to that, the procedure is less invasive (9,10).

Catecholamines: adrenaline and noradrenaline are secreted by the adrenal medulla in response to the stimulation of the sympathetic nervous system. The blood peak is recorded 60 minutes after the stressful event. The measurement of catecholamine levels in saliva requires immediate processing, as catecholamines are rapidly oxidized (9).

Salivary alpha amylase represents the major salivary protein (40-50% of the total proteins) and it is considered a marker of the sympathetic nervous system activity. The determination of salivary alpha amyl-

ase has a number of advantages: the sensitivity of determination, the immediate response to stress, the diurnal variation favorable to collecting morning samples and the low cost. However, the enzyme's levels vary with age, medication, diet, intake of caffeine or alcohol, physical exercise and associated pathologies (9, 10).

Chromogranin A is a protein with a single polypeptide chain, rich in acidic amino acid residues, which belongs to a family of neuroendocrine secretory protein named cromogranins / secretogranins. These proteins are located in the secretory vesicles of the neurons and the endocrine cells. The largest amount of chromogranin A is found in the granulations of the adrenal chromaffin vesicles and of the sympathetic nervous system. Chromogranin A is considered a stress hormone and the precursor of a number of peptides with biological functions (vasostatin, pancreastatin, catestatin, parastatin) with roles in inflammations and oxidative processes. It is associated with increased sympathetic nervous system activity induced by psychological stress (11).

Submandibular salivary glands secrete Chromogranin A, making it possible for it to be measured in saliva. The correlation between blood and salivary concentrations is not yet fully understood. A circadian rhythm has been described for human subjects, with peaks around 11 pm and minimum levels around 8 am.

Salivary chromogranin A level changes faster and is more sensitive to psychological stressors compared to salivary cortisol. After the cessation of the stress factor, chromogranin levels quickly return to the basal state (12).

Cytokines. Initially, it was assumed that stress had an immunosuppressive effect, thus explaining the infections and malignancies reported in people subjected to chronic stress. Recent studies have changed the concept of stress seen as an immunosuppressive factor, considering stress as an immunomodulatory process. These studies have focused on measuring the levels of cytokines secreted by Th1 and Th2 lymphocytes. Th1 cytokines (IL-2, TNF alpha, interferon gamma) are involved in cell-mediated immunity, while Th2 (IL 4, IL 5, IL 9 IL10) are involved in the humoral-mediated immunity. Stress affects the secretion of both types of cytokines; these cytokines are involved in the pathophysiology of inflammatory diseases (rheumatoid arthritis, asthma). Studies have proved that stress does not suppress, but acts differently on the cytokines secreted by Th1 and Th2.

Acute stress events determine a decrease in Th1 levels and an increase in Th2 cytokines levels; since Th1 cytokines are involved in cell-mediated immune response, this would increase the vulnerability to viruses and bacteria. Chronic stress leads to decreased Th1 and Th2 levels. Stress hormones, especially cortisol, affects the differentiation of naive Th0 cells into Th1 and Th2 with inhibitory effects on Th1 and potentiating effects on Th2 cytokines. Glucocorticoids suppress the production of IL12 by antigen-presenting cells (IL12 is necessary for the transformation of Th0 into Th1) and decrease the expression of IL 12 receptors by T cells and natural killer lymphocytes. They prevent the production of IFN gamma by Th1 lymphocytes stimulated by IL12. Glucocorticoid hormones also induce the production of IL 4 and IL 10. Studies have reported that chronic stress may lead to low levels of cortisol. A possible explanation could be the cortisol resistance theory: high levels of cortisol determine a decrease in the number of receptors (down regulation), which leads to

the inability of the immune system to respond to chronic stress.

It is difficult to determine the levels of cytokines, because they degrade rapidly in the tissue, thus reaching the bloodstream in small amounts. Ongoing infections and exposure to allergens also influence the basal cytokine concentrations (13, 14, 15).

CONCLUSIONS

Following the evaluation of the bibliographic information, which added to our clinical experience, we have concluded that children's admission to hospital involves changing their familial and familiar environment. Both the hospital and the illness are major stressors for children as well as for their caregivers, as stress can interfere with the normal development of pediatric patients and it can affect them in the long term. Admitting a child to hospital means interrupting his or her normal daily life and changing the environment that is familiar to him or her. Therefore, the involvement of the family doctor is very important, as many conditions can be solved by visiting his or her office and thus eliminating the need for hospitalization in a pediatric hospital. If, however, the nature of the condition requires that the child should be seen by a pediatrician, the period of hospitalization should not be much extended so as to prevent the occurence of other possible problems that might influence the child's status.

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NEWS

MYOEPITHELIAL CELLS (MEC) OF THE SALIVARY GLANDS IN HEALTH AND TUMOURS

In many exocrine organs, the secretory end pieces and the ducts are partly covered by cells with long processes that form an interlacing network. These cells resemble smooth muscle cells in several important aspects, yet clearly are epithelial cells, and thus are referred to as myoepithelial cells (MEC). The MEC were first discovered in the breast tissue by Krause in 1865. Since then it has been observed in the terminal end pieces and ducts of most of the exocrine glands such as salivary, mammary, sweat, lacrimal and bronchial glands. The description of these cells is diverse. Various authors have described them as "spindle shaped cells", "star shaped cells" or "basket cells" until the term "myoepithelial cells" was conferred to it. Most of the authors have studied this cell in detail in the mammary glands only until it was Tamarin in 1966 that provided a vivid image of a typical acinus-associated MEC in the salivary gland. He typically described the appearance of the cell on the acinar unit as being "like an octopus sitting on a rock". These cells lie between the basal lamina of the acinar and ductal cells at the terminal portion of the salivary glands. Positive identification of salivary gland MEC on routine microscopic preparations is very difficult. Fortunately modern sophisticated microscopic techniques have resulted in a surge of new information on this cell such as exposure of MEC by chemical removal of periacinar connective tissue and basement membrane deposits. MEC have many cytoplasmic processes which embrace glandular cells. Their nuclei are localized in the cell body. Most of the cytoplasmic organelles are found in the small areas around the nucleus. The remainder of the cytoplasm is filled with filaments and vesicles which are morphologically similar to those found in smooth muscle cells. After studying the structure of MEC in detail researchers focussed on identifying the physiological functions of these cells. The function of MEC was identified by an essential term in biologic kinetics (kinesiology) which states "form defines the function". The shape of the MEC suggested that its contraction might reduce the luminal volume in glandular endpieces, and these cells may play a role in expelling secretory products from glandular endpieces to the excretory duct system which was later proved experimentally. Thus one of the chief functions of MEC was determined. Association of tumours with MEC was determined when scientists found that a variety of tumours occurred in salivary glands and breast as compared to pancreas and concluded that this was because of presence of MEC in the former two glands. In conclusion, this paper brings about a thorough description of this cell in both physiological and pathological aspects (Chitturi RT, Veeravarmal V, Nirmal RM, B. et al.Myoepithelial Cells (MEC) of the Salivary Glands in Health and Tumours. *Journal of Clinical and Diagnostic Research*. 2015 Mar, Vol-9(3): 14-18).

Doina Butcovan

ANTIINFLAMMATORY ACTIVITY OF AN N, N'-DISALICYLIDENEMETHYLENDIAMINE-DERIVED SCHIFF BIS BASE AND ITS COPPER(II) COMPLEX

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ANTIINFLAMMATORY ACTIVITY OF AN N, N'-DISALICYLIDENEMETHYLEN-DIAMINE-DERIVED SCHIFF BIS BASE AND ITS COPPER(II) COMPLEX. (Abstract) **Aim:** The cooper(II) complex combination of N, N'-disalicylidenemethylenediamine and the Schiff bis base were investigated for anti-inflammatory activity. **Material and methods:** *In vivo*, the anti-inflammatory activity of the metallic complex in comparison with the activity of the Schiff bis base was tested by the method of Winter and co-workers using the Levy technique. **Results and discussions:** Our study on the anti-inflammatory activity of a new Schiff bis base and its complex cooper(II) combination showed that the Schiff bis bases exhibited significant anti-inflammatory action in acute experimental inflammation when compared to the control group. The copper cation from the complex combination enhanced the anti-inflammatory effect of the Schiff bis base, the effect being stronger at doses of 10 mg/kg cooper(II) complex. **Conclusions:** The Schiff bis base and its cooper(II) complex had an anti-inflammatory effect comparable to that of indomethacin. **Keywords**: COPPER, SCHIFF BIS BASE, COMPLEX, ANTI-INFLAMMATORY ACTIVITY.

The Schiff bis base compounds represent an important class of ligands that have been extensively studied in coordination chemistry, mainly due to their simple synthesis and easy tunable steric, electronic and catalytic properties (1-6). The complex combinations of Schiff bis bases with various cations represent a class of compounds with very important properties from a chemical and biological point of view (7).

There have already been reported many complex combinations of Schiff bis bases with different cations that showed antimicrobial, anti-inflammatory and antioxidant effect (8-11).

This paper describes the evaluation of the anti-inflammatory action of a new Schiff bis base derived from N,N'-disalicylidenemethylendiamine and its copper (II) complex that was previously synthesized and characterized (10-11).

MATERIAL AND METHODS

For the study of the anti-inflammatory activity of the Schiff bis base and its cooper(II) complex (fig. 1) the following

reagents were used: carrageenan kalium salt, indomethacin and carboxymethyl cellulose purchased from Merk - Germany. The method used to test the inflammation was edema experimentally induced through carrageenan in rats (adult Wistar rats, male of 180-200 g purchased from Cantacuzino Institute - Bucharest).

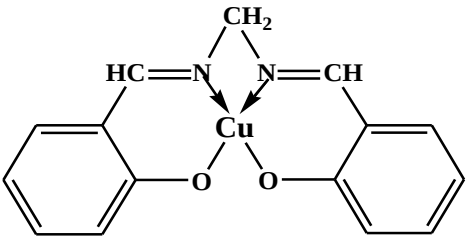

Fig. 1. Structure of cooper(II) complex combination

The evaluation of the anti-inflammatory effect was performed using the carrageenan-induced rat paw edema assay (14, 15). For each substance we used six groups of adult male Wistar rats, 180-200 g, bred in laboratory condition and identically fed. Acute inflammatory paw edema was induced by injecting 0.2 mL carrageenan 2% solution in the left posterior paw of the rat. Paw volume was determined before and after the test substance was administered and also after 1h, 2h, 4h, 6h, 8h or 24h. A control group of six Wistar received an identical carrageenan injection in order to achieve acute inflammatory paw edema, but no any other substance. Paw volume of the control group was determined at the same intervals as the test groups.

We used the following groups of rats: Group I was the control group, group II received indomethacin sodium salts 10 mg/kg i.p., group III received 10 mg/Kg of Schiff bis base, group IV received 10mg/kg cooper(II) complex and group V received 5 mg/Kg cooper(II) complex. The anti-inflammatory activity was evaluated as the variation of inflammation volume (tenths of mL). The average sum and standard deviation of that parameter were calculated for every group of rats and then they were compared to those of the control group. The obtained data were statistically analyzed using ANOVA one way test.

In *vivo*, the anti-inflammatory activity of the cooper(II) complex in comparison with that of the Schiff bis base was tested using 2% (w/v) carrageenan potassium salt solutions and 2% (w/v) indomethacin in 0.1% sodium carboxymethyl cellulose solution. The stock solutions of the complex and ligand were prepared in concentrations of 0.1% (w/v) in methanol.

RESULTS AND DISCUSSION

Several studies proved that Schiff bis bases and their complexes with various cations (Cu(II), Mg(II), Ni(II), Co(II) etc.) reduce the synthesis of some chemical mediators of acute inflammation such as leukotrienes which are involved in the formation of free radicals (9, 12, 13).

The data showed that the anti-inflammatory effect of the Schiff bis base (10 mg/kg) was statistically significant compared to the control group, after 4h ($P<0.05$). The effect increases at 6h and 8h, respectively ($P<0.001$) (tab. I).

The cooper(II) complex presented anti-inflammatory activity compared with control group, the effect being significantly stronger at a dose of 10 mg/kg, beginning with 4h ($P<0.001$). The cooper(II) complex at a dose of 5 mg/kg also presented strong anti-inflammatory effect at 4h, 6h and 8h ($P<0.001$), compared with the Schiff bis base (10 mg/kg).

TABLE I

Influence of the Schiff bis base (10mg/kg) and cooper(II) complex (10mg/kg and 5mg/kg) on carrageenan-induced inflammatory edema in rats (mean edema volume variation (tenths of mL) ± SD)

3	0	1h	2h	4h	6h	8h	24h
Control	18.17± 1.02	27.83± 0.62	30.17± 0.32	35.17± 1.06	38.83± 2.36	38.5± 1.80	23.67± 1.18
Indome-thacin 10mg/kg	22.3±4.05	25.3±2.71	26.9±2.04	25.02±3.21	24.8±2.25	25.32±3.61	24.55±2.77
Schiff bis base 10mg/Kg	18.50 ± 0.62	26.33 ± 1.42	28.33 ± 0.80	30.83 ± 0.63 * P<0.05	31.00 ± 3.20 * P<0.001	29.83 ± 3.62 * P<0.001	20.83 ± 0.86 * P<0.01
cooper(II) complex 10 mg/Kg	19.67± 1.18	28.67± 0.86	31.83 ± 1.72	27.17 ± 3.76 * P<0.001 ** P<0.001	22.67 ± 7.14 * P<0.001 ** P<0.001	21.5 ± 7.54 * P<0.001 ** P<0.001	21.33 ± 1.56 * P<0.001 ** P<0.05
cooper(II) complex 5 mg/Kg	18.83 ± 0.78	25.83 ± 0.47	26.50 ± 0.80	32.50 ± 0.95 * NS ** NS	31.33 ± 7.78 * P< 0.05 ** NS	30.67 ± 9.96 * P< 0.001 ** NS	22.50 ± 0.08 * NS ** NS

* P values compared with control group

** P values compared with the group receiving Schiff bis base

CONCLUSIONS

Our study on the anti-inflammatory activity of a new Schiff bis base and of its cooper(II) complex combination showed that the Schiff bis base exhibited significant anti-inflammatory action in acute experimental inflammation when compared to the control group.

The copper ions enhanced the anti-inflammatory effect of the Schiff bis base in its complex combination, the effect being stronger at doses of 10 mg/kg cooper(II) complex.

The Schiff bis bases and its cooper(II) complex had an anti-inflammatory effect comparable to that of indomethacin.

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NEWS

INFLAMMATION AND HEART DISEASES: ROLE OF TOLL-LIKE RECEPTOR SIGNALING

Myocardial inflammation is a common theme of a group of diseases that include severe sepsis, ischemic myocardial injury and acute myocarditis, and contributes to their pathogenesis. Under these critical conditions, foreign pathogens (e.g., bacteria or virus) or endogenous danger molecules (e.g., heat shock proteins [HSPs] or RNA) released from injured tissues induce local and systemic innate immune responses that lead to myocardial inflammation. Such innate immune responses could be both detrimental and beneficial in nature. Toll-like receptors (TLRs) are a critical part of human innate immune system. TLRs recognize pathogens via pathogen-associated molecular pattern (PAMP) (1) and endogenous danger molecules via danger-associated molecular pattern (DAMP) (2). TLRs also contribute to the development of inflammation in atherosclerosis that leads to myocardial ischemic injury and acute coronary syndromes. By mediating the complex cardiac inflammatory signaling, either beneficial or deleterious in nature, TLRs play a pivotal role in the development of these cardiac diseases. Several important future directions should be considered. While numerous studies have indicated the contributory role of TLRs in the development of septic cardiomyopathy, ischemic myocardial injury and myocarditis, there are many unanswered questions (including TLRs involvement in polymicrobial sepsis) that are critical for our ultimate understanding of the role of TLR signaling (Wei Chao. Inflammation and heart diseases: role of toll- like receptor signaling. J Anesth Perioper Med 2014; 1: 104-17).

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ETHICAL INTERPRETATIONS OF PATIENT AUTONOMY IN CHRONIC CARE WITH SPECIAL REFERENCE TO CANCER CARE

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ETHICAL INTERPRETATIONS OF PATIENT AUTONOMY IN CHRONIC CARE WITH SPECIAL REFERENCE TO CANCER CARE (Abstract): The present review analyzes the ethical interpretations of patient autonomy in chronic care with special reference to the advanced cancer patient and to the transition from curative to palliative. Thus, in the context of chronic care, studies indicate that a positive conceptualization of autonomy, which is compatible with the assumption that people are interdependent, is preferred over a negative conceptualization that presents autonomy as non-interference. Moreover, it has been shown that the concept of "Socratic autonomy", which promotes an ethics of care and of interdependence, represents the standard for assessing autonomy in chronic care. With regard to cancer care, several studies illustrate that most patients decide to delegate the decisional responsibility completely to their physician and such evidence support the interpretation of autonomy as critical reflection. This paper also reviews the ethical challenges that may derive from delivering bad news to advanced cancer patients, pointing out that disclosure of a cancer diagnosis or of a poor prognosis can facilitate a so-called conspiracy of silence. **Keywords:** PATIENT AUTONOMY, CHRONIC CARE, SOCRATIC AUTONOMY, ADVANCED CANCER, CONSPIRACY OF SILENCE.

Studies note that Beauchamp and Childress' liberal individualist concept of autonomy seems to be the prevailing interpretation of autonomy in the medical literature. Stiggelbout and collaborators (1) provide, from the ethics literature, five moral alternative concepts to the one of patient autonomy defined by Beauchamp and Childress, namely, the liberal legal concept of respect for autonomy based on Berlin's negative concept of freedom, autonomy as critical reflection, actual autonomy as identification, Socratic autonomy, and autonomy as negotiated consent, but underline that "the medical literature seldom refers to these concepts of autonomy, although they are potentially of great use".

Moreover, the concept of psychological autonomy has been defined as the capacity of a person to become informed so that he/she can exercise his/her right to self-determination (2). Respect for patient autonomy can be promoted by understanding the patient's values and goals by firstly exploring patient preferences for information sharing and decision making, but also who should be involved in these processes (3).

However, Mars and collaborators (4) have emphasized that it remains unclear how the different conceptualizations of autonomy should be understood with respect to chronic diseases.

In this regard, this review explores the ethical interpretations of patient autonomy in chronic care with special reference to the advanced cancer patient and to the transition from curative to palliative care in cancer, as studies point out that cancer implies making complex and difficult decisions (5). It has also been noted that cancer-related mortality remains high, as more than 50% of cancer patients develop incurable, advanced forms of cancer, which constitute a major challenge (6).

AUTONOMY INTERPRETATIONS IN CHRONIC CARE

The liberal individualist interpretation of patient autonomy identifies autonomous decisions as those made intentionally and with substantial understanding and freedom from controlling influences (1, 7). However, Entwistle and collaborators (7) highlight the significance for autonomy of our interactions with other people and sociocultural systems, focusing on relational understandings of patient autonomy which imply that our individual capability for autonomy is socially and situationally shaped, and which thus support the value of good patient-physician relationships. In addition, Baumann (8) reconsiders the concept of relational autonomy by acknowledging the social dimension of autonomy in such a way that certain relationships and social environments can have an impact on the autonomy of a person. In this regard, Mars and collaborators (4) plead for a relational conceptualization of autonomy in the context of chronic care. The relational understandings of autonomy are thought to deemphasize independence and thus enrich the concept of autonomy (7).

The distinction between autonomy as negative liberty and autonomy as positive liberty also appears to be important in the context of chronic physical illnesses. In this regard, a negative conceptualization of autonomy seems unsatisfactory in the context of chronic care, because a person is considered autonomous if no one interferes with his/her actions. In such a context, a positive conceptualization of autonomy would be preferred, as it takes into account people's actual needs, being compatible with social models of disability and with the assumption that people are interdependent (4).

Furthermore, Naik and collaborators (9) have indicated that the nature and exercise of patient autonomy in chronic care cannot be adequately conceptualized or clinically assessed by appealing to a concept of autonomy that includes only decisional autonomy, which refers to the patient's capacity to understand information and to make voluntary decisions, pointing out the need for an expansion of the concept of patient autonomy to also include the patient's capacity to play an essential role in executing the plan of care, namely, the executive autonomy. Nevertheless, the presence of decisional autonomy with lack of executive capacity is met in the routine care and management of persons with chronic disease.

Studies also draw attention to "actual autonomy as identification", an interpretation according to which it is more important for a patient "to identify him or herself with the decision made" than to make the decision him or herself. In this view, dependence does not seem to be problematic, as identity

refers to identity as a social being in interaction with others rather than to identity as an independent being. With respect to this interpretation of autonomy in chronic care, it has been noted that "People have to integrate the course of illness into their biography", "they must reconstruct their identity, and finally, they must give their biography a new direction" (1, 4).

The "normative value", the "standard for assessing actual autonomy in chronic health care", is considered to be represented by the concept of "Socratic autonomy" which links autonomy with the fragility of human existence and patients' vulnerability by promoting an ethics of care and of interdependence, as "a human being is not only free and autonomous, but also vulnerable and dependent" (1, 10).

SPECIAL FEATURES OF ADVANCED CANCER PATIENT AUTONOMY

Decision making for cancer treatment provides challenges as it is made under high levels of distress and uncertainty and often under perceived or real time pressure (11).

Studies have outlined that telling about poor prognosis to a cancer patient may cause psychological harm to him/her (12, 13). In this regard, physicians may limit prognostic information to preserve hope. However, Mack and collaborators (14) have noted that prognostic disclosure with sensitivity and realism can sustain hope and can increase trust in the physician, even when the prognosis is poor. Thus, their study shows that honest disclosure by the physician supports patient autonomy, desire for information and abilities to make appropriate decisions. Furthermore, studies have pointed out that the breast cancer patients who were more communicatively involved in treatment decision making reported significantly less decision regret (15).

It has also been noticed that advanced cancer patients prefer to get information about their condition directly from their physician and that accepting information about imminent death would be easier if presented with sensitivity and compassion (16). Moreover, Rosner (17) emphasized that disclosure of a fatal disease diagnosis needs to be done "with compassion, sensitivity and hope thus giving the patient an opportunity to «set his house in order»".

In addition, studies underline that, when discussing a bad prognosis, the physician should firstly assess how much information the cancer patient wants to know. In this regard, the following situations have been described in the literature: the cancer patient wants to know about prognosis, but the desired amount of information varies among patients; the cancer patient does not want to know about prognosis – the right not to know has been described in the literature as "an enhancement of autonomy", being based on the idea that individuals should be free to make their own decisions (to know or not to know) with regard to information; the patient is ambivalent about knowing prognosis – the patient seems to be involved in "an ambiguous process of knowing and not knowing at the same time" (18, 19, 20, 21).

Billings & Krakauer (3) have also noted that, in order to respect patient autonomy in end-of-life care, the physician should share only as much information as the patient wishes, pointing out that forcing information or forcing decision making on a patient or family member does not promote autonomy, but it rather overrides it.

Moreover, a qualitative study on advanced cancer patients' preferences for

participation in treatment decision making has shown that all interviewed patients wanted their physician to participate in the decision making process because of his/her knowledge and clinical experience, but there were differences with regard to the extent to which patients themselves wanted to participate in treatment decision making, being emphasized that individual patients' preferences may change along with the illness trajectory (22).

A study assessing satisfaction with oncology care has also revealed that the caregivers of patients with advanced cancer seem to desire greater involvement in treatment decisions (23).

AUTONOMY AS CRITICAL REFLECTION

One of the moral concepts that has been presented in the literature as an alternative to the Beauchamp and Childress' liberal individualist concept of autonomy is the interpretation of autonomy as critical reflection, which has been defined as follows: after a critical reflection on his/her desires and preferences, the patient might decide to leave all the decisions to his/her physician (1).

In this regard, a systematic review on decision making and information provision in patients with advanced cancer has supported this interpretation by illustrating that "almost all patients expressed a desire for full information, but only about two-thirds wished to participate actively in decision making" (24).

Another study that examined the relationship between cancer patients' desire for information and their preference for participation in treatment decision making has shown that, although most of the patients actively sought information, a majority preferred the physician to assume the role of primary decision maker. Therefore, these cancer patients have chosen to express their autonomy by authorizing their physicians to make the decisions – patients "decide not to decide" (2).

Moreover, a cross-sectional study assessing Greek breast cancer patients' preferences for participation in treatment decision making and their information needs has revealed that the majority of patients wanted to play a passive role in decision making, with most of them choosing to delegate decisional responsibility completely to their physician (13).

Another study on cancer patients has emphasized that "Being freed of responsibility for making treatment decisions can produce an immense sense of relief, with treatment failures becoming the responsibility of the practitioner rather than the patient." (25, 26)

Therefore, Gessert (27) underlines that autonomy at the end of life is not universally valued by patients and their families, suggesting that "an appeal to autonomy may actually be a disservice to the peace of mind and well-being of the family".

CONSPIRACY OF SILENCE IN ONCOLOGY

It has been noted that the communication of a poor prognosis is considered one of the most difficult moments to handle in the relationship between physician and advanced cancer patient (28). Moreover, it has been pointed out that the duty of a health care professional to break bad news by giving information about disease and prognosis to terminally ill cancer patients becomes more difficult when family members ask to hold back the diagnosis from these patients (12).

Studies have emphasized that the issue of disclosure of a cancer diagnosis or of a poor prognosis facilitates a "conspiracy of silence", also known in the literature as "collusion", which is considered a universal phenomenon and which has been defined as "a secret agreement between two or more people who are trying to deceive" (29, 30). In this regard, the physician often colludes with the patient's relatives to not inform the patient about his/her diagnosis and poor prognosis, or the physician colludes with the patient to not inform the family about the patient's diagnosis and prognosis. Moreover, it has been noticed that the collusion may be complete or partial – for instance, the physician discloses the diagnosis to the patient, but withholds information about the prognosis (30). The result of withholding information from patients consists of a "false optimism" about recovery, patients becoming dominated by their recovery story and thus focusing on the present and avoiding thinking about the future. Studies also present examples of "unintentional ambiguity" generated by the physician, such as when the physician stated that "this tumor can be treated", but the patient heard that he/she can be "cured" (20).

In addition, it has been shown that close relatives frequently demand the diagnosis of malignancy to be concealed from the patients (13). Similarly, another study on cancer care highlights that a common challenge derives from the relatives seeking to avoid disclosure of bad news to patients (31). With regard to breaking bad news, it has also been noted that informing the relatives before the patient and without his/her consent still represents a common practice that can prove to be really damaging (32). It has thus been outlined that "the long-term consequences of denying patients autonomy at the end of life can be harmful to patients and can leave doctors and nurses distressed and confused" (33). Although the initial purpose is to protect a loved one from bad news, the collusive situation is observed to cause psychological suffering for all the protagonists involved (29).

Studies have thus indicated that it is important to break collusion by initiating a dialogue (29, 30). In this regard, the solution proposed at the end of an ethnographic study on patients with small lung cancer would be to implicate "treatment brokers" – people who are trusted by both the physician and the patient (20).

TRANSITION FROM CURATIVE TO PALLIATIVE CARE IN CANCER

There are only a few studies that approach the transition from curative to palliative cancer care. However, it has been noticed that, from the perspective of both patient and physician, the transition from curative to palliative is one of the most challenging phases of cancer care (34).

A study on women with advanced breast cancer has illustrated two main types of transitions: a personal transition including physical, emotional and social changes that occur over time, and a care transition reflecting shifts in cancer status, treatment and approach to care (curative, palliative or hospice care) (35).

With regard to communicating bad news in the transition from curative to palliative cancer care, studies point out that the information should be passed slowly to the patient and family without destroying hope, but also without giving false hope, and the physician should also be careful not to convey to the cancer patient a sense of

abandonment or failure (18, 34).

Another study which focused on breaking bad news in the transition from curative to palliative care in cancer has identified six types of physicians, as described by their patients, and these types have been named in a suggestive manner, as follows: "the inexperienced messenger", "the emotionally burdened", "the rough and ready expert", "the benevolent but tactless expert", "the «distanced» doctor" and "the empathic professional" (36).

Additionally, it has been indicated that the transition from curative to palliative care is a topic that often involves collusive situations (30). On the other hand, studies have emphasized that the reluctance to communicate the real situation of an incurable stage constitutes a significant barrier in implementing a transition from curative to palliative care in cancer (34, 37).

Furthermore, Jeffrey (38) outlines that a model of care based on respect for patient autonomy will allow choosing the right timing of the switch from curative to palliative care.

CONCLUSIONS

The present review provides evidence that, in the context of chronic disorders, the liberal individualist concept of autonomy has proven to be problematic, so autonomy must be re-conceptualized, the Socratic autonomy representing the normative value.

In this regard, papers on chronic care suggest that the relationship between autonomy and dependence has to be reconsidered and that these ethical concepts should not be referred to as two mutually exclusive categories.

With respect to cancer care, this review emphasizes that autonomy as critical reflection seems to be the prevailing interpretation of autonomy in several studies.

Moreover, truth telling about diagnosis and prognosis, how to break bad news, how much to tell and to whom (to the patient and/or to the relatives) represent common ethical dilemmas that health care professionals must face when interacting with advanced cancer patients.

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NEWS

COPEPTIN IS AN INDEPENDENT PREDICTOR OF DIABETIC HEART DISEASE AND DEATH

It is known that high copeptin is associated with incidence of diabetes mellitus (diabetes), abdominal obesity, and albuminuria. Furthermore, copeptin predicts cardiovascular events after myocardial infarction in diabetic patients, but whether it is associated with heart disease and death in individuals without diabetes and prevalent cardiovascular disease is unknown. Vasopressin (AVP), also called antidiuretic hormone, is a peptide involved in diverse physiological functions and released from the posterior pituitary gland in conditions of decreased blood volume or high plasma osmolality. Vasopressin exerts its antidiuretic effects through the AVP 2 receptor in the kidney. The AVP 1a receptor is involved in blood platelet aggregation and vasoconstriction in the vessels and gluconeogenesis and glycogenolysis in the liver, whereas the AVP 1b receptor is found in the anterior hypophysis and the Langerhans islets of the pancreas, where it mediate secretion of adrenocorticotrophic hormone, insulin, and glucagon. Thus, AVP may affect glucose metabolism through several different mechanisms. Vasopressin is a small, short-lived peptide, and most assays measuring AVP have relatively limited sensitivity. An assay has been developed to measure plasma copeptin (copeptin), the C-terminal portion of the AVP precursor. Copeptin is considered to be a stable, reliable, and clinically useful surrogate marker for AVP. Recently, was found elevated copeptin to be associated with incident diabetes mellitus (diabetes) independently of a range of different clinically used diabetes risk factors including plasma glucose and insulin, as well as independently associated with incident abdominal obesity. Furthermore, copeptin is proposed as a useful biomarker in cardiovascular and renal disease, and is suggested to contribute to the progression and predict prognosis in heart failure (HF) and to predict prognosis in stroke and myocardial infarction. However, it is not known whether copeptin predicts heart disease or death in unselected diabetic and nondiabetic individuals in the population. Based on the previous findings that copeptin predicts diabetes, and its predictive role in diabetes patients with hemodialysis and myocardial infarction, the authors hypothesized that elevated copeptin predicts coronary artery disease (CAD), HF, and death differentially in individuals with diabetes and individuals without diabetes. The results suggested that copeptin is a promising prognostic marker for diabetic heart disease and death. Furthermore, the pharmacologically modifiable AVP system may be a new therapeutic target in the prevention of secondary diseases and premature death among individuals with diabetes (Enhörning S, Hedblad B, Nilsson PM, et al. Copeptin is an independent predictor of diabetic heart disease and death. Am Heart J 2015;169:549-556.e1).

Doina Butcovan

RETROSPECTIVE EPIDEMIOLOGIC RESEARCH ON PREVALENCE OF INFECTIONS IN SURGICALLY TREATED ONCOLOGIC PATIENTS

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RETROSPECTIVE EPIDEMIOLOGIC RESEARCH ON PREVALENCE OF INFECTIONS IN SURGICALLY TREATED ONCOLOGIC PATIENTS (Abstract): The **aim** of this work was the retrospective study of the frequency of infections in surgically treated oncologic patients. **Material and methods:** Our research included 753 oncologic patients treated at the Second Surgical Clinic of the Regional Institute of Oncology, Iaşi, between January 2013 and December 2014, with the selection of cases which had infectious complications. **Results:** The lot of patients was structured in 7 age groups and the most frequent oncologic patients had ages between 60-79 years. The most frequent malignancies were large bowel, breast, rectal, esophageal cancers, malignant hemopathies, gastric and liver malignant tumors. In our lot of 753 oncologic patients we depicted 113 (15.01%) infections. **Conclusions:** Our study proved that the anatomic location of the cancer, the hospitalization duration and the number of the hospitalizations has an important role in appearance of the infectious complications. The gastrointestinal malignant tumors were the most frequent malignancies associated with postoperative infections. The patients with gastrointestinal cancers from urban area, with ages between 50 and 79 years had the highest risk of infectious complications. For main causative organism was proved a high sensitivity for colistin. Clinical, paraclinical and epidemiological exams did not emphasize any decisive factors; we took into account the risks of infection during multiple pre-, intra- and postoperative procedures; all these have been associated with increased receptivity of immunosuppressed oncologic patients. **Keywords**: INFECTION, ONCOLOGY, EPIDEMIOLOGY, GASTROINTESTINAL NEOPLASM.

Oncologic pathology produces severe changes in patient's life; the immunosuppression associated with the malignancy favors the infections with various germs, the most dangerous of all being the ones given by organisms with high aggressiveness (1, 2). For oncologic patients with advanced disease, the most frequent complication with vital risk and the main cause of death are the infectious complications

(3, 4). The causative organisms determining the infections in case of patients with cancer are the opportunist organisms. The risk of selection of antibiotic resistant germs increases along with the hospitalisation duration and antibiotherapy (5, 6). The risk factors which favor the infections of an immunocompromised host are the dysfunctions of the immune system, neutropaenia, misbalances of central nervous system, the obstructive phenomena, medical invasive maneuvers, splenectomy, dysbacteriosis and the presence of microorganisms (7, 8).

MATERIAL AND METHODS

Our research included 753 patients with malignancies (354 in 2013 and 399 in 2014), treated at Second Surgical Clinic of the Regional Institute of Oncology, Iaşi, between January 2013 and December 2014. The information was obtained in collaboration with the Department of Prevention and Control of Nosocomial Infections and by studying the registers containing surgical protocols. The data were processed and interpreted with epidemiologic (prevalence, inquiry), statistic and computerized methods In order to calculate the quantitative differences we used $\chi 2$, p and t-student tests. The linear regression was calculated with the following formula y=a+bx and had underlined the evolution of the phenomenon.

RESULTS AND DISCUSSION

The distribution according to the age groups. The lot of patients was structured on 7 age groups: 20-29 years (13 patients, 1.72%), 30-39 (32; 4.24%), 40-49 (76; 10.09%), 50-59 (169; 22.44%), 60-69 (256; 33.99%), 70-79 (165; 21.91%) and over 80 (42; 5.57%) (fig. 1).

The evaluation of the oncologic patients according to the age groups. We observed that 18.06% of the patients were from the 60-64 years group, followed by 65-69 (15.93%) and 55-59 (14.20%), which shows the socio-economic and familial implications (fig. 2).

The structure of the lot of patients according to the gender. The differences of prevalence between the two genders were not significant ($p>0.05$), 50.19% of patients were males and 49.80% females.

According to the residence, 61.08% of patients lived in urban area and 38.91% in rural area, with a difference statistically significant ($p<0.05$). This situation can be explained by the differences of education and lack of accessibility to the medical assistance in case of the rural residents.

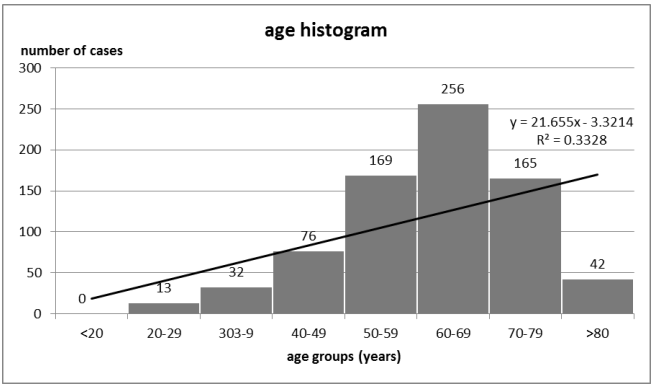

Fig. 1. The distribution by age groups of the oncologic patients included in the study

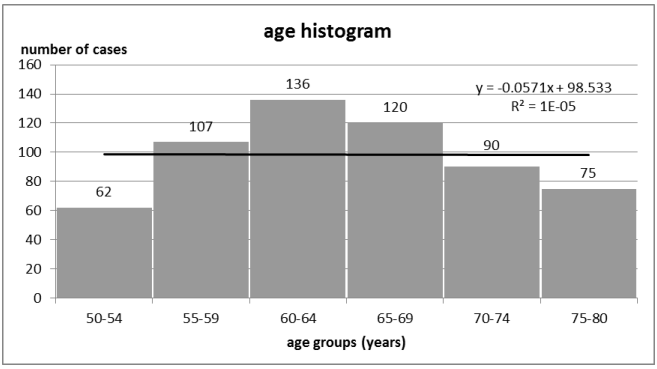

Fig. 2. The age groups distribution of the patients with frequent malignancies

Evaluation of the prevalence of certain forms of cancer. The patients we had studied had the following malignancies: large bowel (96 cases; 12.75%), breast (88 patients; 11.68%), rectum (87; 11.55%), esophagus (64; 8.49%), malignant hemopathies (59; 7.83%), gastric (55; 7.31%), liver (34; 4.51%), uterus (34; 4.51%), ovary (29; 3.85%), pancreas (27; 3.58%), skin (23; 3.05%), soft tissue (23; 3.05%), rectosigmoid (23; 3.05%), pharynx (19; 2.52%), lung (18; 2.39%) (fig. 3).

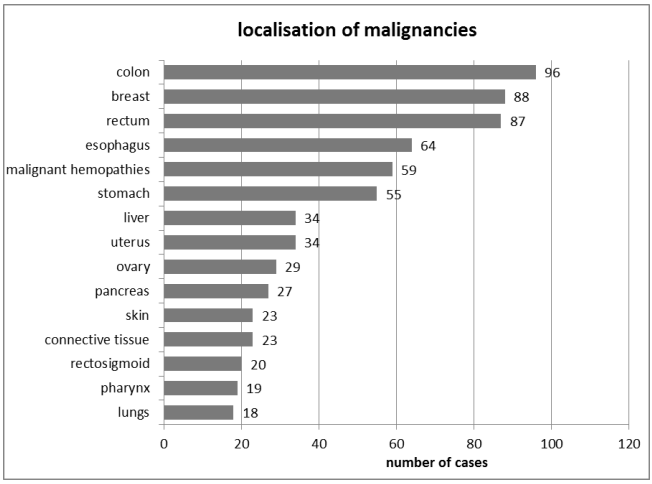

Fig. 3. The patients' distribution related to frequent malignancies

Comparing the frequency of different malignancies in 2013 and 2014, we observed a significant increase in number of various cancers in 2014: esophageal (40 cases in 2014 compared with 24 in 2013, meaning an increased percentage of 3.25%), breast malignant tumors (51 cases in 2014 compared with 37 in 2013, meaning an increased percentage of 2.33), large bowel – percentage difference of 2.20, pharynx (2.09%), thyroid (1.19%).

In 2013, we encountered some other malignancies with a higher frequency: gastric (37 cases in 2013 compared with 18

in 2014, meaning a significant percentage difference of 5. 94), malignant hemopathies (32 cases in 2013 compared with 18 in 2014, difference of 2.27%), skin - 1.70%, lung 1.35%, ovary 1.25%. In case of kidney, tongue, soft tissue, recto-sigmoid and pancreatic neoplasm we found a stationary tendency (percentage difference of less than 1) (fig. 3).

Infections encountered in case of oncologic patients. In our study, according to the literature, the infections prevalence was related to the characteristics of the primary tumor and to different co morbidities of each patient (8). The oncologic patients, secondary to immunosuppression, have an increased receptivity to various endogenous and exogenous organisms, inside and outside the hospital; the microorganisms which determine the infections are, most of the times, various species of bacteria with multiple antibiotic resistance.

Distribution of the infections related to the malignant diseases. Between January 2013 and December 2014, among the 753 patients included in the lot of study, 113 (15.01%) suffered from various infections; the most frequent of all infected patients were de ones with rectal cancers (23.0%), followed by: large bowel (16.81%), breast (11.50%), gastric (8.84%), uterine cancers (6.9%). Far less frequent were the infections appeared in case of ovary, skin, liver, biliary ducts, recto-sigmoid, lung, pharynx, esophagus, pancreatic, urinary bladder and small bowel cancers (fig. 4).

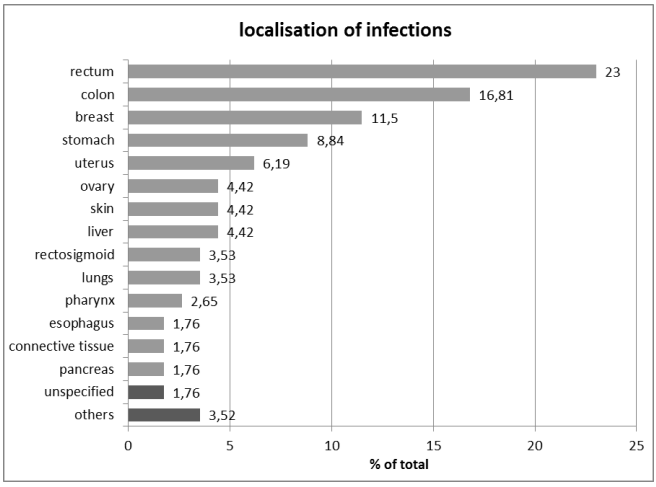

Fig. 4. Distribution of the infections related to the malignant diseases between 2013 and 2014

Mean hospitalization duration (number of days). Among the oncologic patients there was a significant difference ($p \leq 0.05$) between the mean hospitalizations duration in case of patients with infections (24.025 days) compared with those without infections (9.76 days). The hospitalization duration before surgery was longer in case of the patients with postoperative infections (5.88 days) than for the patients without infections (2.72 days), which proves that the risk of infection increases with prolonged preoperative hospitalization ($p \leq 0.05$).

Bacteria's frequency in case of oncologic patients' infections. Among the 113 patients with infections, we have encountered 204 determinations of 23 bacteria: for 63 (55.75%) cases there was only one causative organism and for 50 (44.24%) cases there were two or more causative organism; the difference among this two categories has a mean statistical significance ($p<0.05$) and can be explained by the various sites of infection and the multiple surgical procedures performed on immunosuppressed patients. The most frequent causative organism was *E. coli (*27.94%), followed by *Klebsiella pneumoniae* (18.62%), *Pseudomonas aeruginosa* (8.33%), *and Staphylococcus aureus* (7.84%). We noticed that *Staphylococcus aureus* is now on the fourth place, according to the data from the literature, standing for a decreased involvement of this bacteria community and hospital infections (fig. 5).

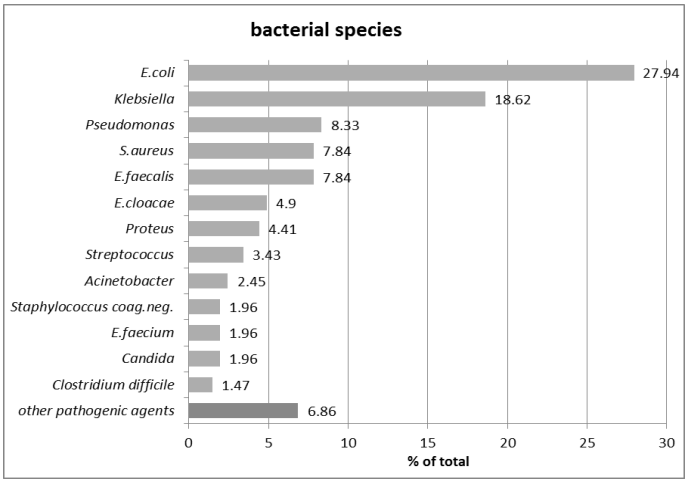

Fig. 5. Bacteria's frequency in case of oncologic patients' infections

Among the 53 cases of nosocomial infections, the most were isolated from the postoperative wounds (27: 50,95%), followed by acute enterocolitides (13: 24.53%), urinary infections (6 : 11.32%), pneumonia (5: 9.43%), and sepsis (2: 3.77%).

Causative organism observed in various biologic products. Among the 27 probes from the postoperative wounds we had isolated: *S. aureus* (33.33%), *K. pneumoniae* (14.28%) *Pseudomonas aeruginosa* (10.47%), *E. coli* (10.47%), *Enterococcus faecalis* (8.57%) and associations in other cases – 22.88% and in case of 6 positive urine cultures, *E. coli* (47.91%) and *K. pneumoniae* (27.08%) had been dominant. We had identified in haemocultures: *E. coli* and *Pseudomonas aeruginosa*; in coproculture: *Clostridium difficile*.

The interval between surgery and the occurrence of infection. The infection began during the first 48 hours in 3 cases, after 72 hours in 3, during the first week in 20, during the second week in 10, during the third week in 10, during the fourth week in 4 and in 3 cases after more than 1 month since surgery. Most of the infections appeared in the first two weeks after surgery (fig. 6).

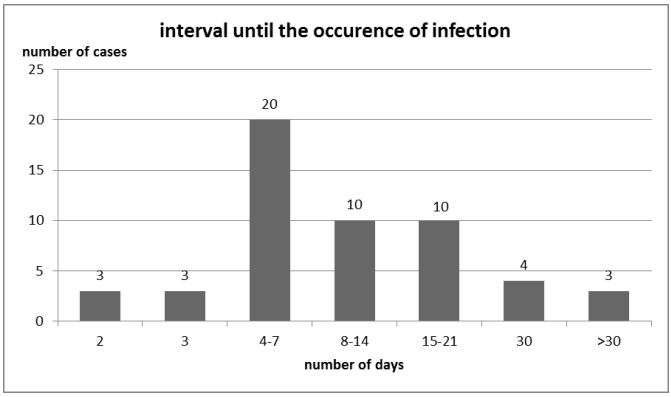

Fig. 6. The interval (days) between surgery and the occurrence of infection

The analysis of the antibiotic sensitivity of the bacteria isolated from the cultures showed the following aspects: 38 (97.43%) of 39 strains of *E. coli* had sensitivity for carbapenem (imipenem / meropenem) and all 33 (100%) strains for polypeptide (colistin); 24 (97.43%) of 33 strains of *K. pneumoniae* had sensitivity for carbapenem (imipenem/meropenem), 31 (96.87%) of 32 strains for polypeptide (colistin); 10 (83.33%) of 12 strains of *Pseudomonas aeruginosa* had sensitivity for aminoglycoside (gentamicin) and fluoroquinolone (ciprofloxacin), all 12 (100%) strains for polypeptide (colistin); all 11 strains of *S. aureus* had sensitivity for fluoroquinolone (ciprofloxacin), glycopeptide (vancomycin), sulfonamide (sulfamethoxazole), 11 (91.66%) of 12 tested strains had sensitivity for rifampicin; all 11 tested strains of *Enterococcus faecalis* had sensitivity for glycopeptide (vancomycin) and ampicillin, 10 (90.90%) of 11 strains for aminoglycoside (gentamicin). The appearance of the resistance of some strains of *K. pneumoniae* for carbapenem is of great concern. An efficient measure to limit the antibiotic multiresistance is avoiding unjustified treatments and implementing programs of good practice of antibipotherapy. *Pseudomonas aeruginosa* had resistance for most antibiotic classes because the difficulties of passing the cellular membrane; we remarked also resistance for spare antibiotics (carbapenem); 3 (27.27%) of 11 strains tested had resistance for imipenem. All tested strains of *S. aureus* had resistance for ampicillin and 50% for cephalosporines, macrolide (erythro-mycine) and tetracycline.

CONCLUSIONS

Our study showed that the anatomic location of the cancer, the hospitalization duration and the number of the hospitalizations have an important role in appearance of the infectious complications. The digestive malignant tumors were the most frequent malignancies associated with postoperative infections. The patients with digestive cancers from urban area, with ages between 50 and 79 years had the highest risk of infectious complications. For main causative organism was proved a high sensitivity for colistin. Clinical, paraclinical and epidemiological exams did not emphasize any decisive factors; we took into account the risks of infection during multiple pre-, intra- and postoperative procedures; all these have been associated with increased receptivity of immunosuppressed oncologic patients.

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DEGREE OF PATIENT SATISFACTION WITH HEALTH CARE PERFORMANCE ASSESED BY MARKETING SURVEYS

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DEGREE OF PATIENT SATISFACTION WITH HEALTH CARE PERFORMANCE ASSESED BY MARKETING SURVEYS (Abstract): **Aim.** Marketing surveys of the health system collect useful information to develop effective management strategies. The research aim consisted in measuring patient satisfaction with health care quality. **Material and methods**. The qualitative research was based on an online SurveyMonkey open-ended questionnaire. The analysis of patient satisfaction/dissatisfaction with healthcare professionals was performed in 1838 patients. Correlation analysis allowed the identification of some determinants associated with patient satisfaction**. Results.** The variable most commonly associated with satisfaction was "I got adequate information about procedures/treatment" according to 32.2% of respondents. The patients who were dissatisfied most commonly complained that they were "Not adequately informed about maneuvers and treatment", reported by 40.0% of respondents. **Conclusions**. This study provides a basis for building an original model for determining the variables of an efficient healthcare system which to ensure a high degree of patient satisfaction. **Keywords:** PERFORMANCE, HEALTH CARE, MARKETING.

In recent years the Romanian health system takes steps toward improving the quality of healthcare services while respecting the rules underlying the accession to the European Union (EU) (1). Even in the context of important variation in health service performance, the decision makers are committed to implementing a monitoring system which is considered essential in the development of quality improvement program (2). Patient satisfaction, or health care practitioner satisfaction, respectively is one of the key criteria in quality assurance. In recent years marketing surveys have been used for assessing, among others, patient satisfaction with the quality on health care in health institutions. According to the modern marketing concept, any activity in the health system must be carried out with the knowledge of patients' needs. Thus, marketing surveys collect process and analyze information about the patients, providing evidence to support the marketing and management strategies (3).

The aim of this study was that by addressing issues of patient satisfaction to determine the necessary and useful direction in the development of management

process in health care system. The aim of this research objective was the measurement of patient satisfaction with health care performance within the context of: diagnostic characteristics, length of hospital stay, satisfaction factors and factors that could lead to dissatisfaction of patients included in the study.

MATERIAL AND METHODS

The qualitative marketing survey was based on data collected with an online SurveyMonkey questionnaire (4). For the analysis of patient satisfaction with healthcare professionals a total of 1838 subjects from university hospitals in Bucharest, Iasi, Cluj Napoca, Târgu Mures, Timisoara and Craiova were included in the study. The questionnaires included open-ended questions aimed at identifying the degree of satisfaction with the result of treatment underwent during hospital stay from both professional and medical point of view and if the patient was allowed or not to stay in touch with next of kin during hospital stays. Thus, 10 important issues were addressed: care, humanism reflected in the treatment, absence of pain, prompt attention to patient requests. Respondents' opinions on the variables that have bothered them most were expressed as 30 possible factors that contribute to a degree of dissatisfaction during hospital stay. Also, a correlative analysis was performed to identify issues that might be associated with the assessment of patient satisfaction in this study. The rate of non-responders the all questions on the questionnaire was 0.1%, not significantly influencing the quality of results. Data were processed according to the studied qualitative variables the results being considered statistically significant within a confidence interval of 95%.

RESULTS

1. Demographic and medical characteristics of the study subjects. The surveyed population was composed mostly of females (58.2%), age-group distribution showing that most subjects were in the 40-50 years (19.7%), 50-60 years (28.2%), and 60-70 years (29.5%) age-groups. Distribution of participants according to the variable "level of education", defined according to the following categories: elementary education, secondary education, post-secondary vocational education, higher education, and postgraduate education, revealed that of the surveyed population 40.6% had a higher education. The length of hospital stay was 3 days in 49.5% of the respondents, 7 days in 19.6%; 14 days in 20.0% and a maximum of 21 days in 10.7%.

2. The results of questionnaire responses in hierarchical order of their frequency were classified as follows: 1. *"I got adequate information about the procedures/treatment"* for 32.2% of respondents, but 39.1% of respondents ranked it 2nd; comparative and cumulative analysis of the results allowed the ranking of this item as 1st; 2. the variable *"I was looked after well"* according to 50.9% of respondents; 3. *"I was treated with consideration"* for 39.4% of respondents; 6. *"I was treated with warmth/kindness"*, 81.6%; 7. *"I had no pain"* according to 53% of respondents; 8. Shared by 3 variables: *"Prompt attention to all my requests"* for 38.3% of respondents, *"I had enough contact with my family"* for 33.8% of respondents and *"I had a good contact with the nursing and medical staff (they introduced themselves, joked, provided adequate psychological support)"* according to 26.6% of respondents; 9. *"I rested properly"*, 47% of respondents; 10. *"Sufficient, good quality food*" for 56.3% of

respondents (tab. I).

Based on the obtained responses the most disturbing 10 dissatisfaction factors during hospital stay in hierarchical order were the following: 1. "I did not get adequate information about the procedures/treatment" for 40.0% of respondents; 2. *"The medical staff did not introduce themselves"* according to 49.1% of respondents; 3. 45% of respondents selected the variable *"I did not know when certain procedures were to be performed";* 4. *"I was aware of odors around me"* for 44.8% of responders*;* 5. *"Mixed wards (men and women)"* according to 44.7% of respondents)"; 6. *"I was regarded as an object"* for 44.7%; 7. "*The medical staff uses words that I do not understand"* according to 45% of respondents; 8. *"In the room you are in is too cold or too hot"* in the opinion of 44.8% of respondents; 9. *"I am disturbed by the reactions of neighbor patients"* for 44.7% of respondents; 10. *"I was treated without consent"* for 35.2% of respondents.

TABLE I

Respondents' opinion on satisfaction during hospital stay

No	Treatment during hospital stay	Place/percentage of the variables that contributed to satisfaction during hospital stay									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1.	I was looked after well	29.7	50.9	10	8.4		1.1				0.1
2.	I was treated with warmth/kindness	0.1	0.9	0.2	16.9	0.2	81.6		0.1	0.1	
3.	I had no pain	0.2	0.2	0.2	29.6	7.6	9.3	53			
4.	Prompt attention to all my requests	0.9	0.2	22.3	0.2	18.7	0.1	19.3	38.3		
5.	I had enough contact with my family	9.9			20.3	0.3	7.6	19.6	33.8	8.4	0.1
6.	I got adequate information about the procedures/treatment	32.2	39.1	27	0.1	0.2	0.2	0.1	0.9	0.1	0.1
7.	I was treated with consideration	18.7	8.5	39.4	0.1	0.1	0.1	7.7	0.3	25.1	
8.	I had a good contact with the nursing and medical staff (they introduced themselves, joked, provided adequate psychological support)	8.4	0.2	0.1		20.3	0.1	0.1	26.6	19.2	25.1
9.	I rested properly				9.8	24.6		0.1	0.1	47	18.4
10.	Sufficient, good quality food			0.9	14.6	28		0.1		0.1	56.3

3. Correlational analysis of the results regarding patient satisfaction with health care performance showed that: "Patient age" correlates with the variables: *Appropriate care* [r (1838) = - 0.76**, p = 0.001]; *Satisfaction with how their requests were resolved* [r (1838) = 0.50*, p <0.05]; *Respect for privacy* [r (1838) = 0.72**, p <0.001] and *Fear of communicable diseases* [r (1838) = - 0.69**, p <0.001]. "Education level" was correlated with *"Perception of the level of pain experienced"* [r (1838) = 0.47*, p <0.05], and *"Modality of importance assessment"* [r (1838) = 0.53**, p <0.05].

The variable "Length of hospital stay" was strongly significantly correlated with the following variables: *Feeling that nurses are focusing more attention on devices than on patient*[r (1838) = - 0.65**, p <0.001)],

Use by the medical staff of specific medical terms [r (1838) = 0.6**, p <0.001], *Perception of ambient temperature adequacy* [r(1838) = 0.63**, p<0.001] and *Explanation of procedures/treatment* [r (1838) = - 0.75**, p <0.001]. "The level of satisfaction with the level of care received" correlates directly and inversely with the following variables: *Perception of how the patients have been treated* [r (1838) = 0.67**, p <0.001]; *Level of promptness to patient requests* [r (1838) = - 0.95**, p<0.001]; *Permission to have contact with the family* [r (1838) = 0.97**, p <0.001]; *The degree of explaining the procedures/drug treatment* [r (1838) = 0.42**, p <0.001]; *The degree of consideration with which the patient is treated"* [r (1838) = 0.321 **, p<0.001]; *Appropriate approach* (r (1838) = - 0.315**, p <0.001]; *Food quality* (r (1838) = - 0.6**, p <0.001), *Disturbed sleep* [r (1838) = - 0 5 **, p <0.001], *Brightness* [r (1838) = - 0.7 **, p <0.001]; *Degree of privacy* [r (1838) = - 0.33**, p<0.001]; *Care by unknown doctors* [r (1838) = - 0.9**, p<0.001]; *Degree of discretion* [r (1838) = - 0.32**, p <0.001] and *Fear of communicable diseases* [r (1838) = - 0.9**, p <0.001].

DISCUSSION

The results obtained allow us to state the level of satisfaction with the quality of hospital care decreases with the increasing age of patients who answered the questionnaire. We found that as the subjects were older they considered that the level of privacy was lower. Also noted was a highly significant relationship between ages and fear of communicable diseases, the older the patient the higher the fear. A relevant aspect concerns the fact that although they declared these attitudes, all subjects admitted the prompt attention to all their requests, the relationship between these variables being statistically significant. Also an increase in the number of hospital stay days was a cause of patient dissatisfaction due to the fact that nurses were more focused on devices than on patient, the patients also becoming more aware of the fact that the medical staff uses specific medical terms that patients do not understand. Also, as the length of hospital stay increased the subjects deemed inappropriate the ambient temperature and believed that they did not get appropriate information about the procedures/treatment they underwent. We believe that as the level of satisfaction with the level of care received is higher, the level of satisfaction with how the patients were treated increases, the patients believe that the procedures/treatments underwent had been clearly explained, increase the satisfaction with the consideration given and permission to be in contact with family. It was found that between the level of satisfaction with the care received and the other above mentioned variables there is an inverse relationship. Thus, as the level of satisfaction with the level of care received is higher, the level of satisfaction with the prompt attention to patient requests, the quality of contact with the medical staff, food services, quality of sleep, brightness of hospital wards, the level of privacy, and the fear of communicable diseases decrease. Also, subjects were more satisfied with the level of care received when not cared for by unknown physicians, and their right to confidentiality is not violated (5, 6, 7).

CONCLUSIONS

1. Quality of patient care largely depends on health system performance. To improve patient satisfaction a system with measurable performance goals for improved patient care should be developed.

2. A code of ethics which to take into account the results of this research is the first step in implementing a health care system. The code will be based on the above analyzed variables, complemented periodically with other variables, the research being repeated every three months.

3. The study provides a basis for the creation of an original model for determining the variables of a performant health care system, which to ensure a high degree of patient satisfaction.

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NEWS

ISOQUINOLINE ALKALOIDS WITH ANTI-HEPATITIS B ACTIVITY

Hepatitis B virus (HBV) is the causative agent of B-type hepatitis in humans. Despite the fact that the disease is vaccine-preventable, about 350 million people worldwide are infected with HBV. Natural compounds are very promising for the treatment of B-type hepatitis. Eighteen isoquinoline alkaloids were isolated from sixteen *Fumaria* and six *Corydalis* species growing in Turkey. They were tested *in vitro* for anti-HBV activity using HBV-expressing cell line HepG2.2.15. Fifteen compounds showed potent anti-HBV activity in the initial screening and six of them (sibiricine acetate, berberine, α-hydrastine, oxosarcocapnidine, fumarophycine, bicuculline) demonstrated consistent antiviral activity in subsequent tests with an efficacy similar to that of tenofovir, a HBV antiviral agent. Sibiricine acetate, fumarophycine and bicuculline were more than 90-, 250- and 150-fold more potent, respectively, than tenofovir in reducing the level of HbsAg. All six compounds targeted the secretion ability of the host cell and reduced the promoter gene pSEAP secretion in a dose-dependent manner. The antiviral activity was also associated with the induction of antiviral genes; berberine and α-hydrastine induced the expression of TNF-α RNA (Aljofan M, Netter HJ, Aljarbou AN et al. Anti-hepatitis B activity of isoquinoline alkaloids of plant origin. *Arch Virol* 2014; 159: 1119–1128).

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MAXILLARY SINUS ANATOMIC AND PATHOLOGIC CT FINDINGS IN EDENTULOUS PATIENTS SCHEDULED FOR SINUS AUGMENTATION

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MAXILLARY SINUS ANATOMIC AND PATHOLOGIC CT FINDINGS IN EDENTULOUS PATIENTS SCHEDULED FOR SINUS AUGMENTATION (ABSTRACT) Objectives**:** An aberrant sinus anatomy or pathology can influence the sinus lift technique if it is encountered during surgical exposure. The purpose of the present study was to determine the prevalence of sinus disease and variations of normal anatomy among edentulous patients prior to sinus augmentation. **Material and methods:** 120 patients attending a private surgical practice (53 women and 67 men, with a mean age of 62.02 years, ranging between 34 and 81 years old) for whom treatment was planned for sinus augmentation were evaluated - medical history, radiographic and clinical examinations. Computerized tomograms (CT) and 3D reconstructions from 240 sinuses were analyzed using Dental CT. **Results:** Abnormalities were diagnosed in 74.2% of cases. Mucosal thickening was the most prevalent abnormality (68.3%), followed by retention cysts (15.8%) and opacification (9.2%). The diagnosed conditions included mucosal thickening, chronic sinusitis, sinus cysts and polyps. The prevalence of one or more septa per sinus was found to be 30%. Males present pathology more often than females (p=.028). No statistical correlation could be found between age and pathology (p>0.05) No significant differences in age or gender between patients with and without sinus septum were found (p>0.05). **Conclusions:** The results reinforce the importance of thorough history taking and clinical and radiographic assessment prior to performing sinus augmentation, to prevent the probability of complications. **Keywords:** SINUS DISEASE, MAXILLARY SINUS SEPTA, SINUS AUGMENTATION, COMPUTER TOMOGRAPHY

The maxilla consists of a variety of anatomic structures, including the maxillary sinus, the lateral nasal wall, the pterygoid plates, associated vasculature structures, and teeth. The maxillary sinus maintains its overall size while the posterior teeth remain in function. It is, however, well known, that the sinus expands with age, and especially when posterior teeth are lost. The average volume of a fully developed sinus is about 15 ml but may range between 4.5 and 35.2 ml. The sinus cavity expands both inferiorly and laterally, potentially invading the canine region. This phenomenon is the possible result of atrophy caused by reduced strain from occlusal function. One or more septa, termed "Underwood's septa", may divide the maxillary sinus into several recesses. The overall prevalence of one or more sinus septa is between 26.5%

and 31% and is most common in the area between the second premolar and first molar. Edentulous segments have a higher prevalence of sinus septa than dentate maxillary segments (1).

The maxillary posterior edentulous region presents more challenging conditions in implant dentistry than any other region of the jaws. However, treatment modalities have developed for this area and allow it to be predictable as any intraoral region. Most noteworthy of these treatments are subantral augmentation to increase available bone height (2).

Diseases associated with the maxillary sinuses include both intrinsic diseases (originating primarily from tissues within the sinus) and those that originate outside the sinus (most commonly diseases arising from odontogenic tissues) that either impinge on or infiltrate the sinus. These types of diseases include inflammatory odontogenic disease (thickened sinus mucosa, polyps, empyema, and retention pseudocysts), odontogenic cysts, benign and malignant odontogenic neoplasms, bone dysplasias, and trauma.

CT has become increasingly important for evaluation of sinus disease and has virtually replaced plane radiography and conventional tomography for investigations of the paranasal sinuses. Because CT provides multiple sections through the sinuses in different planes, they may contribute significantly to delineating the extent of disease and the final diagnosis. High-resolution axial and coronal CT examinations are the most revealing imaging techniques for the paranasal sinuses and the adjacent structures and areas (3).

Previous studies reported the prevalence of incidental abnormalities in CT or CBCT scan in patients' referred to as orthodontic and maxillofacial purposes, the occurrence of abnormalities is not known in samples of patients who underwent the exam for sinusal augmentation procedures (4, 5, 6, 7, 8).

The aim of the study was to determine the prevalence of sinus disease and abnormalities among edentulous patients scheduled for sinus augmentation.

MATERIAL AND METHODS

The study included 120 patients attending a private surgical practice (53 women and 67 men, with a mean age of 62.02 years, ranging between 34 and 81 years) for whom treatment was planned for sinus augmentation. The patients are edentulous in the posterior area of the maxilla. Maxillary computed tomography evaluation is performed for each patient. For statistical analysis, the subjects were divided into the following age groups: 34 – 60 and 61 - 81 years. Computerized tomograms (CT) and 3D reconstructions from 240 sinuses were assessed using Dental CT

CT was performed by using a Tomoscan SR-6000, Philips Medical Systems, Eindhoven, Holland, under the following conditions 1.5 mm slice thickness, 1 mm table feed, 120 kV, ≤25–50 mA/s, 2 s scan time/slice, 512 matrix, native and bone window.

Each observer performed all the measurements separately by using the same computer (17.3-inch diagonal HD+ Bright View LED-backlit Display (1600 x 900), HP Pavilion dv7) and screen resolution (brightness, 300 cd/ m^2; resolution, 1,920 × 1,080).

A screening procedure was engaged to identify the presence or absence of sinus abnormalities using the orthogonal views of coronal, axial and sagittal scans.

We considered deviations from normali-

ty partial or complete opacification of the maxillary sinus and increased thickening of the mucosa greater than 1 mm. We considered cases as having a sinusal abnormality when both examiners identified at least one type of abnormality and excluded cases in which both examiners diagnosed the absence of an abnormality.

The identification of an abnormality was registered separately for the right and left sinuses of each patient. Selected cases were independently reevaluated by the examiners in order to diagnose and classify the cases into different abnormality subtypes, such as: mucosal thickening, retention cysts, opacification, sinus polyps, osteoma and odontogenic cysts. The examiners noted the presence of sinus septa.

Assessment of maxillary sinus septum

Every bony prominence more than 4mm wide or 4mm high was considered because a bony prominence is thought to have an impact during elevation of the sinus floor under local anesthesia. The bony septa were transversal, horizontal, sagittal, or atypical, and were complete or incomplete (partial) (3) (fig. 1).

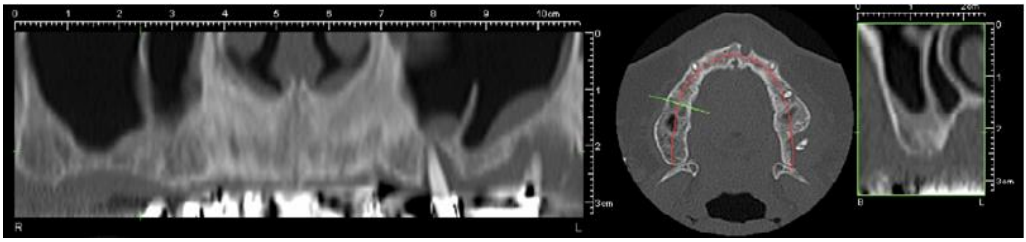

Fig. 1. Bilateral maxillary sinus septum

Assessment of pseudocysts

Whites (3) considers that pseudocysts usually form on the floor of the sinus, although some may form on the lateral walls or the roof. Retention pseudocysts may vary in size from that of a fingertip to completely filling the sinus and making it radiopaque. Retention pseudocysts usually appear as well-defined, non corticated, smooth, dome-shaped radiopaque masses. Because the lesion originates within the sinus, no osseous border surrounds it (fig. 2).

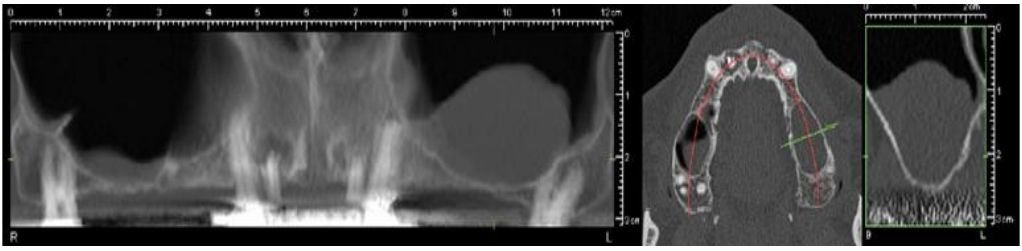

Fig. 2. Left maxillary sinus retention pseudocyst

Assessment of mucosal thickening

The image of thickened mucosa is readily detectable in the radiograph as a non corticated band noticeably more radiopaque than the air filled sinus, paralleling the bony wall of the sinus. Mucosal thickening was

considered present when the thickness of the sinus mucosa was ≥ 1 mm as measured from the floor of the sinus to the highest border of the mucosa(3) (fig. 3).

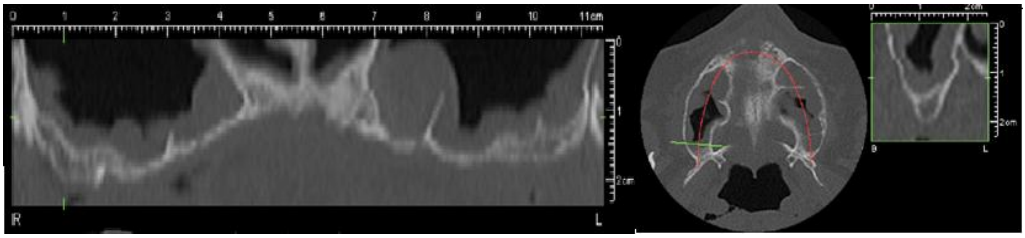

Fig. 3. Bilateral mucosal thickening of the maxillary sinuses

Assessment of sinusitis

Thickening of sinus mucosa and the accumulation of secretions that accompany sinusitis reduce the air content of the sinus and cause it to become increasingly radiopaque. The most common radiopaque patterns that occur are localized mucosal thickening along the sinus floor, generalized thickening of the mucosal lining around the entire wall of the sinus, and near-complete or complete radio opacification of the sinus (3) (fig. 4).

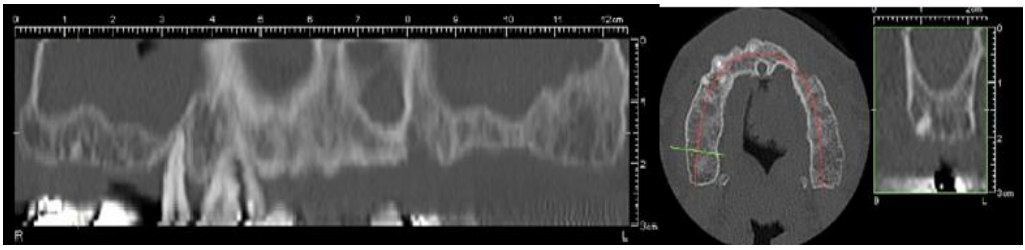

Fig. 4. Bilateral complete opacification of the maxillary sinuses

Assessment of polyps

A polyp may be differentiated from a retention pseudocyst on a radiograph by noting that a polyp usually occurs with a thickened mucous membrane lining because the polypoid mass is no more than an accentuation of the mucosal thickening. In the case of a retention pseudocyst, however, the adjacent mucous membrane lining is not usually apparent (3) (fig. 5).

Fig. 5. Polyps in the right maxillary sinus

Assessment of odontogenic cysts

The most common are radicular cysts, followed by dentigerous cysts and odontogenic keratocysts. A thin radiopaque line divides the contents of the cyst from the sinus cavity. In the case of a radicular cyst, the lamina dura of the involved tooth or teeth is not intact in the apical area (3) (fig. 6).

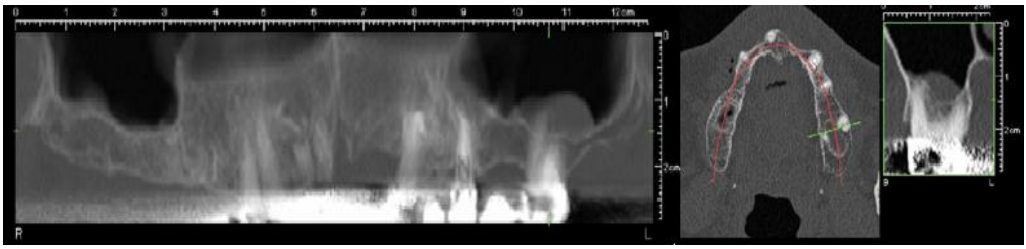

Fig. 6. Odontogenic cyst in relationship with the first left molar

Calibration and reliability

The CT scans were reviewed independently by 2 oral surgeons and 2 maxillofacial surgeons trained by a radiologist with experience of over 1,000 scans at the time of the study. The diagnostic criteria were developed on the basis of the published literature, and in the case of a disagreement, a consensus was reached after a discussion between the observers. Each examiner measured and assessed all the CT images twice.

Statistical analysis

Obtained patient data and scan data were recorded in a *SPSS* database (IBM). Statistical analysis was performed using *SPSS version 20.0* for Windows 7 (IBM). Logistic regression analysis was performed to determine the effects of gender and age on the abnormalities. The level of significance was set at 5%. The chi-square test was used to compare groups. An error probability of <0.05 was accepted as significant.

RESULTS

Mucosal thickening was the most frequently observed abnormality (68.3% of the sinuses). It is generally associated with some kind of irritation, such as odontogenic pathology or allergic phenomena.

Either one or both sinuses of 9 patients showed total opacification, which would suggest an occurrence of sinusitis. However, sometimes opacification can also be found in abnormalities other than sinusitis, such as mechanical trauma, barotraumas and hemorrhage (9). In addition, a final diagnosis of sinusitis may also be considered when clinical signs and symptoms are present and such factors were not evaluated in this study.

Mucous retention cysts were the second most frequently found inflammatory abnormality (15.8% of the sinuses). This result is similar to other studies (9).

4 of these patients showed polypoidal mucosa.

One patient showed an odontogenic cyst in relationship with the first left molar that evolved towards the maxillary sinus, one patient had a tooth in the sinus and another patient had an osteoma (fig.7). These cases were not considered statistically significant.

In most people the maxillary sinus develops as a single space with various compartments. Usually the septa are incomplete,

but in rare cases, complete septa can divide the maxillary cavity. 21.7% of the patients had unilateral maxillary septum and 8.3% had bilateral maxillary sinus septum.

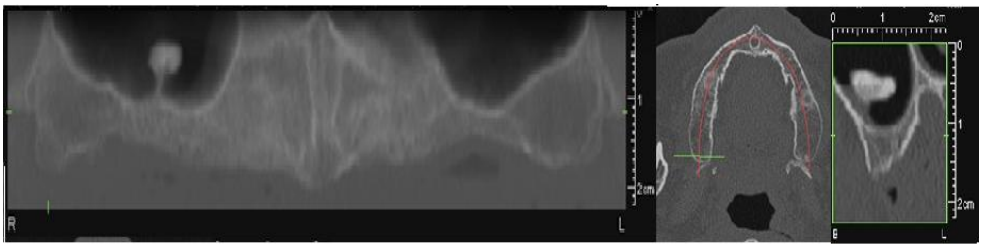

Fig.7. Osteoma in the right maxillary sinus

Pathologic findings in either one or both sinuses were found in 89 patients (74.2%). 49 patients (40.83%) showed pathologic signs in both and 40 patients (59.16%) in either one of their sinus. 31 (25.8%) of the 120 investigated patient scans showed no pathologic signs within either left or right maxillary sinus (tab I).

TABLE I
Findings tabulated for both sinuses, unilateral and bilateral

Findings	Unilateral	Bilateral
Normal	57	31
Mucosal thickening	24	29
Septum	26	10
Pseudocyst	13	3
Complete opacification	7	2
Polyp	2	2
Odontogenic cyst	1	0
Tooth in sinus	1	0
Osteoma	1	0

Out of 67 male patients 44 (65.67%) had at least some pathology in either of the sinuses, whereas only 30 of the 53 investigated scans of female patients (56.6%) showed pathologic signs in either sinus.

Fisher's Exact Test (p=0.029) showed a statistical correlation between gender and pathology. Males tend to present pathology more often (66.2%) than females (54.7%). The logistic regression results showed an association between gender (p=0.028) and pathology. In terms of odds ratio, the odds of having pathology as a male is higher by 62.4%. There was no correlation between age and pathology (p=0.847)

The logistic regression results showed no association between age (p=0.068) and gender (p=0.773) with the maxillary sinus septa for the study group.

DISCUSSION

Several studies have reported a great variability in the prevalence of incidental findings in the maxillary sinuses of asymptomatic subjects when multiplanar images are used. CT scanning studies found abnormalities in approximately 30% of cases (5, 6) and CBCT studies reported a prevalence ranging from 24.6% to 56.3%. Also, gender and age were significantly associated with pseudocysts (p=0.027) and mucosal thickening (p<0.001), respectively (4, 7, 8). In our study, we detected incidental abnormalities in 74.2 % of the cases and no association between age, gender and sinus conditions. Variations in image interpretation and diagnostic criteria and influence of the climate among differences geographical areas can influence abnormality rates (4, 5, 6, 10, 11, 12).

Differences in prevalence found in this study compared with the known literature could be explained by several factors. First, different age and patient groups were in-

vestigated in the various studies. One study investigated 30 patients with paranasal problems determining a prevalence of up to 63% (13), and another study reported on 202 patients having different indications for CT scanning with a prevalence of 82.2% for mucosal abnormalities (14).

Moreover, the definitions for pathologic changes and the applied classification systems vary among the studies. Another factor could be the visualization quality of the different imaging modalities.

Other studies that evaluated the patients about to undergo sinus augmentation observed that 98% were diagnosed with chronic periodontitis, 51% were smokers, and 27% reported a history of symptoms indicative of sinus disease. Eighteen subjects were diagnosed with sinus disease and/or abnormalities (chronic sinusitis, sinus cysts, nasal septum deviation, and ostium stenosis). No significant differences in age, gender, or smoking status between patients with and without sinus conditions were found (15).

The sinus lift technique may be difficult to perform if an aberrant sinus anatomy is encountered during surgical exposure. Previous studies that evaluated the sinus septa found a prevalence of one or more septa per sinus to be 26.5%. Another research team from France found that forty-six subjects (61%) had no bony septa or had septa less than 4 mm (16, 17). A study made on edentulous subjects found a prevalence of maxillary sinus septa of 21.58% and no association between age and gender with the maxillary sinus septa (18).

The present study is limited by several factors. The two observers in this study were not trained radiologists but they were clinically experienced oral and maxillofacial surgeons using CT in clinical routine. Blood and pus appear radiological identical and cannot be discriminated.

Inflammation in the paranasal sinus is a frequent disease and can result from a variety of causes such as infection, chemical irritation, allergy, introduction of a foreign body, or facial trauma. Periodontal disease or rarefying or sclerosing osteitis may cause a localized mucositis in the adjacent floor of the maxillary antrum. Malignancies, also, have to be identified. Incidental findings were the main focus of our study and it provides information on the frequency of anatomic and pathologic findings in the maxillary sinus in CT images of edentulous patients.

CONCLUSIONS

The vast majority of the patients had at least one pathological finding. CT is the most appropriate exam to evaluate the maxillary sinus disease and anatomical variations, before sinus floor elevation.

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CLINICAL EPIDEMIOLOGICAL STUDY ON THE INCIDENCE OF *ESCHERICHIA COLI* INFECTIONS IN THE CANCER PATIENTS ADMITTED TO SURGERY DEPARTMENT II OF THE IASI REGIONAL ONCOLOGY INSTITUTE IN 2013

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CLINICAL EPIDEMIOLOGICAL STUDY ON THE INCIDENCE OF *ESCHERICHIA COLI* INFECTIONS IN THE CANCER PATIENTS ADMITTED TO SURGERY DEPARTMENT II OF THE IASI REGIONAL ONCOLOGY INSTITUTE IN 2013 (Abstract). **The aim** of study was to identify pathogens involved in hospital-acquired or community-acquired infections in patients suffering from various types of cancers. **Material and methods**. A clinical epidemiological study included a group of 355 cancer patients admitted and operated at the Surgery Department II of the Iasi Regional Oncology Institute in 2013 was performed using data from hospital statistical service and the surgery and laboratory records, then processed by epidemiological and statistical methods. **Results**. In cancer patients the pathogen most commonly involved in infections was *E. coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*, *Enterobacter cloacae* and *Acinetobacter*. Analysis of bacterial resistance to antibiotics of the pathogen most commonly involved revealed that of the 29 *E. coli* strains 22 were tested for resistance/sensitivity to ampicillin; of these, 19 strains showed resistance, and only 3 were sensitive; 14 *E. coli* strains of were resistant to cephalosporins and 8 sensitive; all strains tested were sensitive to carbapenems, imipenem or meropenem and polypeptides (colistin); the 10 tested strains showed resistance to monobactams (aztreonam). **Conclusions**. *E. coli* was incriminated in most urinary tract infections, the other pathogens being identified sporadically. Surgical wound infections were caused mostly by *E. coli* and *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*. In our study we encountered increased resistance of *E. coli* to penicillins, cephalosporins, monobactams (aztreonam), fluoro-quinolones and tetracyclines. **Keywords**: HOSPITAL-ACQUIRED INFECTION, CANCER, SURGERY, ESCHERICHIA COLI.

The incidence of infections associated to diseases requiring hospital care varies highly in relation to many factors of which the peculiarities of the underlying disease and

the presence of co morbidities rank first. Not always the medical act itself creates risks for infection. A number of causes or mechanisms may be involved, accounting for the pronounced epidemiological and clinical polymorphism of hospital-acquired infections, and implicitly for the major difficulties in their prevention and treatment.

Many pathogens can cause infections in hospitalized patients, often highly susceptible due to immunosuppression, as often is the case of cancer patients.

The origin of infection can be both endogenous, self-infection, the organisms responsible originating from the patient itself, and exogenous, through various ways and routes of transmission. Hospitalized patients, cancer patients included, are at high risk of infection which can be acquired both during hospital stay, when it occurs some time after admission or after a short or longer period of time after discharge, depending on the incubation period. The pathogens capable of causing infections in cancer patients are most often bacteria resistant and multi-resistant to antibiotics.

Colonization of patients during medical-surgical procedures occurs due to unhygienic lifestyle and as a result of long-term multiple investigations or treatments.

MATERIAL AND METHODS

This clinical epidemiological study included a group of 355 cancer patients admitted and operated at the Surgery Department II of the Iasi Regional Oncology Institute in 2013, of which 53 (14.92%) were detected to have hospital-acquired (43: 12.11%) and community-acquired infections (10: 2.8%). Data were obtained through cooperation with the hospital statistical service, and the study of the surgery and laboratory records. The results were processed and interpreted by epidemiological and statistical methods.

RESULTS AND DISCUSSION

In the 53 cancer patients with infections, the laboratory investigations done on various pathological samples identified 18 bacterial species involved in infectious episodes (tab. I, fig. 1).

In the 53 patients with infection, 93 identifications were obtained because some patients had multiple co-infections and some infections were caused by several pathogens.

In cancer patients the pathogen most commonly involved in infections was *E. coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*, *Enterobacter cloacae* and *Acinetobacter*.

The underlying disease of the patients who contracted hospital-acquired infections with *E. coli* varied widely, the most common being cancers of the rectum (4 cases), lung (3), colon (2), and ovary, uterus, skin, sarcoma, liver, stomach one each.

Surgical wound infections were most often caused by *E. coli* and *Staphylococcus aureus*, 9 identifications each, followed, in order, by *Pseudomonas aeruginosa* (7 identifications), *Klebsiella pneumoniae* (6), *Acinetobacter* (4), *Enterococcus faecalis* (4), *Enterobacter cloacae* (3).

E. coli was incriminated in most urinary tract infections (14 % patients of the 22 patients from whom urine samples were collected had urinary infection with *E. coli*), the other pathogens being identified sporadically.

E. coli, pathogen with an increasing frequency, was found in 9 of the 49 examined wound secretions, equaling *S. aureus*. Of the nine wound samples, 7 were superficial and 2 deep.

TABLE I

Bacterial species involved in the infections detected in cancer patients

No.	Bacterial species	No. of infections	% infections
1.	*Escherichia coli*	29	31.86
2.	*Klebsiella pneumoniae*	16	17.58
3.	*Pseudomonas aeruginosa*	10	10.98
4.	*Staphylococcus aureus*	9	9.89
5.	*Enterococcus faecalis*	5	5.49
6.	*Enterobacter cloacae*	5	5.49
7.	*Acinetobacter*	4	4.39
8.	*Coagulase-negative staphylococcus*	2	2.19
9.	*Morganela morganii*	2	2.19
10.	*Candida albicans*	2	2.19
11.	*Enterococcus faecium*	2	2.19
12.	*Proteus mirabilis*	1	1.09
13.	*Haemophilus spp.*	1	1.09
14.	*Providencia stuartii*	1	1.09
15.	*Streptococcus pyogenes*	1	1.09
16.	*Streptococcus viridans*	1	1.09
17.	*Citrobacter freundii*	1	1.09
18.	*Clostridium difficile*	1	1,09

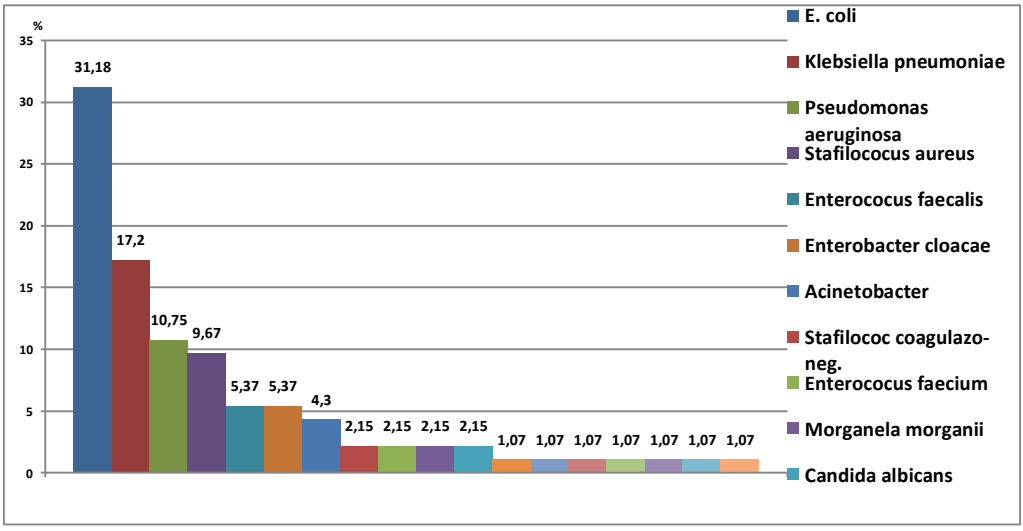


Fig. 1. Frequency of bacterial species involvement in causing infections

TABLE II

Correlations between the examined biological sample and the identified pathogen

Pathogen	Wound secretion		Urine	Catheter	Arterial embolus	Blood culture	Peritoneal fluid	Bronchial aspirate	Bile culture	Copro-culture	Pharyngeal exsudate
Escherichia coli	7s 2p	9	14	1		1	2	1	1		
Klebsiella pneumoniae	5s 1p	6	4	2		1	1	1		1	
Staphylococcus aureus	8s 1p	9									
Pseudomonas aeruginosa	6s 1p	7	1		1	1					
Enterococcus faecalis	3s 1p	4							1		
Enterobacter cloacae	2s 1p	3	1						1		
Acinetobacter	3s 1p	4									
Coagulase-negative staphylococcus				2							
Candida albicans			1	1							
Morganela morganii	1s 1p	2									
Proteus mirabilis	1s	1									
Clostridium difficile										1	
Streptococcus viridans	1p	1									
Haemophilus spp.							1				
Providencia stuartii	1s	1									
Enterococcus faecium	1s	1	1								
Streptococcus pyogenes											1
Citrobacter freundii	1p	1									

s=superficial; p=deep

E. coli caused a total of 29 hospital-acquired infections, being also isolated from blood cultures, bile culture, peritoneal fluid, and catheter.

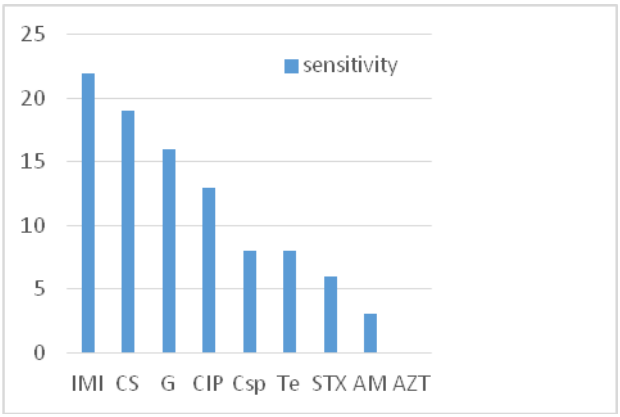
Analysis of bacterial resistance to antibiotics of the pathogen most commonly involved in infections in our cancer patients revealed the following (tab. III): of the 29 *E. coli* strains 22 were tested for resistance/sensitivity to ampicillin; of these, 19 strains showed resistance, and only 3 were sensitive; 14 *E. coli* strains of were resistant to cephalosporins and 8 sensitive; all strains tested were sensitive to carbapenems, imipenem or meropenem and polypeptides (colistin) (22 to carbapenems and 19 to colistin, respectively); the 10 tested strains showed resistance to monobactams (aztreonam).

The 2012 antibiotic use report of the Institute of Public Health, based on data obtained by IMS Health and published on its website, *E. coli* resistance to cephalosporins (in agreement with the result of our study), fluoroquinolones and aminoglycosides is mentioned. In our study, the strains circulating in the Iasi Regional Cancer Institute showed sensitivity to aminoglycosides.

TABLE III

Analysis of bacterial sensitivity to antibiotics

Antibiotic	Tested strains of *E. coli*	Sensitive strains *E. coli*	
		No.	%
Ampicillin	22	3	
Cephalosporins	22	8	
Carbapenems – imipenem, meropenem	22	22	
Monobactams - Aztreonam	10	0	
Gentamicin	22	16	
Tetracycline/doxycycline	21	8	
Ciprofloxacin	21	13	
Colistin	19	19	
Sulfamethoxazole	9	6	



Legend
Ampicillin - AM
Cephalosporins -Csp
Imipenem - IMI
Aztreonam -AZT
Gentamicin - G
Tetracycline/doxycycline - Te
Ciprofloxacin - CIP
Colistin - CS
Sulfamethoxazole – STX

Fig. 2. Sensitivity to antibiotics of *E. coli* strains isolated from cancer patients with infections

CONCLUSIONS

Our research showed that the pathogen most commonly involved in nosocomial infections in the studied cancer patients was *E. coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterobacter cloacae*, and *Acinetobacter*.

E. coli was incriminated in most urinary tract infections, the other pathogens being identified sporadically. Surgical wound infections were caused mostly by *E. coli* and *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*. In our study, we encountered increased resistance of *E. coli* to penicillins, cephalosporins, monobactams (aztreonam), fluoro-quinolones and tetracyclines. The tested *E. coli strains* were 100% susceptible to carbapenems and polypeptides. Of the 22 *E. coli* strains tested for resistance/ sensitivity to aminoglycosides, 16 were sensitive to gentamicin, and 6 exhibited resistances to it, but showed sensitivity to amikacin.

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PERSONALITY PROFILES AND RISK DIET BEHAVIORS – A CASE-CONTROL STUDY ON TEENAGERS FROM TIMIS COUNTY, ROMANIA

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PERSONALITY PROFILES AND RISK DIET BEHAVIORS – A CASE-CONTROL STUDY ON TEENAGERS FROM TIMIS COUNTY, ROMANIA (Abstract): In the study we conducted we aimed at investigating the relation between personality profiles and risk diet behaviors in teenagers. This study was a case-control one and we applied 2 questionnaires (Freiburg Personality Inventory – FPI with 212 items) and CORT 2004 (items Q94-Q116 of diet behavior) on a sample of 2908 teenagers (51.5% girls and 48.5% boys). Cronbach`s alpha index was 0.802 for FPI and 0.730 for items Q101-Q109 of CORT. Personality profiles were built by an Excel 2003 Program. Statistical analysis was realized with SPSS 16 program applying Chi square ($\chi^2$) and gamma ($\gamma$) correlation. Personality features of teenagers` with high and without risk diet behavior were analyzed. Results obtained: personality profiles and statistical results indicated the existence of a significant statistical difference of aggressiveness and domination between teenagers with high and no consumption of butter and/or lard (Q103) (χ^2=6.872, Sig. 0.032 and χ^2=6.922, Sig. 0.031 respectively), of juices from the market (Q106) (χ^2=9.055, Sig. 0.011 and χ^2=14.571, Sig. 0.001 respectively). Aggressiveness correlated with consumption of fried potatoes (Q109) (χ^2=6.144, Sig. 0.046) too. Correlation γ indicated direct proportional relations of aggressiveness with: Q103 (γ=0.215, Sig. 0.017), Q106 (γ=0.224, Sig. 0.004), Q109 (γ=0.242, Sig. 0.012); and of domination with: Q103 (γ=0.234, Sig. 0.008), Q106 (γ=0.073, Sig. 0.000). In conclusion, there is a direct proportional relation between consumption of: butter/lard, juices from the market and teenagers` aggressiveness, domination; and a similar relation between consumption of fried potatoes and aggressiveness. **Key words:** PERSONALITY PROFILES, TEENAGERS, RISK DIET BEHAVIORS

Risk diet behaviors met in teenagers represent the rule rather than exception (1). Prevention strategies consist in healthy nutritive behavior promotion in schools; like fresh fruits or bread and milk consumption (2). On the other hand personality profiles established for the teens` age group present specific personality features, like high nervousness, high aggressiveness, high excitability and high domination (1).

In our study we aimed at investigating the relationship between teenagers` personality profiles and their diet behaviors with high risk and no risk, in the Timis County, Romania.

MATERIAL AND METHOD

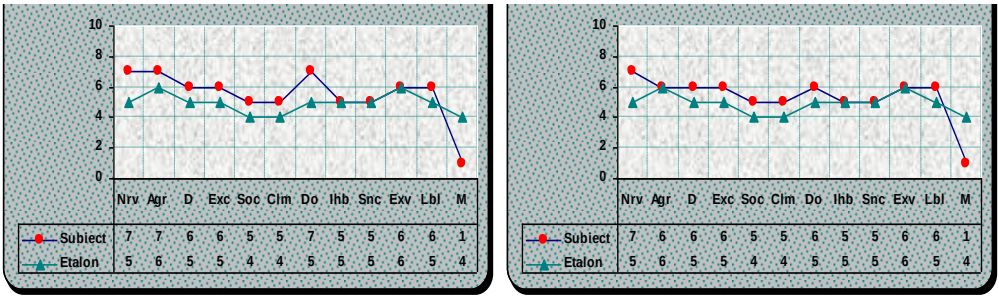
The study was performed on a statistically representative (2908 teenagers, with ages between 15 and 19 years) and homogenous (51.5% girls and 48.5 % boys) sample, provided by secondary schools from the urban area of the Timis county (Timisoara, Lugoj and Deta). The sample was stratified in nests, the sample unit being the class group. This study was a case-control one and it consisted of applying 2 questionnaires: Freiburg Personality Inventory (3) (212 items with Cronbach`s alpha index = 0.802) normalized for Romanian population and CORT 2004 questionnaire (1) (23 items of diet behavior with Cronbach`s alpha index 0.551; Q101-109 with Cronbach`s alpha index = 0.730). The 2 questionnaires were administered individually and anonymously. Work time was 60 minutes. The answering proportion was 74.6%. Depending on risk level we found 5 groups of teenagers, and chose for study the groups with risk 0 (control) and 4 (case with highest level of risk).

We established and compared the personality profiles of the 2 groups of teenagers for certain foods found with high risk: butter/lard (Q103), juices from the market (Q106) and fried potatoes consumption (Q109). Personality profiles consisted in

Nervousness (Nrv), Aggressiveness (Agr), Depression (D), Excitability (Exc), Sociability (Soc), Calm (Clm), Domination (Do), Inhibition (Inh), Sincerity (Snc), Extraversion (Exv), Emotional lability (Lbl). They were built by an Excel 2003 Program and considered the median frequency value of all the 9 ranks of each personality dimension intensity. Statistical work was performed with SPSS 16 Program. In order to avoid small number of cases (under five) we reduced the 9 intensity ranks of the personality profiles to 3 (1 = 1-3 - low intensity; 2 = 4-6 - the average and 3 = 7-9 - high intensity of the feature).

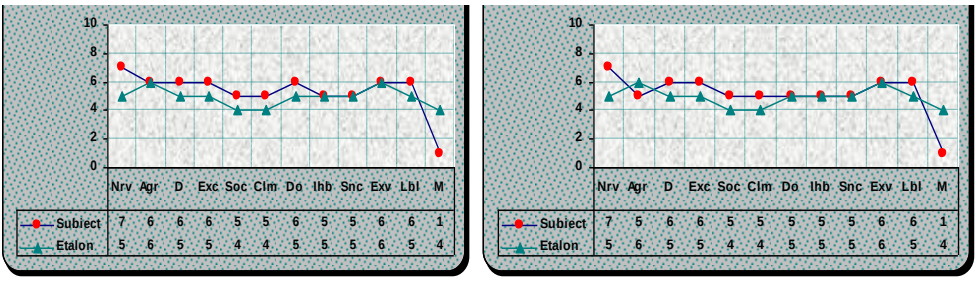
RESULTS

When we analyzed comparatively the personality profiles of the teenagers with high and no risk diet behaviors we obtained the following profiles for: **Q103** (consumption of butter and/or lard, Q103-4 high risk behavior and Q103-0 no risk behavior) (fig.1); **Q106** (consumption of juices from the market, Q106-4 high risk behavior and Q106-0 no risk behavior) (fig.2); **Q109** (consumption of fried potatoes, Q109-4 high risk behavior and Q109-0 no risk behavior) (fig. 3).



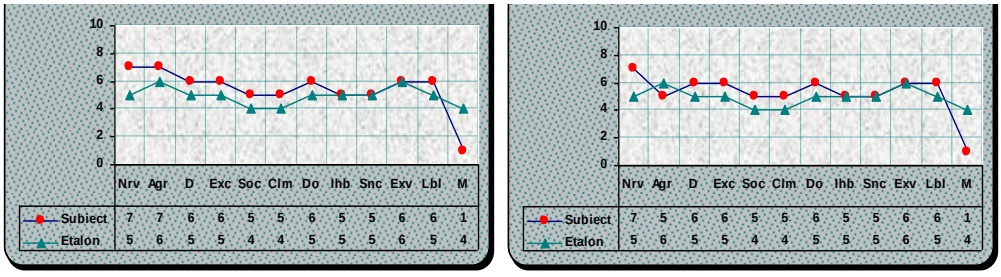
Q103-4 **Q103-0**

Fig. 1. The personality profiles of teenagers with high and no consumption of butter and / or lard



Q106-4 **Q106-0**

Fig. 2. The personality profiles of teenagers with high and no consumption of juices from the market



Q109-4 **Q109-0**

Fig. 3. The personality profiles of teenagers with high and no consumption of fried potatoes

Applying chi square test (χ^2) we found a significant statistical difference between the two personality profiles referring to aggressiveness and domination as personality features in teenagers with high and no consumption of butter and/or lard (Q103) (χ^2=6.872, Sig. 0.032 and χ^2=6.922, Sig. 0.031 respectively) and high and no consumption of juices from the market (Q106) (χ^2=9.055, Sig. 0.011 and χ^2=14.571, Sig. 0.001 respectively) (tab. I). Aggressiveness was different for personality profiles of teenagers which consumed or not fried potatoes (Q109) (χ^2=6.144, Sig. 0.046) (tab. I). Correlation gamma (γ) indicated direct proportional relations: aggressiveness – Q103 (γ=0.215, Sig. 0.017), aggressiveness – Q106 (γ=0.224, Sig. 0.004), aggressiveness – Q109 (γ=0.242, Sig. 0.012), domination – Q103 (γ=0.234, Sig. 0.008, domination – Q106 (γ=0.073, Sig. 0.000).

DISCUSSION

Previous researches identified the utility of personality questionnaires on the adolescents` personality in correlation with their dietary behavior (2). Freiburg Personality Inventory is an efficient tool in building individual personality profiles valuable in clinical diagnosis and in characterizing healthy individuals (3, 4). In our research studies we discovered the possibility to use FPI in building personality profiles specific to an age group (e.g. teenage) (1). Risk diet behaviors in teenagers are well known and frequently investigated in many studies.

TABLE I

Comparative analytical data (A), χ^2 test (B) and γ correlation (C) for Q103, Q106 and Q109 in relation with Aggressiveness, Domination and their statistical significance

A				Aggressiveness			Domination		
			1	2	3	1	2	3	Total
Q103	0	Count	130	558	456	128	573	433	1144
		Expected count	127.7	547.4	468.9	122.2	567.4	454.3	1144
		Residual	2.3	10.6	-12.9	5.8	5.6	-11.3	
	4	Count	10	42	58	6	49	55	110
		Expected count	12.3	52.6	45.1	11.6	54.6	43.7	110
		Residual	-2.3	-10.6	12.9	-5.8	-5.6	11.3	
Total		Count	140	600	514	134	622	498	1254
		Expected count	140	600	514	134	622	498	1254
Q106	0	Count	26	91	62	30	93	56	179
		Expected count	18.1	84.7	76.2	19.5	85.8	73.7	179
		Residual	7.9	6.3	-14.2	10.5	7.2	-17.7	
	4	Count	40	218	216	41	220	213	474
		Expected count	47.9	224.3	201.8	51.5	227.2	195.3	474
		Residual	-7.9	-6.3	14.2	-10.5	-7.2	17.7	
Total		Count	66	309	278	71	313	269	653
		Expected count	66	309	278	71	313	269	653
Q109	0	Count	23	93	69				185
		Expected count	18.9	86.7	79.4				185
		Residual	4.1	6.3	-10.4				
	4	Count	11	63	74				148
		Expected count	15.1	69.3	63.6				148
		Residual	-4.1	-6.3	10.4				
Total		Count	34	156	143				333
		Expected count	34	156	143				333

B	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square Q103 - Aggressiveness	6.872(a)	2	0.032
Pearson Chi-Square Q103 - Domination	6.922(a)	2	0.031
Pearson Chi-Square Q106 - Agressiveness	9.055(a)	2	0.011
Pearson Chi-Square Q106 -Domination	14.571(a)	2	0.001
Pearson Chi-Square Q109 - Aggressiveness	6.144(a)	2	0.046

[a] 0 cells (,0%) have expected count less than 5. The minimum expected count is 12.28 and 11.75 respectively for Q103; the minimum expected count is 18.09 and 19.46 respectively for Q106;
The minimum expected count is 13.33 for Q109.

C		Value	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
Ordinal by Ordinal Q103- Aggressiveness	Gamma	0.215	0.089	2.391	0.017
Ordinal by Ordinal Q103 - Domination	Gamma	0.234	0.086	2.65	0.008
Ordinal by Ordinal Q106- Aggressiveness	Gamma	0.224	0.075	2.896	0.004
Ordinal by Ordinal Q106-Domination	Gamma	0.281	0.073	3.685	0.000
Ordinal by Ordinal Q109 - Aggressiveness	Gamma	0.242	0.094	2.513	0.012

[a]Not assuming the null hypothesis [b] Using the asymptotic std. error assuming the null hypothesis.

Special programs were created in order to change a risk diet behavior into a healthy one (5, 6).

In our study we analyzed together behaviors determined by personality features and diet behaviors.

Teenagers with high risk diet behaviors (7) presented different personality profiles in comparison to teens without these behaviors. When we analyzed the statistical difference (chi square) and gamma correlation we found significant values. Certain diet behaviors (consumption of butter or lard – Q103, consumption of juices from the market – Q106 and consumption of fried potatoes Q109) proved to be connected with teenager aggressiveness and/or domination.

A limit of our study was work with ordinal variables which diminished the possibilities of statistical analysis and of prediction of diet behaviors.

CONCLUSIONS

There is a direct proportional relation between consumption of: butter/lard, juices from the market and teenagers' aggressiveness, domination; and a similar relation between consumption of fried potatoes and aggressiveness.

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SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SOME NEW THIAZOLIDIN-4-ONE DERIVATIVES

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SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SOME NEW THIAZOLIDIN-4-ONE DERIVATIVES (Abstract): **Aim:** To design new thiazolidin-4-ones derivatives and to evaluate their potential antioxidant effects using *in vitro* methods. **Material and methods:** New ethyl esters of the 2-(R-phenyl)-4-oxo-thiazolidin-3-yl propionic acid were synthesized using "one step reaction" between different aromatic aldehydes, thioglycolic acid and beta-alanine ethyl ester hydrochloride. The antioxidant potential of the synthesized compounds was evaluated using the DPPH radical scavenging assay and phosphomolybdenum method. **Results:** Eight thiazolidine-4-one derivatives were obtained in good yields and high purity. The structure of the synthesized compounds was confirmed using IR spectroscopy. The evaluation of antioxidant activity showed that 2-[(4-NO_2)-phenyl]-4-oxo-thiazolidin-3-yl propionic acid ethyl ester (compound 16) was the most active compound. For this derivative the DPPH radical scavenger activity (I% = 91.63%±0.77) and the total antioxidant capacity (absorbance = 1.0691±0.0763) were similar with that of ascorbic acid used as standard antioxidant. **Conclusions:** The antioxidant activity of the thiazolidine-4-one derivatives depends on the nature of the phenyl ring substituents, the NO_2 and OH radicals having the most significant influence. **Keywords:** SYNTHESIS, THIAZOLIDIN-4-ONE, ANTIOXIDANT ACTIVITY.

The concern to design thiazolidine derivatives started in 1928 when penicillin was discovered, and since then the development of new derivatives kept increasing. So far, the thiazolidin-4-ones derivatives had been found to posses various biological actions: anti-HIV1 (1, 2), antibacterial (3, 4), antitubercular (5), antioxidant (6, 7), anti-inflammatory (3, 8) and antitumoral (7, 9, 10).

On the other hand, it is known that oxidative stress is involved in the occurrence and development of many diseases, there are more than 200 diseases caused by oxidative stress and 39 deadly diseases for which there is evidence validating their positive link with oxidative stress. Antioxidants inhibit the oxidative damage induced by radicals and reactive oxygen species (ROS) (11). Thus, the therapy with antioxidants has gained ground in the last years.

Furthermore, commercial availability of

a drug is depending on the cost of production line. A simple and efficient method, a small number of synthetic steps or selectivity in a reaction of many components is desirable for obtaining the drug.

The aim of this study was the synthesis of new thiazolidin-4-one derivatives using an one- step reaction ("domino" reaction) and evaluation of their potential antioxidant effects.

MATERIAL AND METHODS

Reagents. Aromatic aldehyde (4-bromo/4-fluoro/4-chloro/2-methoxy/4-methoxy/4-nitro/2-hydroxi/4-hydroxi-benzaldehyde), thioglycolic acid, beta-alanine ethyl ester hydrochloride, *N,N*-diisopropyl-ethylamine (DIEA), and standard reagents for antioxidant assays were purchased from Sigma Aldrich or Flucka. The toluene was freshly distilled and other solvents were used without further purification. TLC silica gel 60 F_{254} from Merck was used to monitor the reactions.

Methods

Chemistry. The aromatic aldehydes (**1-8**), beta-alanine ethyl ester hydrochloride (**9**) and thioglycolic acid (**10**) were mixed in the presence of *N,N*-diisopropyl-ethylamine (DIEA) used as catalyst. The reaction was carried out by adapting similar methods used for synthesis of other thiazolidine derivatives (12).

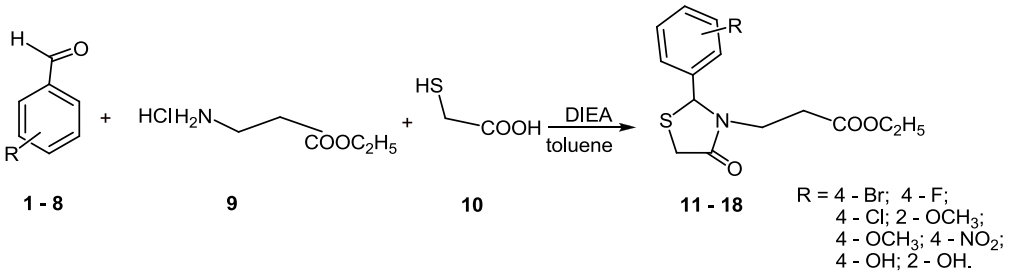


Fig. 1. The synthesis of new ethyl esters of the 2-(R-phenyl)-4-oxo-thiazolidin-3-yl propionic acid

General procedure for synthesis of thiazolidin-4-one derivatives. To beta-alanine ethyl ester hydrochloride (0.01 mol) in 10 ml toluene the aromatic aldehyde (0.015 mol) was added. The mixture was stirred for 5 min and then mercaptoacetic acid 98% (0.02 mol, 1.25 mL) was added. The mixture was stirred again for 5 min and after that *N,N*-diisopropyl-ethylamine (DIEA) (0.013 mol, 2.26 mL) was added and then the mixture was heated to 110-115°C under reflux for 24-30 h under argon. The water was removed by colleting it in a Dien Stark apparatus. The reaction was monitored by TLC using as eluent ethyl-acetate/petroleum ether = 4:6.

The reaction mixture was neutralized with saturated solution of sodium bicarbonate and the organic phase was extracted using ethyl acetate (2 x 25 mL). The organic layer was successively washed with 1M HCl solution and saturated sodium chloride solution, dried over anhydrous $MgSO_4$ and finally filtrated. The filtrate was evaporated and the residue was passed through a silica gel column.

Physico-chemical characterization

Fourier transform infrared (FTIR) spec-

tra were recorded on a Nicolet iS50 FT-IR Spectrometer. All spectra were recorded in the 4000-600 cm^{-1} range at resolution of 4 cm^{-1} over 16 scans. Spectroscopy data were processed with an Omnic Spectra Software. The value of logP (logarithm of partition coefficient between n-octanol and water) as a measure of compound hydrophilicity was calculated using Osiris Property Explorer software (clogP)

Antioxidant assays

The total antioxidant capacity of the tested compounds was evaluated using the phosphomolybdenum method according to the standard procedure of Pietro *et al.* (1999) with some modifications. Briefly, the sample solution (300 µL, 5 mg/mL in methanol) was mixed with 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM amonium molybdate). The samples were incubated at 95°C for 90 min. After the mixture has cooled to room temperature, the absorbance of samples was measured at 695 nm against a blank (300 µL methanol mixed with 3 mL reagent solution) using the GBC-Cintra 2020 UV-VIS Spectrophotometer. Ascorbic acid was used as standard antioxidant.

The DPPH radical scavenging activity. DPPH is a stable nitrogen-centered free radical that is scavenged by antioxidants, changing its color from violet to yellow upon reduction by either the process of hydrogen or electron donation. The sample solution (300 µL, 10 mg/mL in methanol) was added to 2.7 mL solution of DPPH in methanol (dilution 1% from a solution of 0.043%).

The mixture was left for 30 min in the dark and then absorbance was measured at 515 nm against a blank using the UV-VIS spectrophotometer. Ascorbic acid was used as standard antioxidant.

The scavenging capacity was calculated according to the following equation:

$I\% = (Abs_{t=0} - Abs_{t=30min}/Abs_{t=0}) \times 100$

where:

$Abs_{t=30min}$ is the absorbance of the tested compound after 30 minutes.

$Abs_{t=0}$ is absorbance of DPPH methanol solution without sample.

Statistical analysis. The antioxidant assays were carried out in triplicate. Data were analyzed by the analysis of variance (ANOVA) ($p < 0.05$) and were expressed as means ± SD.

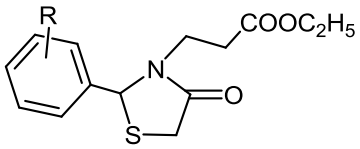
RESULTS AND DISCUSSION

Chemistry. The physical characteristics of the compounds are presented in Table I. The compounds looked like colorless or yellow liquids, excepted for the compound (17) that was solid. The value of *clogP* calculated using a free software showed a high lipophilicity (value of the *clogP* is more than 2.5) for thiazolidin-4-one compounds substituted on aromatic ring with 4-Br (*clogP* = 2.76), 4-Cl (*clogP* = 2.67) and 4-NO_2 (*clogP* = 2.60).

The chemical structure of the compounds was confirmed using FT-IR spectroscopy (tab. II). In the IR spectra the absorption band of CO from ester group appeared in the range of 1726-1728.87 cm^{-1}. The thiazolidine ring was identified by the bands characteristic of CO group (1673-1676 cm^{-1}) and C-S bond (622-644.5 cm^{-1}). The characteristic bands of aromatic ring appeared in the range of 2979-2981 cm^{-1} and the phenyl ring substituents appeared at: 1155.62 cm^{-1} (C-F), 767.93 (C-Cl), 1520.4 and 1344.87 cm^{-1} (NO_2), 3270.59 (O-H), 3233.78 (O-H). The C-Br bond had a absorption less than 650 cm^{-1}.

TABLE I

Physical characteristics of thiazolidin-4-one derivatives



Comp.	R	Physical characterization					clogP
		Molecular Formula	Molar Mass	Aspect	Yield (%)	Rf*	
11	4-Br	$C_{14}H_{16}BrNO_3S$	358.25	light yellow liquid	67%	0.64	2.76
12	4-F	$C_{14}H_{16}FNO_3S$	297.08	colorless liquid	42%	0.62	2.12
13	4-Cl	$C_{14}H_{16}ClNO_3S$	313.80	colorless liquid	46%	0.61	2.67
14	4-OCH_3	$C_{15}H_{19}NO_4S$	309.38	light yellow liquid	71%	0.50	1.95
15	2-OCH_3	$C_{15}H_{19}NO_4S$	309.38	yellow liquid	82%	0.51	1.95
16	4-NO_2	$C_{14}H_{16}N_2O_5S$	324.35	yellow liquid	54%	0.47	2.60
17	4-OH	$C_{14}H_{17}NO_4S$	295.35	white solid**	24%	0.2	1.76
18	2-OH	$C_{14}H_{17}NO_4S$	295.35	brown yellow liquid	37%	0.3	1.76

Rf*: ethylacetate: petroleum ether = 4:6; white solid**: m.p = 98°C

TABLE II

IR Spectral characteristic bands of thiazolidin-4-one derivatives

Comp.	R	IR characteristic band (cm^{-1})				
		Ar-H	C=O ester	C=O Ring	C-S	R
11	4-Br	2979.88	1727.42	1674.19	625.07	<650 (Br)
12	4-F	2981.63	1727.38	1674.28	636.25	1155.62 (F)
13	4-Cl	2980.48	1727.40	1674.06	622.07	767.93 (Cl)
14	2-OCH_3	2979.74	1727.16	1674.34	644.94	2838.23 (C-H)
15	4-OCH_3	2979.52	1728.11	1673.79	628.68	2837.11 (C-H)
16	4-NO_2	2981.11	1726.37	1675.79	631.98	1520.4; 1344.87 (NO_2)
17	4-OH	2980.25	1727.87	1650.39	632.68	3270.59 (OH)
18	2-OH	2980.80	1728.72	1645.82	640.57	3233.78 (OH)

Antioxidant assays.

The total antioxidant capacity. The assay for total antioxidant capacity was based on the reduction of Mo^{+6} to Mo^{+5} by the tested

compounds followed by formation of a green phosphate/Mo^{+5} complex at acid pH. The results of the tested compounds are listed in table III. The most active compound was thiazolidin-4-one derivative with NO_2 on the phenyl ring (compound 16), for which the absorbance (1.0691±0.0763) was similar with that of ascorbic acid used as reference (1.00±0.4052). The compound (11) with Br as substituent on aromatic ring also had important activity (0.8467±0.0406), the value of the absorbance being comparable with that of ascorbic acid.

The DPPH radical scavenger activity. The results of the DPPH radical scavenger assay are presented in fig. 2. The most active compound was compound 16 with NO_2 as substituent on aromatic ring for which the inhibition (91.63%±0.77) was very close to that of ascorbic acid (AA) (99.01 ± 0.32). Compounds 17 and 18 with OH radical on aromatic ring also showed important scavenger activity, the inhibition exceeding 60%.

TABLE III

Absorbance of the tested thiazolidin-4-one derivatives (5 mg/ml)

Sample	R	Absorbance	Sample	R	Absorbance
11	4-Br	0.8467±0.0406	15	4-OCH_3	0.4622±0.0221
12	4-F	0.5408±0.0171	16	4-NO_2	1.0691±0.0763
13	4-Cl	0.3980±0.0124	17	4-OH	0.4955±0.0119
14	2-OCH_3	0.6006±0.0519	18	2-OH	0.47283±0.003
Ascorbic acid (AA)*			1.00±0.4052		

*Ascorbic acid 0.125mg/mL

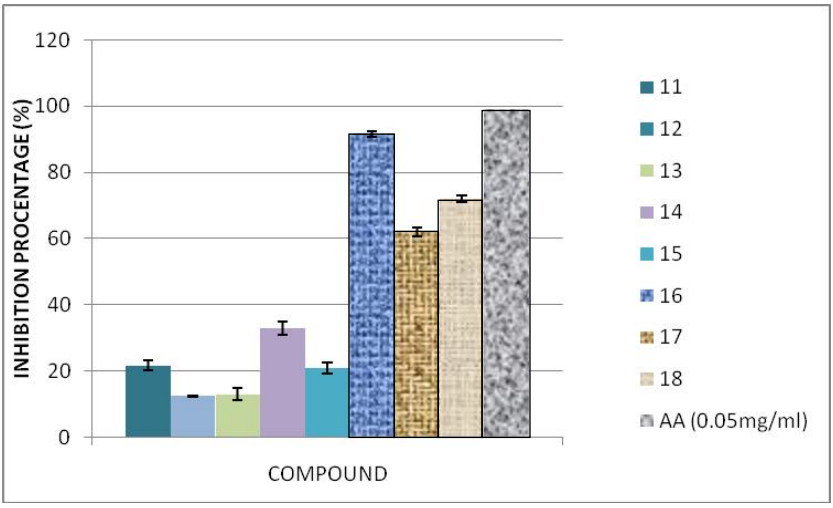


Fig. 2. Antioxidant activity – DPPH scavenger radical

CONCLUSIONS

Some new 1,3-thiazolidin-4-one derivatives have been synthesized. The synthesized compounds have been physically characterized (yield, molecular formula, molecular weight, solubility in different organic solvents, clogP) and their chemical structure was confirmed by FT-IR spectroscopy. The

antioxidant potential of the synthesized compounds has been evaluated using the DPPH radical scavenger assay and phosphomolybdenum method. The results suggest that the most active compound is 2-[(4-NO_2)-phenyl]-4-oxo-thiazolidin-3-yl propionic acid ethyl ester (compound 16) the DPPH radical scavenger activity and total antioxidant capacity of which was comparable to that of ascorbic acid.

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CONTRIBUTIONS REGARDING THE LEAF HISTO-ANATOMY OF SOME PELARGONIUM SPECIES

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CONTRIBUTIONS REGARDING THE LEAF HISTO-ANATOMY OF SOME PELARGONIUM SPECIES (**Abstract**): *Pelargonium* L. genra (Fam. *Geraniaceae*) includes over 250 species, most of it native to southern Africa. Nowadays, four perennial species are cultivated in Romania as ornamental plants. Our **aim** was to establish the main characteristics and differences that occur between *Pelargonium zonale, P. hispidum, P. grandiflorum, P. peltatum* and *P. radens*. All in all, the quantitative differences (regarding the leaf-stalk, blade and trichomes) have a taxonomical value for all five species included in the research. **Materials and methods:** The plant material was represented by fresh leaves harvested from 5 species of *Pelargonium*: *P. peltatum* (L.) Aiton, *P. radens* H.E. Moore, *P. grandiflorum* (Andrz.) Willd., *P. hispidum*. (anthesis phase) and preserved in ethanol 70%. Using a dissecting microscope, sections were made through leaf blade surface and cross sections of/ for leaf stalk and blade. **Results:** The common characteristics of the leaf is the palmatilobate blade with long petiole. Although many studies regarded glandular trichomes from *Pelargonium* leaves, most of the leaf structures are still unknown. The most important for the quality and quantity of essential oil extracted from *Pelargonium sp.* are trichoms. **Keywords**: *PELARGONIUM SPECIES*, ANATOMY, MEDICINAL PLANTS, DECORATIVE PLANTS.

Pelargonium genre represents a large number of species that were brought to Europe from southern Africa. Species of Pelargonium have long been cultivated due to their attractive scents and bright colors (1).

At the beginning the most common were the species with red flowers. Ever since, their popularity increased slowly along with the horticultural enhancement until 1830. The abundant foliage form and color were especially valued.

Starting with 1870 horticultural improvement of pelargonium reaches the highest peak (a larger variety of colors, flowers, forms and a longer flowering season).

Nowadays, the majority of pelargonium species stand out through vigorous plants, with variated colors (red, scarlet, white, pink, purple etc.), fragrant, simple, double and sometimes very large flowers (2). It is among the top 20 aromatic plants that are particularly rich in essential oils; therefore most of the chemical studies were concentrated on this fraction.

Our study goal was to establish the existent hysto-anatomical differences between five species of *Pelargonium* considering that, such variations will be notable

in the chemical composition of the volatile oil. Therefore, the emphases of this paper especially regarded the structure, place and density of the glandular trichomes.

The present research is part of a larger study, the chemical analysis and biological potential of the essential oil will be included in a following paper.

MATERIAL AND METHODS

1. Plant material. The plant material was represented by fresh leaves harvested from 5 species of *Pelargonium*: *P. peltatum* (L.) Aiton, *P. radens* H.E. Moore, *P. grandiflorum* (Andrz.) Willd., *P. hispidum*. (anthesis phase) and preserved in ethanol 70%. The plants were cultivated in the greenhouse of the Botanical Gardens in Iaşi.

2. Preparation of the microscopic sections. Using a dissecting microscope, sections were made through leaf blade surface and cross sections of/ for leaf stalk and blade. The microscopic preparations were colored with iodine green and ruthenium red and then, analyzed with NOVEX (Holland) photonic microscope. The photographies were taken with Olympus photo camera.

RESULTS AND DISCUSSION

Petiole. Cross sections did not show significant discrepancies between the basis and the top of the leaf blade. It was noted that the number of conducting fascicles was higher at the basis than for the rest of the leaf. Thus, in accordance with other researchers (3,5) we analyzed sections from the middle of the leaves.

The cross section had a circular shape excepting *P. Radens* and *P. grandiflorum* with semicircular-elyptic shape and the plane adaxial surface, whereas the second species has the thickest leaf stalk (4).

Epidermis consisted of isodiametric cells with external and internal wall of cells slightly thicker than the lateral ones; the external wall was couvered by a thin cuticle.

External collenchymatous parenchyma was typically lacunar, hypodermic cell layer was much smaller and tangentially collenchymatised. The number of cell layers in the anatomical area ranges from 4 to 6 and only the external parenchyma of *P. hispidum* is thicker (8-9 layers), many cells containing a single or a few crystals of calcium oxalate.

In the epidermis of the 5 species included in the study glandular and surface trichomes were found; their number, thickness and dimensions variates from one species to another (tab.I, fig.1-5).

Sclerenchyma ring bylayer is thicker (three rows) near the vascular bundle; the polygonal cells present thickend walls that are lignified. *P. hispidum* presented sclerenchyma (2-4 rows), with fibres that have thick and lignified walls and very large cells, whereas the structure of *P. grandiflorum* sclerenchyma was still primary with weak lignified walls.

Lacunar parenchyma was uncolored with bigger cells than those of the external layer, especially in the median part of the petiole. It was noted that the cells from *P.hispidum* sample contained 1-2 oxalate druses similar to external parenchyma (5).

The vascular bundles bear both xylem and phloem vessels next to the sclerenchymatic fibers. For all five species, the vascular fascicles are in great number and various dimensions alternately, the biggest of them representing central isles in the parenchyma. On the external layer phloem consisted of sieve elements, companion cells and few

large parenchyma cells, whereas the xylem (the vessels disorderly dispersed with parenchyma cells) was situated inside the bundle. Internal layer of xylem had a group of small polygonal cells with moderately thickened walls (tab. II).

TABLE I

Structural differences in petiole epidermis

Species	Surface trichomes		Glandular trichomes	
	Number	**Characteristics**	**Number**	**Characteristics**
Pelargonium peltatum	Dense	- uni/bicellular - long	Sparce	-short: -one basal cell -basal podium (2-3 short cells overlapping) -unicellular gland
Pelargonium zonale	Sparce	- long, thin şi thick - multicellular bunt tipped	Dense	-short: basal podium (2-4 cells) -long: basal podium (3-4 sharp point cells) -globular or pear-shaped gland
Pelargonium radens	Very dense	- short - unicellular - long – bicellular; thick	Dense	-short: basal podium (2-4 cells); globular gland
Pelargonium hispidum	Abundant	- uni/bicellular; rarely with 3 cells - variable dimensions	Dense	-short -medium: basal podium (3-4 cells) -large globular gland
Pelargonium grandiflorum	Very dense	- unicellular - variable dimensions -pointed tip	Very dense	- short: basal podium (3-4 cells) - pear-shaped gland

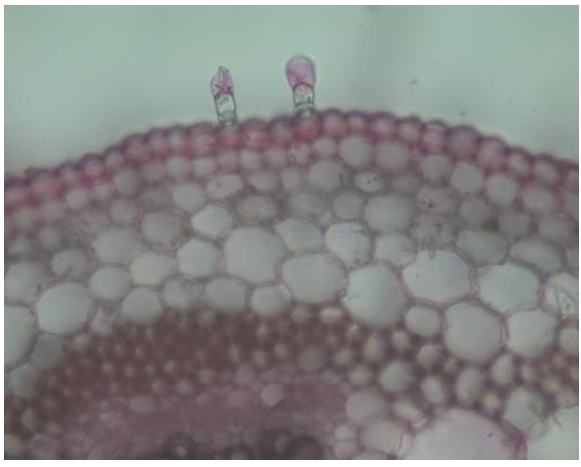

Fig.1. *P. radens* petiol

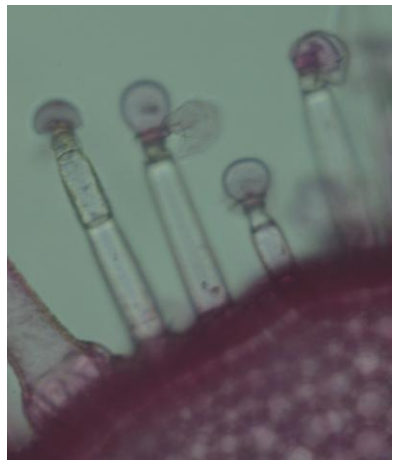

Fig.2. *P. hispidum* petiol

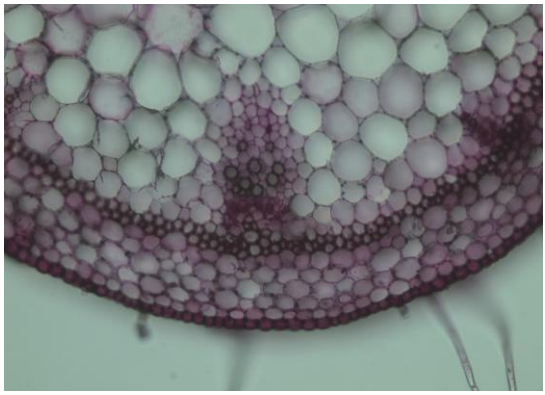

Fig.3. *P. peltatum* petiol

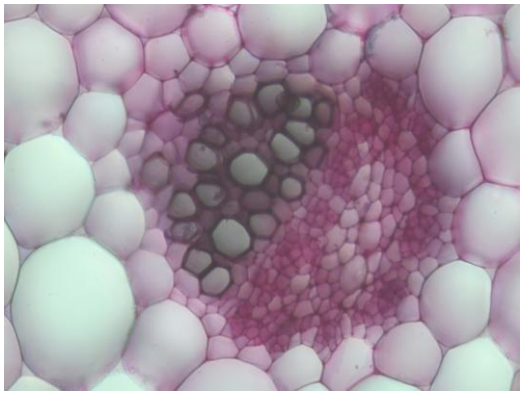

Fig.4. *P. zonale* petiol

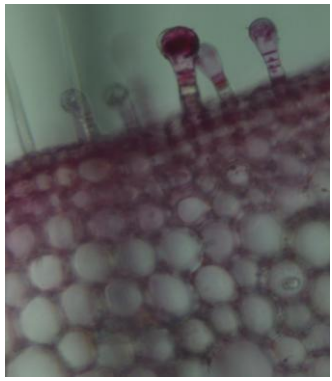

Fig.5. *P. grandiflorum* petiol

TABLE II

Structural differences of vascular bundles for all samples

Species / **Characteristics**	***P. peltatum***	***P. zonale***	***P. radens***	***P. hispidum***	***P. grandiflorum***
Fascicle number	10	11	10	8	19-21
Fascicle type	4 thick 6 thin 1-2 xylem vassels	5 large 6 small 14 at the basis of petiole	4 thick 6 thin	4 large (abundant xylem) 4 small	4 large 4 small 11-13 very small (only phloem)
Central vassel	large, thick	1 large 1 small	1 large	1 large	1 large suround-ed by 2-3 smaller
Sclerenchyma cells	absent	absent	absent	peripheric	peripheric

In *the surface view of the lamina* we noticed the upper epidermis had cells with irregular outline and slightly waved side-walls, whereas the lower epidermis had

very sinuated walls.

In *cross section*, the leaf blade looked like a ribbon and the midveins were thinly (*P. peltatum*) and extremly (*P. grandiflorum)* prominent on the abaxial surface.

All the veins contained one libero-ligneous bundle surrounded by a sheath of polygonal cells, smaller than those from the lacunar tissue.

One of the specific characteristics of *P. grandiflorum* was the presence of 3 opposite fascicles (one large and two small) with xylem elements face to face.

The epidermis has small isodiametric cells with internal and external walls thicker than the lateral ones and the external wall is covered by a very thin cuticle.

The epidermis of all selected species presented stomata and trichomes (surface and glandular) (6) (tab. III).

TABLE III

Stomata and trichomes in *Pelargonium sp*.

Species	**Stomata**	**Surface hairs**	**Glandular trichomes***
Pelargonium peltatum	anomocytic amphistomatic	-uni/bi/pluricellular -uniseriate, variated dimensions -dense and long	- dense and thin - bicellular basal podium
Pelargonium zonale	anomocytic amphistomatic	-uni/bi/pluricellular -uniseriate, variated dimensions -abundant, long and very long	- dense - short: bicellular basal podium - long: tricellular basal podium
Pelargonium hispidum	anomocytic hypostomatic	-uni/bi/pluricellular -uniseriate, variated dimensions -abundant, long and very long	- dense - short: bicellular basal podium -medium: tricellular basal podium - long: tricellular basal podium
Pelargonium radens	anomocytic hypostomatic	-uni/bi/pluricellular -uniseriate, variated dimensions -abundant, long and very long	- dense - short: uni/bicellular basal podium -medium: tricellular basal podium
Pelargonium grandiflorum	anomocytic hypostomatic	-uniseriate, variated dimensions -dense and long -abundant on the abaxial surface and on the midvein	- dense - short: bicellular basal podium -medium: tricellular basal podium - long: tricellular basal podium

*variated dimensions and number on the surface unit, with a basal cell, pluricellular basal podium and one globular-shaped gland

The mesophyll had a unistratified palisade and a bistratified lacunar tissue (7). The main differences that were noted between the five samples of pelargonium consisted in: height of the palisade, number of the layers in the lacunar tissue, abundance and layout of calcium oxalated druses (tab. IV).

TABLE IV

Mesophyll characteristics in *Pelargonium species*

Species	Palisade	Lacunar tissue	Druses
Pelargonium peltatum	-short and wide	-4-5 layers, slightly elongated cells, the size decreases towards the abaxial epidermis	-along the mesophyll
Pelargonium zonale	-short	-5 layers, slightly elongated cells	-only in lacunar tissue
Pelargonium hispidum	-high and long	-3-4 s layers, rounded cells	-sparce only in lacunar tissue
Pelargonium radens	-short	-3-4 layers, small, elongated cells	- only in palisade tissue
Pelargonium grandiflorum	-high	- 4-5 layers, elongated cells	- only in palisade tissue

The following images reveal the most important characteristics of the lamina from the five samples included in the study: *Pelargonium peltatum* (fig. 6-7), *Pelargonium zonale* (fig. 8), *Pelargonium radens* (fig. 9), *Pelargonium hispidum* (fig. 10) and *Pelargonium grandiflorum* (fig. 11).

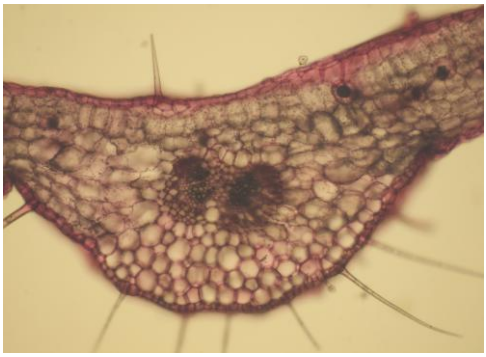

Fig. 6. *P. peltatum* lamina

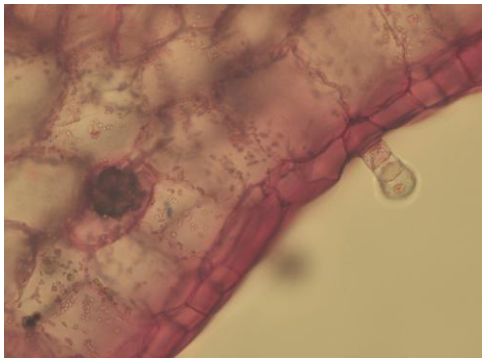

Fig. 7. *P. peltatum* lamina –detail

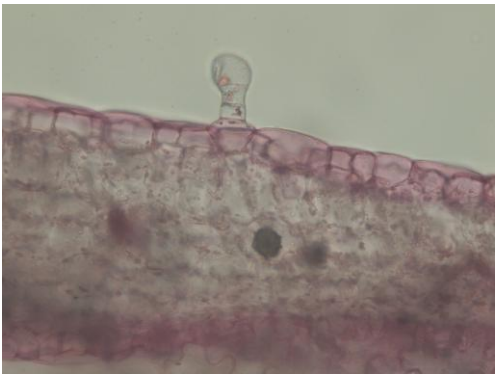

Fig. 8. *P. zonale* lamina

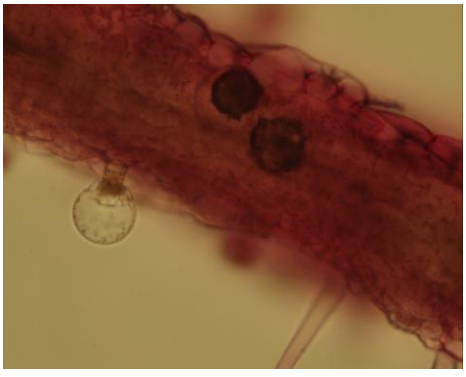

Fig. 9. *P. radens* lamina

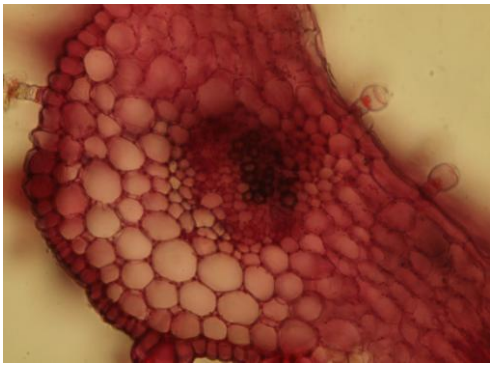

Fig. 10. *P. hispidum* lamina

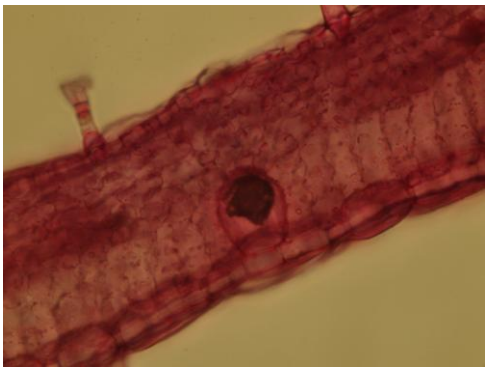

Fig. 11. *P. grandiflorum* lamina

CONCLUSIONS

As we expected, most of the notable differences between the samples were mainly quantitative and have a great taxonomic value. Since the most important for the quality and quantity of essential oil extracted from *Pelargonium sp.* are trichoms, the results showed that *P. peltatum* has a small number of hairs, whereas *P. hispidum* and *P. zonale* have abundant and long hairs. On the other hand, *P. radens, P. hispidum, P. grandiflorum* have the longest trichoms on the petiole. Such differences are to be expected to influence more or less the chemical composition of the plants metabolites. Therefore, our future research is going to establish if there are such correlations.

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SULFADIAZINE MODIFIED CS/HA PEC DESTINED TO WOUND DRESSING

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SULFADIAZINE MODIFIED CS/HA PEC DESTINED TO WOUND DRESSING (Abstract): **Aim:** To present the research results concerning enhanced antimicrobial and release properties of the chitosan derivative with sulfadiazine/hyaluronic acid polyelectrolyte complex (PEC) hydrogel. **Material and methods**: The PECs have been prepared from chitosan of different molecular weight, sulfadiazine chitosan derivative and sodium hyaluronate. The complex structure was assessed by FT-IR spectroscopic method and swelling capacity was followed by weighing measurements. **Results:** It has been establish that chitosan derivative influenced both PEC properties and swelling capacity. **Conclusions:** Incorporation in PEC of the sulfadiazine chitosan is a new way to combine bacteriostatic effect of chitosan with that of sulfadiazine, to control properties, antimicrobial activity in the treatment of the wound.
Keywords: WOUND HEALING, DRESSING, CHITOSAN, HYALURONIC ACID, SULFADIAZINE.

The discovery and development of new antimicrobial drugs is a main goal for scientists because of microbial resistance to a number of antimicrobial agents among a variety of clinically significant species of bacteria (1, 2).

Hydrogels containing grafted chitosan. Both functional groups of chitosan (CS) can be grafted, firstly free amino groups of deacetylated units and then hydroxyl groups of C_3 and C_6 from acetylated and deacetylated units. Grafting is achieved with molecules bearing at least one functional group capable to form covalent bonds with CS as palmitic, lactic or glycolic acids, etc. (3) or aldehydes (4). Multifunctional molecules lead to crosslinking when the concentration of grafts becomes important as in the case of ethylenediaminotetraacetic acid (EDTA) which contains four functional groups (5). Catalysts (sodium borohydride, 1-ethyl-3-(3-dimethyl aminopropyl carbodiimide bromide, cetyl trimethyl ammonium, p-nitrophenyl), initiators (iron or ammonium sulfate, potassium persulfate, ammonium cerium salt) or organic solvents (pyridine, chloroform) employed in these reactions are considered as toxics for drug delivery. Gamma irradiation (^{60}Co) affects also CS. Indirect grafting of CS with p-cresol, hexyloxyphenol or chlorogenic acid can be performed in presence of enzymes as ţyrosinase.

Grafted CS has interesting properties for wound healing when they exhibit bacteriostatic properties, although because of auxiliary substances their biocompatibility should be carefully controlled. Carboxymethyl CS forms an injectable hydrogel for sustain release of morphine and gentamicin which is useful in wound healing and also it increases the antimicrobial activity of CS. Swelling depends on the electrostatic repulsion of the free ammonium groups which leads to the expansion of the chains and the increase of the water uptake by gel, which frequently happens in acidic solutions. Dissolution of the systems containing grafted CS is rare but they show some advantages as improve the solubilization of lipophilic drugs, protect peptidic and proteinic drugs. EDTA grafted onto CS increases its antibacterial activity by complexation with Mg which in normal conditions stabilizes external membrane by Gram negative bacteria. CS can be used to prevent and to treat infection of the wounds and burns not only because of its intrinsic antimicrobial properties but also due to its capacity to deliver antimicrobial agents to wounds and burns being a carrier for retarded release. CS proved to be efficient in hemostasis acceleration favoring rapid wound healing (6, 7).

Antimicrobial activity is higher for CS with high molecular weight and high deacetylation degree and at low pH.*Kulkarniet al.* reported increased 10 times of antibacterial activity of CS by derivatization with thiazolidinone (8). It have been synthesized temperature-responsive hydrogels by grafting with poly(N-isopropylacrylamide) (PNIPAAm) of CS or HA (9). Both hydrogels show higher transition temperatures than that of PNIPAAm and they are good carriers for hydrophilic and lipophylic drugs. Most of hydrogels containing CS are dual or multi stimuli receptive, while PEC containing HA show a pulsatile behavior under electrical field action. CS/HA PECs absorb a high water amount, this increasing with CS content of PEC (10).

Sulfonamides and their derivatives are among the most useful antimicrobial agents because of their low cost, low toxicity and excellent activity against bacterial infections. Most of antibiotics delivered by systemic way are inefficient for wound healing, therefore choosing of an antibacterial topic agent is important to reduce the risk of sepsis and to bring benefits to general treatment.

MATERIALS AND METHODS

Sulfadiazine, also called sulfapyrimidine or 4-amino-N-(pyrimidin-2-yl)-benzen-sulfonamide (fig. 1) is a white or white-yellow powder, sensitive to light with a melting point of 252-256°C, insoluble in water, alcohol, ether, chloroform, soluble in acetone and diluted acidic or alkaline solutions (11).

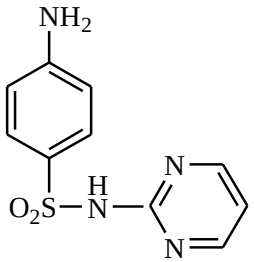

Fig. 1. Sulfadiazine chemical structure

Sulfadiazine is useful in the treatment of meningoccocal, staphyloccocal, streptococcal etc. infections. Based on this action sulfadiazine was selected to modify CS and to obtain formulations with modified and

retarded antimicrobial action.

The procedure of synthesis of the CS derivative with sulfadiazine is described in other paper (12). Chemical structure of the chitosan derivative with sulfadiazine has been confirmed by IR spectroscopy. IR spectrum shows characteristic absorption bands both for sulfonamide unit and also for glucosamine: C=O (amide I) at 1629 cm^{-1}, NH (amide II), 1595 cm^{-1}; phenyl 1546, 839 cm^{-1}, SO_2-N 1259, 1168 cm^{-1}. Degree of substitution of this derivative was 31.64 %. The sulfadiazine CS derivative (SDCS) was used as component in preparation of PEC hydrogels with sodium hyaluronate which was comparatively investigated with PEC containing unmodified CS.

Low (CHL) and medium (CHM) molecular weight chitosan were purchased from Sigma-Aldrich and sodium hyaluronate (NaHA) from *Streptococcus equis* was obtained from Fluka.

Preparation of CS/sodium hyaluronate PEC hydrogels **.**CS/NaHA and SDCS/NaHA PEC hydrogels have been prepared by dissolution of two components in an aqueous solution of formic acid 50% with a polymer concentration of 1 % and then these solutions were mixed in the ratio 1:1 (13). Solutions were continuously magnetically stirred at room temperature ~ 20^oC for 48 hours or 72 hours. The obtained solutions have been filtered and then cast on Petri dishes and dried at oven at 40^oC for 48 hours. It has been obtained thin transparent films in the case of CS/NaHA polyelectrolyte hydrogels and semi-transparent film from SDCS/NaHA polyelectrolyte hydrogels. The films were taken off from Petri dishes and washed with double distilled water for 2 days, recurrently changing the washing water and after that, they were dried at 40^oC for 48 hours.

ATR-FTIR spectroscopy have been recorded at 4 cm^{-1} resolution with 64 scans by means of a spectrometer Bruker VERTEX 70, in absorbance mode, by the ATRFTIR technique with a 45°ZnSe crystal. Penetration thickness was about 100 microns. For each film sample, the evaluations were made on the average spectrum obtained from three recordings. Background and sample spectra were obtained in the 500 to 4000 cm^{-1} wavenumber range. The processing of spectra was achieved using a SPECVIEW program.

Swelling behavior was followed by weighing of the dried PEC hydrogels and swollen ones at predetermined time intervals. Swelling was performed in buffer solution of pH 7.4 and swelling degree (SD) was evaluated using eq. (1) (14):

$$SD\ (\%) = \frac{(m-m_0)}{m_0} \times 100 \qquad (1)$$

where m_0 is mass of dry sample and m is mass at moment "t" of swelling.

RESULTS AND DISCUSSION

ATR-FTIR spectroscopy

CS spectrum shows a wide band at 3320 cm^{-1}, assigned to stretching vibration of OH group while the band centered at 1560 cm^{-1} is assigned to bending vibration of NH group (amide II) (NH_2) (15). The HA spectrum has six main bands (16): 1. a wide band centered at ~ 600 cm^{-1} attributed to rotation vibration, δL of the water molecule; 2. a strong band extended from 950 to 1200 cm^{-1}, corresponding to stretching vibration of COO (-COOH) from acids. Assignment was made on characteristic bands of cellulose with a shoulder at 1160 cm^{-1} for asymmetric vibration of $\nu_{C\text{-}O\text{-}C}$ from glycosidic units; 3. a band around

1400 cm^{-1}, which can assigned both to ν_{COO^-} symmetric vibration from carboxylate group and to $\nu_{C\text{-}O^-}$ asymmetric vibration from the same group, allowing distinction between two parts of bond: C=O and $\nu_{C\text{-}O\text{-}}$; 4. a strong band at 1500 - 1700 cm^{-1} which is an overlapping of the bands of amide I and II and various carbonyl ($\nu_{C=O}$) and carboxyl vibrations; 5. a band at ~ 2,900 cm^{-1}, which is $\nu_{C\text{-}H}$ (C-H stretching); 6. a wide and strong band which extended from 2500 to 3600 cm^{-1}, this being a stretching vibration of $\nu_{N\text{-}H}$ and $\nu_{O\text{-}H}$ groups involved in hydrogen bonding. Shoulder from 3100 cm^{-1} is characteristic to *Fermi* resonance of the νN-H with the band of amide II (17).

In all spectra can be identified the band at 1595 cm^{-1} assigned to NH_2 groups of CS and also very clear and strong bands at 1545 cm^{-1} and 895 cm^{-1} corresponding to N-H from sulfadiazine and two bands centered at 837 and 782 cm^{-1}, assigned to pyrimidinic ring (tab. I, fig. 2).

In ATR-FTIR spectra of PEC hydrogels the bands are wider and are shifted in respect with bands of pure components which is a proof of PEC formation. The shape of the ATR-FTIR spectrum of the SDCS/HA film shows differences comparatively with those described by bands at 1595 cm^{-1} assigned to amine (NH_2) group of CS, at 1545 cm^{-1} a strong band due to amide II (NH) and the band assigned to COO^- (symmetric) from HA at 1402 cm^{-1} is clearly distinct which means that not all of amine groups participate to PEC formation being available to provide antimicrobial activity according to well-known mechanism.

Swelling behavior. The swelling capacity is different from a sample to other, the lowest swelling capacity having PEC hydrogel containing sulfadiazine CS derivative (SDCS). The SDCS sample swells in the first 2-3 min reaching a swelling degree of ~1150%, then equilibrium is reached. PEC containing unmodified CS shows a lower rate of swelling up to 30 min, then a plateau is reached, but the degree of swelling is higher of ~ 2500% and ~3700% for CSL / HA and CSM / HA, respectively, high molecular weight being favorable to swelling (fig. 3).

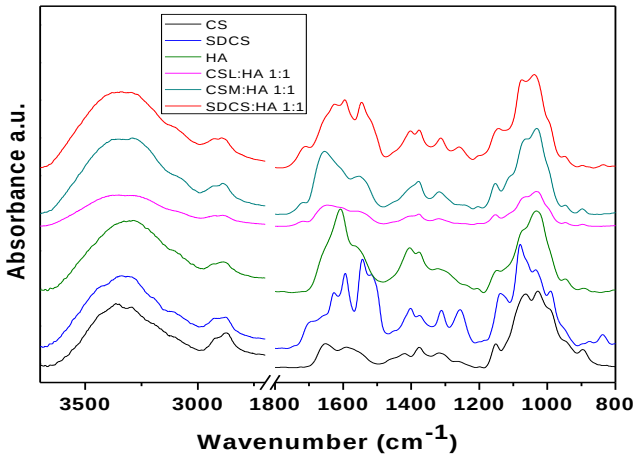

Fig. 2. ATR-FTIR spectra of CS, HA, SDCS and CS/HA PEC

TABLE I
ATR-FTIR bands of the CS/HA and SDCS/HA PEC hydrogels

CSL/HA (cm^{-1})	CSM/HA (cm^{-1})	SDCS/HA (cm^{-1})	Bands assignments
3334	3334	3334	-OH and -NH groups vibrations
2890	2890	2890	vibrations of CH_2 from glucopyranoze ring
1718 (shoulder)	1718 (shoulder)	1710 (shoulder)	C=O
1650(s)	1655(s)	1625	amide I (C=O) overlapped with COO^- (asymmetric)
		1595 (s)	NH_2 group
1554 (shoulder)	1552 (shoulder)		amide II (C-N-H)
		1545	N-H from sulfadiazine
1407 (shoulder)	1405 (shoulder)	1402	COO^- symmetric
1378 (w)	1380 (w)	1377	CH_3 from amide group
1318 (w)	1318	1313	amide III
1245 (w)	1242 (w)	1259 (w)	C-O group
1153 (w)	1153 (w)	1143 (w)	C-O-C (O in glycosidic bond)
1068 (shoulder)	1067 (shoulder)	1075	C-O (exocyclic), C-C
1030 (s)	1030 (s)	1038 (s)	C-OH alcohol groups
895 (w)	896 (w)	895 (w)	N-H
		837 (w), 782 (w)	C-H pyrimidine ring

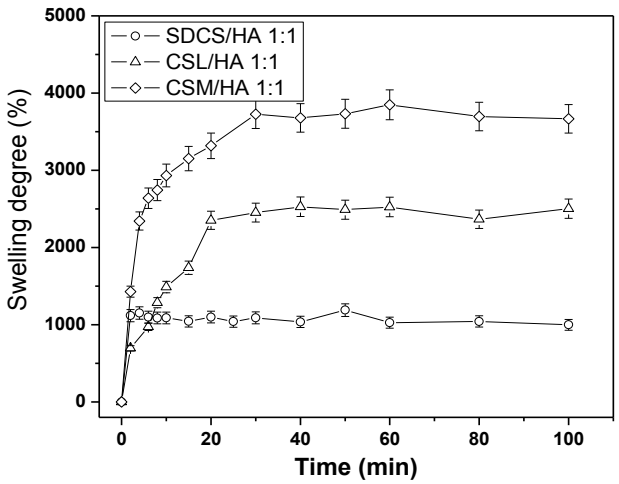

Fig. 3. Swelling profiles of CS/HA polyelectrolyte hydrogels.

CONCLUSION

Grafted chitosan should be considered as a second choice for the preparation of hydrogels, especially for drug delivery systems. Some grafted chitosan hydrogels can be interesting in specific medical or pharmaceutical applications if chitosan is functionalized without auxiliary molecules and if intrinsic properties such as bacteriostatic effect or promotion of wound healing are enhanced by grafting.

Sulfadiazine modified chitosan can be used to prepare polyelectrolyte hydrogels with hyaluronic acid which shows lower swelling capacity which could found potential application in wound healing taking in view their capacity to retain a relatively high quantity of liquid at a low degree of substitution which allow to combine antimicrobial activity of chitosan with that of sulfadiazine.

ACKNOWLEDGMENTS

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NEWS

THE MOLECULAR CHANGES IN MALIGNANT PLEURAL MESOTHELIOMA AND THEIR IMPACT ON PROGNOSIS AND TREATMENT

In recent decades, various studies have been conducted to define the molecular characteristics of malignant mesothelioma (MM) cells. Molecular studies have identified somatic genetic and epigenetic alterations in malignant pleural mesothelioma (MPM) cells, associated with altered expression and activation or inactivation of critical genes in oncogenesis. Deregulation of signaling pathways related to differentiation, survival, proliferation, apoptosis, cell cycle control, metabolism, migration, and invasion has been demonstrated in complementary studies. These changes were found by investigating individual gene status in genomic and transcriptomic studies, and were supported by immunohistologic studies. MPM cells show a large spectrum of abnormalities shared with other malignancies, or more specific alterations such as those of the NF2 gene. Comparative studies of series of MPMs have usually demonstrated that both alterations in a given gene and combined genetic and epigenetic alterations are present in MPM subsets, consistent with interindividual variations of molecular alterations. There are therefore at least 2 levels of heterogeneity, at the genome level and at the gene level, suggesting that identification of patient subgroups would be essential to develop more specific therapies. Moreover, the tumor microenvironment, consisting of a large number of different cell types, adds another level of complexity in identifying the best strategy to improve the outcome of this disease. This tumor heterogeneity could explain differences in patient survival and response to treatments. Bernestein L reviews on genomic abnormalities and signal transduction dysregulation have been summarized the molecular changes in MM, focusing on more recent advances in MPM and discussing the level of confidence and limitations of these results, their impact on prognosis and treatment, and the future research required to fill the gaps and enhance the benefit of basic research to improve patients' outcomes. Demonstration of the multiple alterations present in the tumor should encourage research into combined or more global therapies. (Jean D, Daubriac J, Le Pimpec-Barthes F, Galateau-Salle F, Jaurand M-C. Molecular Changes in Mesothelioma With an Impact on Prognosis and Treatment. *Arch Pathol Lab Med* 2012; 136: 277–293)

Doina Butcovan

HEMANGIOMA OF THE PAROTID GLAND: CASE REPORT

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HEMANGIOMA OF THE PAROTID GLAND: CASE REPORT (Abstract): Hemangiomas account for half of the parotid gland tumors occurring in children. After a rapid increase in the first months of life, some of them gradually regress (until complete disappearance) in a period of several years. Hemangiomas that do not regress over time, grow in size (possibly within a short period of time) or develop complications such as intralesional acute bleeding or thrombosis require aggressive therapy: intralesional corticosteroid injection, sclerotherapy, radiation therapy, laser photocoagulation, embolization, or surgical removal. We present the case of a 12-year-old boy with hemangioma of the left parotid gland diagnosed by CT scan, angio-CT with 3D reconstruction and angiography successfully treated by superselective intraarterial embolization with acrylic glue followed after 4 days by left superficial parotidectomy with facial nerve preservation. **Keywords**: HEMANGIOMA, PAROTID GLAND, SURGICAL TREATMENT.

Salivary gland tumors occurring in children are less than 5% of all tumors, and hemangiomas are the most common (50% *versus* 2% in adults), followed by: pleomorphic adenomas, mucoepidermoid carcinomas, lymphangiomas, acini cells carcinomas (1, 2, 3). The clinical picture of parotid hemangioma is characterized by the presence of a painless swelling, usually confined to the superficial lobe of the gland, but sometimes involving the masseter muscle, bluish discoloration of the overlying skin and a network of vascular capillaries (4). Magnetic resonance imaging is considered to be the first choice in making an accurate diagnosis and investigating the vascular supply of this lesion due to image quality, soft tissue definition, lack of ionizing radiation, additional invasive procedures not being required (5,6). Therapeutically there are two options: a) conservative management and clinical monitoring of parotid hemangioma, given the probability for most of these lesions to resolve spontaneously, and b) a more "aggressive" treatment (systemic corticosteroids or intralesional steroid injection, laser photocoagulation, sclerotherapy,embolization and surgical excision), particularly when parotid hemangiomas are large, deforming, ulcerated or involve neighboring structures, with functional consequences (3, 7, 8, 9, 10, 11).

In this article, we present the case of a 12 years old boy diagnosed at the Department

of Oral and Maxillofacial Surgery with left parotid hemangioma. Management consisted in preoperative embolization and surgical excision of hemangioma by parotidectomy, without postoperative complications, the best morphological, functional and aesthetic results being achieved one year after surgery.

CASE REPORT

A twelve-year-old boy with no associated general diseases was referred to the Department of Oral and Maxillofacial Surgery for the presence of a 5cm x 4cm tumor mass in the left parotid region, poorly defined, soft, compressible, non-pulsatile, painless, and healthy overlying skin; case history revealed that the tumor mass progressively increased in size over the last 10 years (fig. 1). Head and neck CT scan showed a nodular mass located at the superficial lobe of the left parotid gland, 4.4/2.6/4.8 cm in diameter, intensely enhanced (arterial phase) after contrast administration, with a low intensity signal in the middle of the lesion (fig. 2). Angio-CT with 3-D reconstruction showed that the arterial blood supply of the tumor was from the branches of the transverse facial artery and venous drainage into the internal and external jugular vein (fig. 3). Presumptive diagnosis was parotid hemangioma and the therapeutic decision was surgical removal of vascular lesion, due to the lack of natural tumor involution. Angiography (fig. 4) and superselective intraarterial embolization with acrylic glue (Glubran), used in interventional neuroradiology, were performed prior to surgery (fig. 5) in order to reduce tumor vasculature. Four days after arterial embolization, the vascular tumor was removed by superficial parotidectomy, with facial nerve preservation (fig. 6); at 1 year postoperatively the results were very good (fig. 7). Histopathological examination confirmed the mixed type of parotid hemangioma (capillary and cavernous) (fig. 8).

Fig. 1. Left half-profile, showing the tumor developed in the left parotid area

Fig. 2. CT scan with contrast, showing structural details of the left parotid tumor

Fig. 3. Angio-CT with 3D reconstruction to investigate the vascular supply of the parotid tumor

Fig. 4. Angiographic image of the tumor developed in the left parotid gland

Fig. 5. Angiographic image after supraselective intraarterial embolization with Glubran

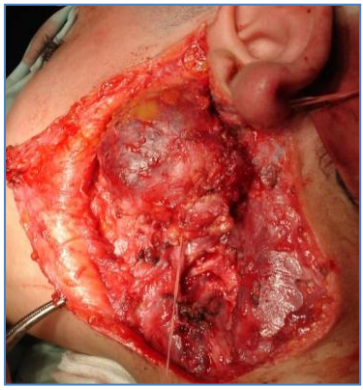

Fig. 6. Intraoperative appearance of the left parotid hemangioma

Fig. 7. 1-year postoperative facial appearance: no evidence of facial nerve paralysis

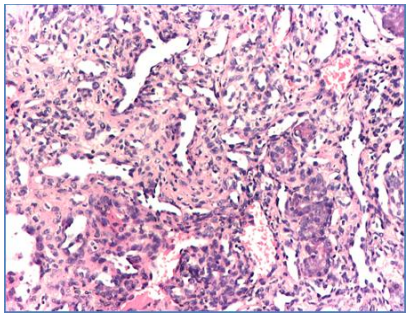

Fig. 8. Histopathologic appearance: capillary and cavernous parotid hemangioma

DISCUSSION

Hemangiomas of the salivary glands are benign vascular mesenchymal tumors and may arise from the gland proper or by secondary invasion of subcutaneous blood vessels into the gland structure (12). Congenital

capillary hemangiomas („true" hemangioma according to the classification proposed by Mulliken and Glowacki in 1982) represent 90% of the parotid gland tumors in infants and young children (2). The course is characterized by a rapid growth phase in early childhood (proliferative phase), followed by a gradual spontaneous involution over a period of several years (5). This type of benign vascular tumor is found more frequently in girls, mean age at presentation 4 months, and the overlying skin has a characteristic bluish tint. Comparatively, cavernous hemangiomas (classified as true vascular malformations) occur as a consequence of the abnormal development of blood vessels in late childhood, are not accompanied by a bluish discoloration of the overlying skin, may contain „phleboliths" (a feature that is not found in true hemangiomas), and are unlikely to regress spontaneously, their surgical removal being the treatment of choice (13). There are also compound types (capillary and cavernous), as the case presented in this paper, in which case the final diagnosis is established histologically.

The clinical description of a parotid hemangioma includes: tumor in the parotid area, slowly progressive, poorly defined, soft, compressible, mobile, painless and the overlying skin has sometimes a bluish tint (14). Deformity and severe pain depend on tumor size and the presence of intralesional bleeding or acute thrombosis. History and physical examination are elements supporting the diagnosis of probability, but imaging is needed to confirm the suspicion.

In children, the initially recommended imaging study to salivary gland lesions is ultrasound examination, since the pathology in these locations is generally benign, ultrasound images have good accuracy and no sedation or anesthesia is required for optimal exploration (3). In particular, color Doppler is recommended to explore salivary vascular lesions. Ultrasound protocol of a parotid hemangioma reveals a homogenous mass that replaces most of the parotid gland, with fine echogenic internal septa and numerous intratumoral vessels (described as non-echogenic areas) (5). Color Doppler shows filling of non-echogenic areas, the high vascular density (> 5 per cm^2) differentiating these vascular tumors from others. At CT scan without contrast material parotid hemangioma has the same density as the muscle structure, the administration of contrast material revealing a homogenous, well defined, high absorption mass (1). An accurate diagnosis of juvenile parotid hemangioma sometimes requires an additional fine needle puncture-aspiration (15). T1-weighted magnetic resonance image shows flow voids within the lesion, thus distinguishing parotid hemangioma from other soft tissue lesions encountered in children. On T2-weighted MRI the vascular lesion appears hyperintense to muscle. During hemangioma involution, there are areas of fat replacement that appear hyperintense on T1-weighted images. Magnetic resonance imaging in cavernous hemangioma shows a heterogeneous T2 signal (due to phleboliths and venous lakes) and no intralesional flow voids (1). More recently, as to differentiate between a high and low flow lesion, or hypovascularized and hypervascularized tumor is not always possible, MRI digital subtraction angiography can be used (6). This technique can be safely performed in children of all ages, without complications, providing information on the vascular supply of the explored lesion, information not available by clinical examination or routine magnetic resonance imaging. Our patient was investigated by CT, angio-CT with 3D reconstruction, and angiography,

useful and sufficient data for determining the vascular supply of the parotid lesion being thus obtained.

Therapeutic approach of parotid hemangioma in children should be individualized, initial therapy being less aggressive and the subsequent ones more aggressive. The spectrum of treatment modalities includes: clinical monitoring of tumor involution over several years, corticosteroids (intralesional or systemic), interferon treatment, sclerotherapy, radiation therapy, laser therapy, cryotherapy, arterial ligatures, and surgical excision (3). Since many parotid hemangiomas are likely to regress, the indications for surgery are: increase in tumor size, rapidly growing lesion, non-involuting hemangiona, or intralesional bleeding. It is recommended that tumor vascular supply to be explored by MR angiography or digital subtraction angiography prior to surgical removal of a parotid hemangioma (6). Surgical excision of parotid hemangioma is performed by superficial or total parotid-ectomy, with intraoperative identification and preservation of the facial nerve. The case presented in this article was managed mainly surgically, but we also resorted to a postoperative "artifice" - superselective intraarterial embolization with acrylic glue, a technique used in recent years in interventional neuroradiology to achieve endovascular embolization stability in the treatment of cerebral vascular tumors or malformations (16, 17); the advantage of this therapeutic "mix" was a decrease in the vascular flow to the parotid lesion, facilitating intraoperative hemostasis and, thus, optimal performance of parotidectomy.

CONCLUSIONS

Surgical removal of parotid hemangioma must be preceded by a thorough imaging study of the lesion aimed at clearly detecting the vascular supply (main and collateral) in order to avoid intraoperative bleeding complications.

Superselective intraarterial embolization with acrylic glue just a few days before parotidectomy is a method that decreases vascular flow of a parotid hemangioma, with positive impact on operative performance.

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NEWS

BIOMARKERS AND SILENT CARDIAC DISEASE IN PRIMARY PREVENTION: TIME TO UNMASK THE DAMAGE?

The aim of this study was to examine whether biomarkers can identify silent cardiac target organ damage (cTOD) in a primary prevention population. The researchers observed that one possible way to improve primary prevention of cardiovascular events is to identify those patients who already harbor silent cTOD (i.e., myocardial ischemia, left ventricular hypertrophy, systolic dysfunction, diastolic dysfunction, or left atrial enlargement). This might be possible by screening with a biomarker (e.g. high sensitivity cardiac troponin T [hs-cTnT] or B-type natriuretic peptide [BNP]). The prospective methods used for identifying silent cTOD were transthoracic echocardiography, stress echocardiography, and/or myocardial perfusion imaging. Few conclusions have to be take in cpnsideration. In asymptomatic treated primary prevention patients, BNP screening is able to identify existing silent cTOD. The performance of hs-cTnT was not as good as that of BNP. B-type natriuretic peptide plus hs-cTnT together performed best. Prescreening with BNP ± cTnT followed by targeted phenotyping is worth exploring further as a possible way to improve primary prevention. (Nadir MA et al. Improving the primary prevention of cardiovascular events by using biomarkers to identify individuals with silent heart disease. J Am Coll Cardiol. 2012; 60 (11):960-968).

Doina Butcovan

DIETARY PATTERNS IN YOUNG ADULTS FROM TIMIS COUNTY

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DIETARY PATTERNS IN YOUNG ADULTS FROM TIMIS COUNTY (Abstract): Dietary patterns present at the age of the young adult have great probability to remain unchanged for the rest of the life and may contribute to eating disorders later in life. **The aim** of this study is the analysis of the intake patterns for fruits and vegetables in students from Timis County. **Material and methods:** We conducted a cross sectional study using a stratified cluster sample design to produce a representative sample of students for the Timis County. The students' sample totalized 2076 students from the universities in Timis County. **Results:** A large percent of the young people participating in the study (41.9%) does not consume fresh juices. For the consumption of fresh fruits, the present results back up the international discoveries, only 32.8% of the young people consuming fruits daily, which is a smaller percent that the medium value reported by other countries. The frequency of consumption for fruits differs according to gender, being higher for girls. For salads, the percent of young people consuming this type of foods at least once a day is 17.0%, much lower compared to 32.8% of the young people consuming fresh fruits at least once a day. **Conclusions:** The examination of the dietary patterns related to the intake of fruits and vegetables helped us to determine priority areas for future interventions. **Keywords:** FRUITS, VEGETABLES, YOUNG ADULTS, DIETARY PATTERNS

Diet and physical activity, alongside genetic factors, have important roles in determining the individual status of health. The habits related to diet and physical activity start to form during childhood and continue to shape until the adult age. While the time of childhood is characterized by resistance to new culinary experiences, the young adults will use more often new culinary products to declare their independence, not always in a beneficial manner.

In the context of the young adult distancing from the known family environment and creating a new social entourage, this will influence in a variable proportion the diet. The frequency of obesity in all age groups increased 2 to 3 times in the last two decades (1). The results of the Behavioral Risk Factor Surveillance System study (BRFSS) conducted between 1991 and 1998 show the most important increase for the obesity rates in the age group 19 to 29 years, also for those with higher educational degree (2).

Overweight and obesity are some of the most challenging and steadily rising public health problems worldwide. Strategies to effectively reduce and maintain a healthy body weight are urgently required. A number of national and international bodies recommend an increased intake of fruits and vegetables in order to decrease the risk of developing lifestyle-related diseases including overweight and obesity.

The risk-reducing effect of fruits and vegetables on overweight and obesity may in part be exerted through their possible reduction upon the total energy intake. This may be explained by various factors. Fruits and vegetables are low in energy density, high in water content and they contain a considerable amount of dietary fibers, soluble dietary fibers in particular (3).

A number of observational and interventional studies have investigated the possible association between fruit and vegetable intake and body weight. Most of these studies find an inverse association. In a review (3), emphasis is given to the role of fruits alone and the risk of developing overweight and obesity. The rationales for this are several: fruits are typically consumed at other occasions than vegetables as they can be obtained in various physical forms, such as fresh, dried, canned, pureed, making them convenient as between-meal snacks, potentially substituting more energy-dense snacks; the culinary use of fruits differs from that of vegetables. For example, because of the various physical forms and commonly sweet taste, they are suitable as deserts. Also, here they may act as the relatively healthier alternative to the traditionally more energy dense deserts and fruits are frequently consumed raw, whereas vegetables are often prepared by addition of fatty substances, which diminishes the low energy-dense characteristics of vegetables. Although, to the best knowledge of the authors, the plausible differential physiological mechanisms of fruit and vegetables have not been explored, fruits possess a distinct physical profile, which may be manifested differently in relation to body weight status. Fructose, the main sugar in fruits, has a relatively low glycaemic index (4), producing a slow increase in postprandial blood glucose followed by a possible increase in satiety. The slow absorption may also increase satiety as a result of extended contact time with the gastrointestinal receptors that produce satiety signals. Another factor that may connect fructose to satiety involves incomplete absorption of fructose with subsequent hyperosmolar environment in the colon (5). This results in attraction of fluids into the gut lumen, causing a feeling of indisposition and lost interest in further food consumption. Based on these and other as yet unknown probable factors, the authors find it appropriate to distinguish between fruits and vegetables in relation to overweight and obesity (3).

Previous literature revealed that young adults tend to consume excess amounts of total fat, saturated fat, cholesterol and sodium. Other studies have found inadequate intakes of essential micronutrients, such as calcium, iron, zinc, folate, and vitamins A, B_6 and C, as well as low consumption of fruits and vegetables. Promotion of dietary change among young adults, who are currently healthy, could be especially challenging. Potential future health benefits of a balanced diet, which are the principal emphases in many of the current guidelines, may not seem as appealing to this particular population as immediate physical and psychological benefits (6).

The aim of this study is to analyze dietary patterns among young adults from the urban area in Timis County.

MATERIAL AND METHODS

This study is based on the grant "The evaluation of risk behavior dimension in high school students and young people from vocational schools and universities in Timis County" (CORT 2004), carried out between 2003-2005, cod CNCSIS 1167, attained at The University for Medicine and Pharmacy Timisoara. The CORT 2004 study is a transversal epidemiologic survey assessing risk behavior in a group of young people attending the colleges and universities in Timis County.

We conducted a cross-sectional study using a stratified cluster sample design to produce a representative sample of students for the Timis County, Romania. A 126-item questionnaire was developed to investigate health-risk behaviors of young adults. Some of the questionnaire items were modified from other instruments, including the 1999 Youth Risk Behavior Survey (YRBS) (7) and the European School Survey Project on Alcohol and Other Drugs (ESPAD) (8). The survey focused on health risk behaviors established during the period of young adulthood that results in the most significant mortality, morbidity, disability, and social problems for youths and, later, adults. The following issues were examined: family environment, out-of-family entourage, tobacco use, alcohol use, illicit drug use, violence-related behaviors and school-related violent behaviors, suicide ideation, attempted suicides, and the seriousness of those attempts, preventive conduct in road traffic, sexual behaviors, dietary behaviors, overweight and weight control, physical activity, medical attendance. In total, 30 items were dedicated to dietary behaviors. A pilot test was conducted to establish the validity and reliability of the instrument.

The participants were included in this study based on their free consent. The questionnaire was administered in a regular classroom setting and took students 60 minutes to complete. No filter questions were used. By doing this, comparable amounts of time are required to complete the questionnaire, regardless of risk behavior status and students cannot detect the risk behaviors of their colleagues simply by looking at the pattern of the responses. Only students present the day of the survey were eligible for participation. Trained public health residents and undergraduate students conducted the survey. The data collectors read aloud scripts that explained the survey procedures. Students were told that they do not have to put their names on answer sheets, and that obtained data would be used only for general assessment of the situation in the county.

The data processing and interpretation used modern methods of advanced medical statistics. Data were filed using *EpiInfo*, version 6.0, 2001 and processed using *EpiInfo*, version 3.5.1, 2008. The values of the statistical significance level $p<0.05$ were considered statistically significant, and $p<0.01$ were considered very significant statistically. The following statistical tests were applied: chi-square test, Mann-Whitney test, Kruskal-Wallis test, Pearson correlation and Kendall correlation. Factorial exploratory analysis was also used in order to group the association of the risk behaviors.

RESULTS AND DISCUSSION

The students sample from Timis County

was composed of 2076 students from universities in the urban environment. Among this sample, the distribution was as it follows: 62.49% (1296) girls and 37.51% (778) boys. The individuals participating in this study were young adults, meaning their age was between 18 years (0.3%) and 25 years (3%).

In Great Britain, in the year 1994, the Cardiovascular Review Group recommended a daily intake of 5 to 6 portions of fruits and vegetables, twice as much as the medium intake of the average British citizen, although the consumption pattern vary in different areas and in different social layers (9). Among multiple factors influencing the intake of these food classes, the pleasure of consuming them is one of the most important internal factors, and their availability, cost and time necessary for cooking them are maybe the most important external factors (10). For people leading an active life reaching the recommended ratio of these foods may be challenging. One way to consume them during the day is in the form of juice.

As to the intake of fresh fruits juices, most of the young people participating in the study declared they do not consume it, 41.9% (858), or they consume it 1-3 times per week, 37.9% (775). Only 7.1% (145) admitted they drink fresh fruits juices once a day, and 5.5% (113) twice or more times per day. The contribution of fruit juices to the fibers intake is very poor and in this aspect it is more indicated the intake of fresh fruits (tab. I).

The studies conducted on national level in the United States showed that young adults with the age between 18 and 24 years consume insufficient quantities of fruits and vegetables (11, 12, 13).

TABLE I

The students' distribution according to the frequency of consumption for juices prepared from fresh fruits

The frequency of consumption for juices prepared from fresh fruits	Frequency	Percent	Valid percent	Cumulated percent
2 or more times per day	113	5.4	5.5	5.5
Once per day	145	7.0	7.1	12.6
4-6 times per week	156	7.5	7.6	20.2
1-3 times per week	775	37.3	37.9	58.1
never	858	41.3	41.9	100.0
Total	2047	98.6	100.0	
Missing data	29	1.4		
Total	2076	100.0		

The studies conducted by WHO uncovered that, on average, only 30% of the boys and 37% of the girls report daily fruits intake. The consumption was highest in Israel (49% of the boys and 54% of the girls) and lowest in Estonia (17% of the boys and 23% of the girls). In 16 different countries and regions, more than 25% of

the young people consume only rarely fruits (once per week or less). In almost all the countries and regions, mostly girls reported daily fruits intake, although there is a considerable geographic variation present (14).

Of the young adults participating in the present study, 41.13% (844) reported fruits intake 1-3 times per week, and 23% (472) consume fruits 4-6 times per week. Only 3.07% (63) of the participants to this study declared they never eat fruits. 16.0% (329) of the young adults included in the study eat fresh fruits 2 or more times per day, intake pattern comparable to the international recommendations (tab. II).

TABLE II

Students' distribution related to the frequency of intake for fresh fruits

Frequency of intake for fresh fruits	Frequency	Percent	Valid percent	Cumulated percent
2 or more times per day	329	15.8	16.0	16.0
Once per day	344	16.6	16.8	32.8
4-6 times per week	472	22.7	23.0	55.8
1-3 times per week	844	40.7	41.1	96.9
Never	63	3.0	3.1	100.0
Total	2052	98.8	100.0	
Missing data	24	1.2		
Total	2076	100.0		

The results of the present study corroborate the results of other international studies showing that most of the young people do not consume the recommended quantities of fruits (15, 16).

The sample of 844 young adults reporting fruits intake 1-3 times per week, is composed of 497 girls (38.7% of the female respondents) and 347 boys (45.4% of the male respondents). For the different fresh fruits intake frequencies, the males and females percents are similar. Nevertheless, the girls' percent, 17.4% (223) is higher than the boys' percent, 15.7% (120), which consume fresh fruits once per day. The same, in case of consumption 2 or more times per day of these aliments, the percent of girls reporting this intake pattern, 18.2% (234), was higher than the boys percent, 12.4% (95) (tab. III). These results suggest that the consumption frequency for fruits differs related to gender, being higher for girls, conclusion reached by other authors too (16).

Salads are consumed by the young adults: 52.0% (1067) 1-3 times per week, 16.8% (345) never, and 6.3% (130) consumes salads 2 or more times per day, but in this case also the intake is low (tab. IV).

In the fast-food restaurants accessible to students, there is a less diversified offer of salads, and for the most part they are missing. As the young adults prefer this way to procure food, the reduced intake of these foods can be explained.

TABLE III

Students' distribution according to the fresh fruits frequency of intake, by gender

Fresh fruits frequency of intake	sex				Total	
	female		male			
	Number	Percent	Number	Percent	Number	Percent
Twice or more times per day	234	18.2	95	12.4	329	16.0
Once per day	223	17.4	120	15.7	343	16.7
4-6 times per week	301	23.4	170	22.2	471	23.0
1-3 times per week	497	38.7	347	45.4	844	41.2
Never	30	2.3	33	4.3	63	3.1
Total	1285	100.0	765	100.0	2050	100.0

TABLE IV

Students' distribution related to the salads intake frequency

Salads intake frequency	Frequency	Percent	Valid percent	Cumulated percent
Twice or more times per day	130	6.3	6.3	6.3
Once per day	219	10.5	10.7	17.0
4-6 times per week	290	14.0	14.1	31.2
1-3 times per week	1067	51.4	52.0	83.2
Never	345	16.6	16.8	100.0
Total	2051	98.8	100.0	
Missing data	25	1.2		
Total	2076	100.0		

CONCLUSIONS

Dietary patterns may seem not as acute a risk behavior compared with behaviors producing more dramatic results (violence, addiction etc.), but on a long term is just as damaging as the others. There are always difficulties in adequate assessing nutrition intake with a limited number of items.

The present study corroborates the findings of other authors, namely the young adults' intake of fruit and vegetables is lower than the recommended ratio. There are a number of reasons we can think off in order to explain this situation: the students prefer to eat in fast-food restaurants, where the offer for fresh salads and fruits is usually not very diversified; the cost of these foods can represent a barrier to higher intake because they are more expensive than highly processed foods; during an active

day it may be difficult to consume these particular aliments (an option is represented by juices); fruits are usually ready to be eaten, but vegetables necessitate more time to prepare; the food choices are usually influenced by entourage and publicity.

The insufficient intake of fruits and vegetables situates the young adults at risk for future chronic diseases (cardiovascular diseases, diabetes, obesity). In the future, strategies to increase the intake of fruits and vegetables must be developed for this age group, taking into account the particularities of the young adults.

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LEFT-SIDED DUPLICATION OF INFERIOR VENA CAVA: CLINICAL IMPLICATIONS IN A PATIENT WITH SIGMOID ADENOCARCINOMA

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LEFT-SIDED DUPLICATION OF INFERIOR VENA CAVA: CLINICAL IMPLICATIONS IN A PATIENT WITH SIGMOID ADENOCARCINOMA (Abstract): Double inferior vena cava is a rare congenital anomaly, usually detected by computed tomography or magnetic resonance imaging. Although asymptomatic, it may have a clinical significance, as it may mimic a para-aortic lymphadenopaty. A case of left-sided duplication of the inferior vena cava in a patient with sigmoid colon cancer is presented. The diagnostic pitfalls and clinical implications are discussed. Accurate preoperative assessment of such an anatomical variant is of utmost importance, this way potentially life-threatening surgical complications, particularly when a minimally invasive approach is planned, are prevented. **Key words**: DOUBLE INFERIOR VENA CAVA, COLON CANCER, LYMPHADENOPATY, COMPLICATIONS.

Congenital anomalies of the inferior vena cava are uncommon findings (1) and usually are identified in asymptomatic patients (2). Although rare, double inferior vena cava may have a significant clinical impact, especially during retroperitoneal surgery (3, 4) or in the treatment of thromboembolic diseases (5). The left inferior vena cava can be misinterpreted as a retroperitoneal tumor or, more frequently, as a para-aortic lymphadenopaty (7, 8). We present the case of a patient with sigmoid colon adenocarcinoma and a double inferior vena cava, the left one mimicking a lymph node metastasis. The clinical implications of this association are also discussed. To the best of our knowledge this is the first case reported in the English literature on an association between colon cancer and double inferior vena cava.

CASE REPORT

A 48-year-old woman with no significant medical history was investigated for alternating constipation and diarrhea along with abdominal discomfort. Clinical examination was unremarkable, except mild skin pallor. Laboratory tests revealed mild anemia (Hb = 9 g/ dl) without other abnormalities. Carcinoembryonic antigen and CA 19-9 serum levels were within normal limits. Colonoscopy emphasized a ulcerate tumor mass at the level of the sigmoid colon, partially obstructing the lumen. Abdominal ultrasonography revealed a para-aortic "tumor mass" suggestive of lymphadenopaty. Axial contrast enhanced computed tomography showed a double inferior vena cava (fig. 1 a), invalidating the presence of para-aortic lymphadenopaty; no distant metastases to the liver, lung or peritoneum

were found. Coronal computed tomography reconstruction revealed a left accessory inferior vena cava lying along the left side of the aorta and draining into the left renal vein (fig. 1 b). The patient was referred to surgery and underwent a left open colectomy. The double inferior vena cava was confirmed intraoperatively (fig. 2). The postoperative outcome was uneventful and the pathological examination of the operative specimen diagnosed a moderately differentiated (G2) sigmoid adenocarcinoma invading through the muscularis propria into the pericolic tissue (pT3), with no regional lymph node metastases (pN0) – stage IIA, Dukes B.

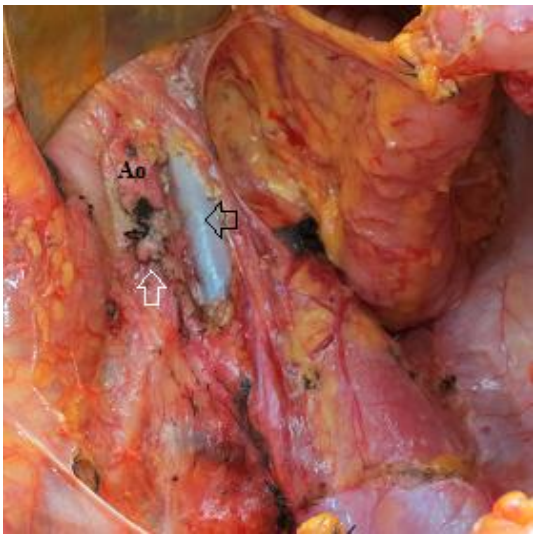

Fig. 1. (a) Axial contrast enhanced abdominal computed tomography showing a pseudotumoral mass on the left side of the aorta (black arrow); (b) Coronal contrast enhanced reconstruction imaging revealed double infrarenal inferior vena cava; the left inferior vena cava (black arrow) continues the left common iliac vein and drains into the left renal vein (Ao, aorta; rIVC, right inferior vena cava; lrv, left renal vein; SMA, superior mesenteric artery)

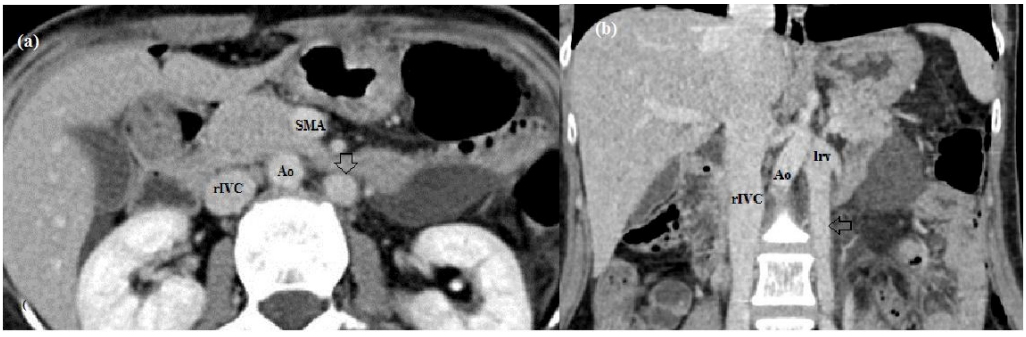

Fig. 2. Intraoperative aspects of the left inferior vena cava (black arrow) after left colectomy with removal of the lymph nodes at the origin of the inferior mesenteric artery from the aorta (Ao); white arrow shows the stump of the inferior mesenteric artery

DISCUSSION

The prevalence of double inferior vena cava in the general population is no more than 3% (1), most of the cases being asymptomatic and diagnosed by radiology (2, 8). However, in a limited number of cases, this rare anatomical variant may have a surgical impact, especially in urology (7, 9) or vascular surgery (4).

The embryogenesis of the inferior vena cava is a complex process. Development of the inferior vena cava is due to appearance, regression, and fusion of three paired embryonic veins: posterior cardinal, subcardinal and supracardinal veins. Normally, the left supracardinal vein regresses, and the right supracardinal vein forms the infrarenal part of the inferior vena cava (1, 2). Persistence of the left supracardinal vein results in a double infrarenal vena cava; usually, the left infrarenal vena cava ends into the left renal vein (1, 2), as seen in the above presented case.

Data in the literature show that double inferior vena cava was misdiagnosed as para-aortic lymphadenopathy in patients with prostate (7), testicular (9), cervical (10) or rectal cancer (8). Thromboses of the double inferior vena cava (6) have been reported. CT scan without the administration of a contrast agent is the most common cause of misdiagnosis (6).

Lymph node metastases are widely recognized as one of the most important prognostic factors after colon cancer resection. Recently it was demonstrated that the presence of lymph node metastases at the origin of inferior mesenteric artery from the aorta strongly correlates with poor prognosis (11). Thus, the identification of a para-aortic lymphadenopathy in sigmoid colon cancer is of utmost diagnostic importance. This is particularly important when a minimally invasive surgery is planned because in these cases there is a poor depth perception, and tactile sensation is diminished (12). Misinterpretating a left inferior vena cava as a lymph node metastasis may lead to such life-threatening complications as severe intraoperative hemorrhages. Nevertheless, laparoscopic approach has been previously demonstrated as feasible and safe in left nefrectomy patients, even in the presence of a double inferior vena cava (3).

A double inferior vena cava was also associated with an increased risk for deep vein thrombosis, especially in young adults, due to inadequate venous return from the lower extremities (5).

The clinical implications of a double inferior vena cava in a patient with colon cancer are related to an increased risk for deep vein thrombosis and misdiagnosis of para-aortic lymphadenopaty. An accurate preoperative diagnosis can be made if computed tomography assessment of vascular structures is made by using intravenous contrast material; during the arterial phase, the veins exhibit little or no contrast (2). Prophylactic use of anticoagulants may theoretically reduce the risk for deep vein thrombosis and pulmonary embolism.

CONCLUSIONS

Double inferior vena cava is a rare congenital anatomical variant that may have significant surgical implications. Awareness of this anomaly is mandatory and the surgeon should be able to distinguish it from para-aortic lymphadenopaty. If a double inferior vena cava is not preoperatively recognized, it can be a source for severe surgical complications.

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EVALUAREA INFLUENȚEI VARIAȚIILOR FACTORILOR METEOROLOGICI ASUPRA STĂRII DE SĂNĂTATE A UNUI LOT DE PACIENȚI DIN MUNICIPIUL IAŞI

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ASSESSMENT OF METEOROLOGICAL FACTORS INFLUENCE ON THE HEALTH OF A GROUP OF PATIENTS IN THE CITY OF IASI (Abstract): **Aim**: Analyzing the meteorological factors influence perception on state of health and evaluation of the awareness of how they act **Material and methods**: The study was carried out between 2010-2011 on a sample of 75 patients from of a cabinet of family medicine în the city of Iasi. The lot included randomly selected persons with age between 18-74 years. They answered a questionnaire with 25 items. The questionnaire Included demographic date (age, education, social, financial situation, the belonging religious) and questions on personal perception and evaluation of the influence of climate and weather conditions on individual state of health, degree to promote beneficial climatic factors în maintaining health and quality of life. **Results**: The istribution of Cases în function of perception implication în meteorological factors în health reveals the following aspects: they showed significantly morecases meteorosensibili from urban areas (45.3%) and meteoro dependentecases from rural areas (10.7%) în our group has revealed several casesmeteorosensibile both females (36%) and the male ones (14.7%) Distribution în the study group depending on the class of diseases revealed predominant rheumatic diseases (36%) and heart disease (33.3%), haematological (20%) mental illness (14.7%) digestive (12%),respiratory diseases and neurological (10.7%). **Conclusions** Meteorological facts does not represent etiological but the favoring or triggering factors în some pathological conditions. Important weather newsletters în informing and educating patients about the risks of meteorological sesnsibili requires that a necessity for maintaining health andquality of life. **Key words**: METEOROLOGICAL SENSITIVE, METEOROLOGICAL ADAPTATIV, ANTICIPATIVE, METEOROLOGICAL DEPENDENT.

Studiile privind impactul factorilor meteorologici asupra stării de sănătate au evidențiat că meteorosensibilitatea interesează aproximativ 1/3 dintre locuitorii zonelor cu climă temperată, manifestând tropism la schimbările vremii. Diagnosticul meteoropatiilor se stabileşte cu dificultate deoarece nu se poate separa acțiunea factorilor meteorologici de întreg complexul de agenți etiologici şi de a separa reacțiile somatice de cele psihoemoționale.

Experții francezi au realizat un studiu de durată, computerizat, care a demonstrat că incidența infarctului de miocard creste în perioadele friguroase, în vreme ce seceta şi canicula din timpul verii favorizează apariția atacului vascular cerebral, exprimat prin pierderea bruscă a cunoştinței şi a

motilității active. De asemenea s-a constatat că atunci când temperatura zilei scade cu 10 grade, frecvența cazurilor de infarct crește cu 13% iar când presiunea atmosferica se modifică cu 10 milibari, rata crește cu până la 12%. Astfel de studii s-au efectuat în mai multe țări din Europa occidentală şi majoritatea au confirmat aceste observații. (1). Cercetări efectuate în Suedia au relevat faptul că migrenele sunt mai accentuate în urmatoarele trei zile după sosirea unui front atmosferic, însoțită de o schimbare bruscă a temperaturii şi presiunii atmosferice iar cercetările efectuate în Japonia au evidențiat faptul că astmul are crize frecvente când vântul îşi schimbă direcția. Un studiu efectuat în Ungaria în cadrul companiei de transporturi auto „Volan Trust", a arătat că variațiile condițiilor atmosferice proprii în special primăverii şi toamnei, generează migrene, oboseală, somnolență sau, dimpotrivă, încordare. Fenomenele constatate la schimbarea vremii afectează în mod nedorit eficiența şi siguranța în trafic a conducătorilor auto. Un studiu similar efectuat la celebrele uzine Mercedes-Benz din Stuttgart, Germania, asupra unui număr de 36.000 de accidente de muncă, a pus în evidență influența fronturilor de aer cald, însoțite de instabilitatea atmosferică, în producerea accidentelor de la locul de muncă. Un alt aspect a fost surprins de únele studii americane efectuate în unitățile de învământ. Concluziilor acestora au arătat că scăderea marcată a presiunii atmosferice este însoțită de diminuarea disciplinei în şcoli, în vreme ce valurile de caldură, merg mână în mână cu creşterea violenței urbane. Studiul unui un front atmosferic care a ucis mii de oameni în Chicago, a evidențiat că nu doar valul de căldură a fost cauza deceselor, ci faptul că a existat o asociere de factori care ar fi putut agrava activitatea cardiacă, concluzionându-se ca fenomenele meteorologice reprezintă factori dinamizatori - favorizanți pentru unele procese patologice (2,3,4).

Studiul nostru a avut următoarele obiective: (i) analizarea implicării factorilor meteorologice în starea de sănătate individuală; (ii) evaluarea percepției subiective, cognitive şi emotionale a influenței factorilor meteorologice în starea de sănătate şi starea de boală individuală şi (iii) evaluarea implicării personale în promovarea factorilor meteorologice în menținerea stării de sanatate şi în prevenirea şi combaterea stării de boala

MATERIAL ŞI METODE

Studiul a fost efectuat în perioada 2010-2011, pe un lot 75 de pacienti ai unui cabinet de medicină de familie din municipiul Iasi. S-au respectat principiile etice de cercetare, pacienții fiind informați cu privire la metoda cercetării şi şi-au exprimat consimțământul. Lotul selectat aleator a cuprins persoane cu vârste între 18 şi 74 de ani. Aceştia au răspuns unui chestionar cu 25 itemi care a inclus date demografice (varsta, sex, studii, mediul social urban/rural, situație financiară, religie) şi întrebări privind percepția şi evaluarea personală a influentei climei şi condițiilor meteorologice asupra stării de sănătate individuală, a gradului de promovare a factorilor climatici sanogeni în mentinerea sănătății şi a calității vieții. Prelucrarea datelor s-a efectuat cu ajutorul programului SPSS 16.0

REZULTATE şi DISCUTII

1. *Caracteristicile lotului de studiu*. Lotul de 75 de pacienti, a inclus 45 (60%) femei şi 30 (40%) bărbați cu un raport F/M= 1,5/1. Au predominat cazurile de sex femi-

nin provenite din mediul urban (48%), însă diferența nu a fost semnificativă statistic față de masculin (32%) comparativ cu distribuția cazurilor din mediul rural (F=12% vs M=8%) (χ^2=0,09; GL=1; p=0,768).

Vârsta medie a pacienților la intrarea în studiu a fost de 42,91 ± 14,50 ani, variind de la 18 la 74 ani: vârsta medie a pentru sexul masculin a fost de 39,67 ani cu variații de la 18 la 68 ani; iar cel feminin de 44,13 cu variații între 19 şi 73 ani.

Nivelul de instrucție determină conştientizarea implicării factorilor sau condițiilor meteorologice în definirea stării de sănătate şi a calității vieții a evidențiat ca 40% au avut nivelul liceal; 33,3% studii superioare; iar 26,7% studii medii. Lotul de studiu a cuprins semnificativ mai multe femei cu nivel mediu de instrucție comparativ cu bărbații (p=0,008); mediul de proveniență a fost preponderent urban la persoanele cu nivel mediu de instrucție (p=0,008); nu s-au înregistrat diferențe cu semnificatie statistica pe grupuri de vârstă (5)

Activitatea profesională a evidențiat existenta a 56% salariati iar 33,3% erau pensionare. Analizarea condițiilor socio-economice din momentul intrării în studiu a evidențiat: condiții nesatisfăcătoare: 15 (20%) - cu venitul lunar mediu de 600 ron;condiții satisfăcătoare: 40 (53,3%) – cu 1000 ron;condiții bune: 15 (20%) - cu peste 1000 ron; 6,7% dintre pacienți nu au precizat venitul familiei.

2. Distribuția cazurilor în relatie cu *percepția cognitiv-emoțională a implicațiilor factorilor de mediu în starea de sănătate* a relevat existenta mai multor cazuri meteorodependente provenite din mediul rural (10,7%) şi meteorosensibili din mediul urban (45,3%); s-au evidențiat mai multe cazuri meteorosensibile atat la sexul feminin (36%), cat şi la masculin (14,7%); la grupul de vârstă de 18- 30 s-au înregistrat diferențe semnificative comparativ cu grupul de grupa de peste 65 ani, unde au fostsemnificative: la grupul 31-65 ani predomină persoanele meteorosensibile (40%) la grupul de peste 65 ani au predominat cazurile meteorodependente (9,3%) (tab. I).

TABELUL I

Semnificații statistice în relatie cu caracteristicile epidemiologice ale lotului de studiu

Răspuns	**Mediul de proveniență**				**Sexul**				**Grupul de vârstă (ani)**					
	Urban[a]		**Rural**		**Masculin**		**Feminin**		**18-30***		**31-65**		**>65****	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Adaptabili	**14**	18,7	**1**	1,3	**5**	6,7	**10**	13,3	**2**	2,7	**10**	13,3	**3**	4,0
Anticipativi	**8**	10,7	**2**	2,7	**3**	4,0	**7**	9,3	**1**	1,3	**8**	10,7	**1**	1,3
Meteoro-sensibili	**34**	45,3	**4**	5,3	**11**	14,7	**27**	36,0	**4**	5,3	**30**	40,0	**4**	5,3
Meteoro-dependenți	**4**	5,3	**8**	10,7	**9**	12,0	**3**	4,0	**1**	1,3	**4**	5,3	**7**	9,3
Semnificație statistică	χ^2=20,13; GL=3; **p=0,0002**				χ^2=8,75; GL=3; **p=0,033**				Comparativ cu 31-65 ani * p>0,05 **** p = 0,003**					

[a]pentru valorile mai mici de 10, proportiile sunt orientative

3. *Analizarea grupurilor de afectiuni* la lotul studiat a evidențiat predominanța bolilor reumatismale 27 (36%) cazuri şi a grupurilor celor cardiace 25 (33,3%), de sange 15 (20%) cazuri, psihice 11 (14,7) digestive 9 cazuri (12%), cu respiratorii şi neurologice câte 8 (10,7%) cazuri (10,7%) (tab. II).

TABELUL II

Diferențe statistice în relatie cu meteotropismul şi categoriile de afecțiuni la lotul de pacienți investigați

Afecțiuni	Meteorotropi				Semnificație statistică
	Meteoro-sensibili		Meteoro-dependenti[a]		
	n	%	n	%	
Cardiovasculare	17	68,0	8	32,0	χ^2=5,47; GL=1; **p=0,019** RR = 2,47
Respiratorii	4	50,0	4	50,0	χ^2=5,13; GL=1;**p=0,023** RR= 5,25
Reumatismale	17	63,0	10	37,0	χ^2=11,55; GL=1;**p=0,0007** RR= 11,55
Psihice	6	54,5	5	45,5	χ^2=5,95; GL=1;**p=0,015** RR= 4,38
Neurologice	4	50,0		50,0	χ^2=5,13; GL=1;**p=0,023** RR= 5,25
Anemie	7	46,7	8	53,3	χ^2=16,1; GL=1;**p=0,00006** RR= 9,51
Metabolice	2	100	0	0	χ^2=0,12; GL=1;p=0,725
Digestive	7	77,8	2	22,2	χ^2=0,29; GL=1;p=0,587

[a]pentru valorile mai mici de 10, proportiile sunt orientative

4. Analizarea *evolutiei simtomatologiei* pe grupuri de afecțiuni corelat cu modificările de vreme diferențele procentuale semnificative au următoarele caracteristici: afectarea cardiovasculară prezintă un risc relativ de 2,47 ori mai mare la pacienții meteoroinfluențabili (p=0,019); afectarea aparatului respirator este asociată semnificativ cu pacienții meteoroinfluențabili (p=0,023); afecțiunile sistemului osteoarticular, în asociere cu factorii de mediu prezintă un risc relativ de peste 11 ori mai mare la pacienții meteoroinfluențabili (p=0,0007); afectarea psihică este dependentă semnificativ de variațiile factorilor meteorologici, cu un risc relativ de aproximativ 6 ori mai mare la grupul de pacienți meteoroinfluențabili (0,015); afecțiunile neurologice sunt asociate pacienților meteoropați, la impactul cu variațiile factorilor meteorologici (p=0,023), riscul relativ fiind de peste 5 ori mai mare la aceştia; sindroamele anemice sunt caracteristice pacienților meteoropați, cu un

risc relativ de 9,51 (p=0,00006); prezența bolilor metabolice, digestive, de piele, renale şi chiar cancerele nu s-au asociat semnificativ cu variațiile factorilor de mediu.

5. Evaluarea *cunostintelor* legate de efectul sanogen (6) al factorilor climatici în mentinerea starii de sanatate şi în prevenirea şi combaterea starii de boala din cei 75 de pacienti intervievati doar 26,7% consulta prognozele meteo; cunoaşterea şi promovarea factorilor climatici în starea de sănătate s-a regăsit la 36% din pacienți; 46,7% dintre cazuri au beneficiat de tratamente în stațiuni balneoclimaterice şi 72% nu cunosc eficiența terapeutică a salinelor.

CONCLUZII

Cercetările noastre au evidențiat ca implicarea factorilor meteorologici în promovarea sănătății şi combaterea stărilor de boala este insuficient cunoscut şi valorificată iar fenomenele meteorologice s-au dovedit a fi factori dinamizatori favorizanți pentru unele procese patologice.

S-a dovedit dificultatea de stabilire a diagnosticului meteoropatiilor existând dificultate în a separa acțiunea factorilor meteorologici de întreg complexul de agenți etiologici şi de a diferenția reacțiile somatice de cele psihoemoționale.

Educarea pacienților asupra riscurilor meteorotrope a fost evidențiată ca principală preocupare pentru medicul de familie.

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LASER MICRODISSECTION – A NOVEL APPROACH FOR ACCESSING THE MOLECULAR BASIS OF CANCER

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LASER MICRODISSECTION – A NOVEL APPROACH FOR ACCESSING THE MOLECULAR BASIS OF CANCER (Abstract): **Background and aim:** It has been known for quite a lot of time that tumours are cellular aggregates of different cells, mainly malignant cells but also immune cells – of which the most well-known are the tumour infiltrating lymphocytes (TIL) and the tumour associated macrophages (TAM). By observing hematoxylin – eosin stained or immunohistochemical stained slides belonging to different areas of the tumour it is clear that there are clusters of malignant cells within the tumour itself that seem to behave differently from the rest of the tumour. Another fact is that different areas of the tumour contain different inflammatory cells which may promote carcinogenesis or may help to confine it. Whereas different immune cells can be recognised by using immunohistochemistry, a satisfactory characterization of the molecular characteristics of the malignant clusters of the tumour cannot be made without further use of different molecular techniques such as different PCR techniques or microarray methods. Laser microdissection thus comes as a valuable aid in choosing exactly which cells will be analyzed further on. **Principle of the method:** Laser microdissection is based on using the energy of a focused laser beam to cut through the thickness of the tissue that is placed on a microscope slide in order to obtain cell samples previously selected by the pathologist through special software. **Conclusion**s: To be able to cut the cells that you want and analyze them further without having them contaminated with other cells means that you can get more insight into the progression of the mutations that occur in these malignant cells, mutations that cause them to become more aggressive or multidrug-resistant. This could in time lead to the discovery of new molecular targets for cancer therapy. **Key words**: LASER MICRODISSECTION, LASER PRESSURE CATAPULTING, CELL CLUSTERS, MOLECULAR ANALYSIS.

Much of the current scientific focus in medical sciences has nowadays shifted on gathering information about the pathogenesis of the disease at a molecular level. The reason for this increasing interest regarding the molecular characteristics of cancer cells has to do with trying to find new approaches for cancer treatment.

A "top-down" strategy for gathering and integrating information about various factors

that could influence the prognosis of the tumour or the treatment should include the assessment of the histological type of the tumour, followed by the analysis of the tumour microenvironment and finally the molecular alterations responsible for the development of the tumour. Such an analysis could lead to a better understanding of the factors that play a role in determining a tumour's outcome and can complete the prognosis issued by the TNM staging thus leading to better patient management.

There has been a lot of struggle to find a good tool for selecting only some of the cells that are of particular interest to the researcher. It is widely known that a tumour mass consists of different cell clusters, belonging to different cellular clones. Sometimes these clusters are clearly defined from one another even on hematoxylin-eosin stained slides and the study of these cells alone, without their neighbouring cells could be interesting from the point of view of discovering the more intimate mechanisms that lead to metastasis, multidrug-resistance, local relapse or more precisely the mechanisms that lead to the selection of clones that induce these effects.

High throughput methods for molecular studies have become available but despite the increasing complexity of these new methods their accuracy is influenced by the selection of the study material (1, 2). For many types of assays the use of homogenised tissue samples can result in evening-out the results because of mixing cells that are heterogeneous in nature. The reasons behind this is the fact that all human tissues and especially malignant tissues are mixtures of different cell types from which procuring a pure sample may help to acquire meaningful data.

The concept of tumor heterogeneity is increasingly being recognized, and it will be important to define and analyze separately subpopulations of cells within a neoplasm to identify properties that might be unique to them.

Some of the techniques that have been used so far in attempting to isolate pure cellular populations are cell cultures, flow cytometry and different microdissection techniques. Each of them has proven to be useful to a certain extent. Flow cytometry for example is a technique that has been used extensively, but it comes with the disadvantage of having to use a suspension of cells and this means it has limited usefulness in studying solid tumors.

Cell cultures are another example that has limited applicability because of the fact that cells being cultivated although belonging to a cell line that is of interest to the researcher do not grow in the exact same environment that the tumor does. This means that they are not subject to the same selection pressures and accordingly they may be unfit for some research purposes. For example there have been reported differences in gene expression between primary colon cancer cells and cultivated cell lines (3).Early research had focused on grafting tumor tissue on laboratory animals but this technique has been abandoned due to it being expensive and time consuming.

We mentioned earlier the use of some microdissection techniques, the most rudimentary of which involved using a fine needle to scrape off the tissue of interest from a histological section (4). An advanced version of this technique is the non-laser based microdissection – a technique that uses a piezoelectric micro-chisel that vibrates to ultrasonic frequencies (5). Yet another improvement to the technique above is a system that uses a micromanipu-

lator for extracting single cells suitable for single-cell PCR (6).

Another technique that was used was coating the cells of interest with a pigment that prevents ultraviolet light from destroying the DNA of the cells of interest and then subjecting the whole tumor section to ultraviolet light (7). By the use of this technique cellular studies could be performed only on the genetic material of the cells that were of interest for the researcher.

The most intensively used microdissection technique currently applied is laser capture microdissection.

THE PHYSICAL PRINCIPLE BEHIND LASER CAPTURE MICRODISSECTION

Laser capture microdissection is "a recently developed technique that allows the rapid and reliable procurement of pure populations of cells from tissue sections, in one step, under direct microscopic visualization" (8). This method was developed at the National Cancer Institute (NCI) for the analysis of populations of cells from tumors. A commercial instrument, developed by NCI and Arcturus Engineering Incorporated (Mountain View, California, USA), became available within 12 months of the first publication that described the method(9).

The principle underlying LCM is simple. It involves cutting the cells that are of interest to the researcher by means of a laser beam coming from a laser generator that is coupled into the microscope. The laser beam cuts away the sections previously selected by the pathologist either by the movement of the laser beam or by the movement of the microscope's stage. The setup can involve either an inverse microscope or a normal one, depending on the means by which the sampled cells are transferred into the PCR tubes (8).

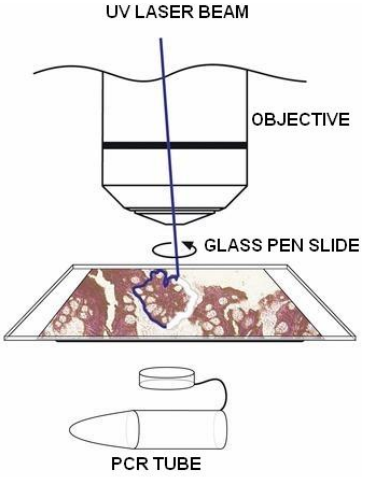

Fig. 1. Schematic representation of a laser microdissection system in which the dissected sample is collected through gravity - adapted from the Leica official website (10).

Lasers used are either ultraviolet (UV) or infrared (IR). The UV spectrum can be split into three regions UV-A, UV-B and UV-C. The absorption peaks of biomolecules such as DNA is located somewhere between 260 and 280 nm – which corresponds to UV-C. This is one reason why UV-A is used in laser microdissection. Another reason for using UV-A laser is better spatial resolution. This why 355 nm is the wavelength of lasers being used in most of the commercially available laser microdissection systems. When compared to IR lasers, UV laser have the advantage of being faster and able to cut through thicker sections. However UV lasers could supposedly produce lesions when very small cells are cut due to the high energy that they produce. Compared to UV laser, IR laser has the major advantage of being less harmful to the dissected cells, but has

the great disadvantage of being slower and requiring special slides.

The energy of the lasers being used is important considering the fact that section thickness or hardness is variable (5 – 20µm sections can be processed by laser microdissection) (8). This is the reason why the newest laser microdissection systems offer adjustable energy lasers. Besides the energy of the laser, the pulse duration is important because shorter pulse duration means faster and more precise cutting by depositing strongly localized energy at well-defined locations. The laser beam is focused through the lens system into a micron-size spot diameter that allows the laser beam to carry enough energy in order to cut through the tissue.

The transfer of the selected cells can be done by so-called contact methods or by contact-free methods. In the first instance histological sections that are to be processed using this method have to be placed on specially designed slides covered with ethylene vinyl acetate (EVA). The reason why the EVA film is used is that when this material interacts with the laser beam it melts and fuses to the area of the slide that is intended to be removed. The selected cell or cell group fused with the EVA film is then removed and further processed. When using this method the EVA film is placed on the cap of a PCR tube. This is the original laser microdissection method described that was using IR laser. The need for these special caps is one of the major disadvantages of using IR laser (8).

There are some other contact methods that use different adhesive plastic films, but all these methods have the disadvantage of possible contamination with foreign material.

Another group of transfer methods are contact – free and they involve using gravity or different "catapulting" technologies. The easiest of these methods is based on letting the sample fall into the cap beneath under the influence of gravity. This method requires the use of an upright microscope and PEN (polyethylene naphthalate), PET (polyethylene terephthalate) or POL (polyester) membrane slides.

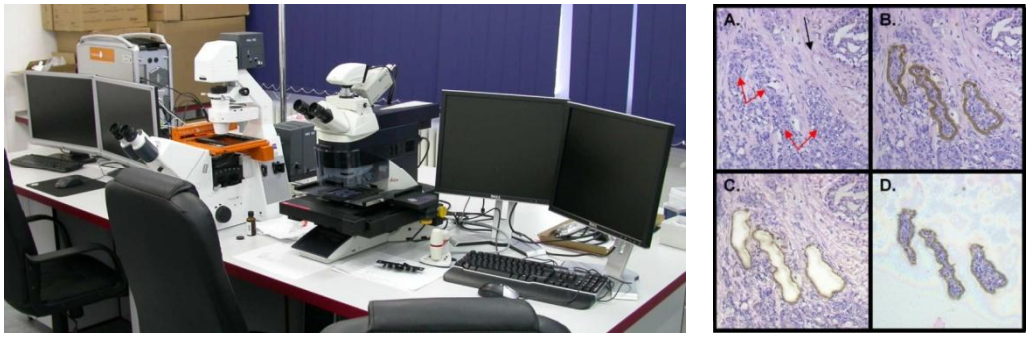

Fig. 2. The Leica LMD6000 microdissection system available in the Genetics and Immunology Laboratory -"Sf. Spiridon" Hospital, Iaşi and the steps involved in laser microdissection (A. visualization of the slide in order to chose the cells that could be of interest; B. selection of the cells; C. view of the slide after microdissection. D. microdissection product) (11).

Other contact-free methods are based on different "catapulting" technologies. The first of them is a method that is already implemented and is based on laser pressure catapulting. The principle behind laser pressure catapulting has been asserted by Schütze et al in 1998 and confirmed by Niyaz et al in 2005 (12,13). To allow the sampled cell or group of cells to be lifted out of the plane of the slide a laser beam is focused below the focal plane of the sample through an objective with high numerical aperture. The power generated by this unfocused laser beam can lift the selected cells out of the object plane and transports them along the axis of the beam (14).

In order to have a more in depth understanding on how laser pressure catapulting works we have to stress the fact that the way in which a laser beam interacts with tissue depends on some characteristics of the tissue and the laser: absorption characteristics of the tissue, wavelength, numerical aperture of the objective, whether or not the laser is pulsed. The way in which laser energy is used to cut tissue has been investigated by Vogel and Vengupalan and they concluded that laser pressure catapulting relies on plasma formation supported by linear absorption of energy that leads to mechanical effects such as cavitation or shock wave formation (15). The process takes a minute interval of time that should be short enough not to affect other cells situated in the proximity of the targeted cells (16, 17, 18).

The newest transfer method that involves the use of laser is LIFT (Laser Induced Forward Transfer) – a method that involves the transfer of minute quantities of material as droplets and their deposition on another surface (19).

APPLICATIONS OF LASER MICRODISSECTION SYSTEMS

By the use of this system cells can be selectedbased on their morphology, on immunohistochemical staining or by using in situ hybridisation methods to select cells with a specific genotype. Immunohistochemistry is the basis of a novel microdissection technique – expression microdissection. After immunohistochemical staining a special film is applied to the slide and the whole slide is then irradiated by a light or laser source. When the film is removed the positively stained cells are removed along with it because they become adherent to the film after the irradiation process. Such a system is virtually an operator-independent system that can be used by a researcher with minimal or no histopathology training (20).

Laser microdissection is currently being used on a variety of substrates: living cells, frozen sections of tissue, but most importantly it can also be used on formalin fixed, paraffin embedded sections. The fact that it can be used on such paraffin embedded sections makes laser microdissection an important tool for analyzing archived pathological material.

After the dissection process takes place the genetic material of the cell has to be extracted. For DNA extraction proteinase K is used. PCR can then be performed on the resulting DNA and finally the DNA can be sequenced or it can be used in Southern blotting (21).

One problem with using DNA material from paraffin embedded tissue is that the use of formalin fixation leads to a certain degradation of the genetic material which is traduced into a smaller amount of amplification product at the end of the PCR process compared to frozen tissue. Because of this a

methanol fixation method was proposed before the paraffin embedding. The results of using methanol instead of formalin have been investigated by Maitra et al who have shown that the DNA obtained from samples fixed by their protocol could amplify products up to 989 base-pairs compared to only 268 base-pairs when the DNA was obtained from formalin fixed samples (22). Such DNA samples can then be used in loss of heterozygosity studies or single strand conformational polymorphism assays.

DISADVANTAGES OF THE METHOD

The major disadvantage especially in the current economic climate of our country is price. Besides the initial sum that has to be invested in buying the system one must take into account the need for special slides which can be considered costly compared to slides that are used in normal microscopy for example.

Most of the other disadvantages come from the fact that the procedure requires a researcher with histopathology training and from the fact that cell selection requires a lot of time from the researcher.

From the point of view of its output the method does not allow for comfortable procurement of large numbers of cells for different array-based methods or proteomic analysis and is hard to use in the case of scattered cells. Sometimes the need may rise for subcellular dissection and currently this technique may not be accurate enough for this purpose.

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CARDIOTOXICITATEA LA ANTRACICLINE. MECANISME, METODE DE DEPISTARE PRECOCE, POSIBILITĂŢI DE INFLUENŢARE TERAPEUTICĂ

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CARDIOTOXICITY OF ANTHRACYCLINES (Abstract): It is a well known fact that the antitumor agents like the anthracyclines induces a dose-related cardiotoxicity and more to add, the new agents used in the treatement of breast cancer may stimulate these cardiotoxic effects, causing in the end an increased morbidity and mortality rate by cardiovascular diseases. Cardiotoxicity caused by anthracyclines can be divided into 3 types: acute (during administration), early (several days to month following administration) and late (years to decades following exposure to anthracyclines). Mechanisms of this cardiotoxicity are very complex: myocyte damage has been attributed to the production of toxic oxygen free radicals, which cause lipid peroxidation of membranes leading to vacuolation, irreversible damage and myocyte replacement by fibrous tissue. Clinical manifestations of anthracycline cardiotoxicity are variable, depending in part upon the time course of appearence (acute, early or late). A number of risk factors for the development of anthracyclines cardiotoxicity have been identified. The strongest predictor is cumulative dose, age, concomitant administration of other chemotherapeutic agents and underlying heart disease. Approaches to reducing anthracyclines cardiotoxicity involved the use of novel infusion protocols, the development of new anthracycline compounds, the use of adjunctive agents, endomyocardial biopsy and noninvasive monitoring of cardiac function. **Key words**: ANTHRACYCLINES, CARDIOTOXICITY, OXYGEN FREE RADICALS

Antraciclinele (AC) sunt printre cei mai activi agenţi utilizaţi în tratamentul cancerului de sân, utilizarea lor în regimuri terapeutice combinate îmbunătăţind atât evoluţia bolii, cât şi supravieţuirea acestor pacienţi (1, 4, 16). Din nefericire, utilitatea clinică a antraciclinelor este limitată de toxicitatea cardiacă, prin efect cumulativ doză-dependent, toxicitate care în final, duce la insuficienţă cardiacă (4, 5, 6, 16).

Paralel cu efectele benefice asupra cancerului creşte şi numărul pacienţilor care dezvoltă fenomene de cardiotoxicitate. Mai mult, noii agenţi utilizaţi în tratamentul cancerului de sân pot potenţa aceste efecte cardiotoxice (ex. trastuzumab).

Antraciclinele au fost izolate din culturi de *Streptomyces peucetius* (specie de actinobacterii) de către Federico Arcamone, în 1960 (4). În timp au devenit componentă esenţială în tratamentul cancerului de sân, în combinaţie cu terapia adjuvantă, reprezentând standardul în stadiile precoce de boală. Mai sunt utilizate în tratamentul anu-

mitor forme de leucemii, limfoame, cancer uterin, ovarian, pulmonar.

Cele mai utilizate sunt doxorubicina şi epirubicina, derivat sintetic de doxorubicină. Alţi agenţi utilizaţi sunt: daunorubicina, idarubicina, valrubicina.

Se cunosc **3 mecanisme de acţiune** (4, 5, 6): 1) Inhibarea sintezei de ADN şi ARN prin intercalarea între lanţurile de ADN şi ARN, prevenind replicarea celulor canceroase cu creştere rapidă; 2) Inhibarea topoizomerazei II prin aceasta blocând transcripţia şi replicarea ADN; 3) Producerea (mediată de fier) a radicalilor liberi de oxigen care lizează ADN-ul şi membrana celulară.

Utilizarea pe termen lung este limitată de apariţia cardiomiopatiei şi a insuficienţei cardiace (4). Efectele toxice ale antraciclinelor la supravieţuitorii de durată cu cancer, pot duce la creşterea mortalităţii şi morbidităţii cardiace. Există dovezi care arată că efectele cardiotoxicităţii cresc la supravieţuitorii pe termen lung, de la 2% după 2 ani la 5% după 15 ani.

Cardiotoxicitatea la antracicline poate fi împărţită în 3 tipuri: acută, precoce şi tardivă, în funcţie de relaţia cu tratamentul.

Forma acută apare în timpul perfuziei cu AC sau în prima săptămînă. Tahicardia sinusală este cel mai obişnuit simptom, însă au fost raportate şi aritmii (tahicardie ventriculară). Au fost descrise: miopericardite şi insuficienţă ventriculară stângă acută. Această formă se poate însoţi de modificări electrocardiografice, cum ar fi: modificări nespecifice ale segmentului ST şi undei T, scăderea amplitudinii complexului QRS, alungirea intervalului QT, aritmii (tahicardie sinusală, aritmii supra-şi/sau ventriculare, tahicardii joncţionale), blocuri atrioventriculare sau de ramură. Incidenţa formei acute este aprox. 1% şi de obicei dispare la oprirea tratamentului. Nu este clară relaţia între toxicitatea acută şi dezvoltarea ulterioară a cardiotoxicităţii întârziate (7).

Cardiotoxicitatea precoce apare în primul an după tratamentul cu antracicline. Pacienţii pot dezvolta modificări electrofiziologice, disfuncţie ventriculară stângă, simptome de insuficienţă cardiacă manifestă. Momentul maxim de apariţie al acestor tulburări este la 3 luni de la ultima doză de antracicline, iar mortalitatea la aceşti pacienţi este ridicată.

Debutul tardiv al cardiotoxicităţii este la peste un an după terminarea tratamentului. Pacienta iniţial asimptomatică poate dezvolta disfuncţie VS, insuficienţă cardiacă, aritmii ventriculare severe şi chiar moarte subită (4).

Unii autori folosesc termenul de cardiotoxicitate „cronică" forma care apare în timpul primului an de tratament şi cardiotoxicitate „cu debut tardiv" forma care apare după un an de la întreruperea completă a tratamentului. Ca manifestări ale acestui tip de cardiotoxicitate s-au descris: scăderea fracţiei de scurtare (cu o incidenţă de 18%) şi creşterea postsarcinii, anomalii care preced o viitoare decompensare cardiacă.

Cardiotoxicitatea tardivă la antracicline se pare că este mai frecventă la vârste tinere şi la sexul feminin (1, 4, 16).

Mecanismul cardiotoxicităţii AC este încă incomplet elucidat. Modificările miocardice care apar după tratament includ: pierderi de miocite prin apoptoză sau necroză, pierderea de miofibrile, distensia reticulului sarcoplasmatic (prin interferarea cu receptorii rianodini), balonizare mitocondrială.

Una din ipotezele cardiotoxicităţii induse de doxorubicină este cea conform căreia doxorubicina ar creşte speciile reactive de oxigen (ROS) în mitocondriile miocitelor,

mai mult decât în alte ţesuturi. AC pot induce generarea de radicali liberi de oxigen pe 2 căi principale: o cale nonenzimatică care utilizează fierul şi un mecanism enzimatic care utilizează lanţul respirator mitocondrial. Radicalii liberi sunt toxici puternici putând provoca leziuni directe ale proteinelor, lipidelor şi ADN. Miocitele adulte sunt cele mai susceptibile. Administrarea doxorubicinei la om este urmată de creşterea nivelelor plasmatice de ROS şi produşi ai peroxidării lipidelor şi scăderea nivelelor plasmatice şi tisulare de antioxidanţi. Nivelul stresului oxidativ indus de doxorubicină este de 10 ori mai mare în cord decât în ficat, rinichi şi splină.

Studiile la nivel de gene pe şoarece au arătat că esenţială pentru apariţia efectelor cardiace ar fi HER2, o protooncogenă, membră a familiei erbB de tirozinkinaze transmembranare, iar deleţia HER2 ar duce la apariţia cardiomiopatiei dilatative (1, 4, 5, 7, 9). La şoarecele deficient în proteina HER2 sau în ligandul său asociat neuregulina (NRG), un peptid paracrin cu rol de mesenger care activează HER 2, antraciclinele au rol în declanşarea disfuncţiei ventriculare stângi. Aceste observaţii au furnizat o explicaţie pentru cardiotoxicitatea crescută apărută după administrarea concomitentă de antracicline şi trastuzumab, un anticorp monoclonal antiHER2. Ca răspuns la expunerea acută la antracicline, calea de supravieţuire a cardiomiocitelor este activată de către neuregulinele care leagă HER2: heterodimerul HER4, prevenind moartea cardiomiocitelor. Traztuzumabul, prin inhibarea receptorului HER2 interferă cu această cale şi favorizează efectele cardiotoxice ale antraciclinelor.

De asemenea, se pare că antraciclinele produc inhibiţia selectivă a expresiei unor gene din miocite, cum ar fi cele pentru actină, troponină, lanţurile uşoare de tip 2 pentru miozină, izoforma M pentru creatinkinază, care pot explica pierderea de miofibrile asociată cardiomiopatiei. Aceste fenomene se adaugă injuriei produse de eliberarea de radicali liberi, supraîncărcării celulare cu calciu, perturbărilor funcţiei adrenergice, eliberării de amine vasoactive şi toxicităţii celulare produse de metaboliţii doxorubicinei. De asemenea, eliberarea de citokine proinflamatorii (implicată şi în alte forme de disfuncţie ventriculară) este un factor adjuvant în apariţia cardiotoxicităţii la antracicline (10).

Supraîncărcarea intracelulară cu calciu poate duce la moarte celulară. Doxorubicina favorizează influxul de calciu în miocit şi eliberarea de Ca din reticulul sarcoplasmic. Injuria membranei celulare de către radicalii liberi pare să favorizeze acest influx intracelular de calciu, sugerând că cele 2 mecanisme ar fi în legătură (5). La acestea se adaugă disfuncţia adrenergică care constă în fenomenul de *downregulation* a receptorilor beta adrenergici din miocard (4).

Studii recente au arătat că citokinele proinflamatorii circulante pot fi legate strâns de evoluţia disfuncţiei ventriculare şi a dilataţiei cardiace (4, 5, 6, 10). Doxorubicina induce eliberarea factorului alfa de necroză tumorală din macrofage şi a interleukinei 2 din monocite, ambii cu efecte cardiotoxice, prin intermediul unor receptori specifici la nivel miocardic. Eliberarea de citokine este diferită însă în funcţie de tipul tumorii (4).

În ceea ce priveşte cardiotoxicitatea tardivă la antracicline, ea apare la pacienţii trataţi cu antracicline, după o perioadă destul de lungă de compensare. Disfuncţia ventriculară progresivă apare la distanţă de injuria miocardică iniţială, fiind posibilă

intervenţia unor factori *trigger*: sarcina, intervenţii chirurgicale, infecţii virale acute etc.

Factori de risc ai cardiotoxicităţii la antracicline:

Cel mai puternic factor de risc se consideră a fi doza cumulativă. Alţi factori de risc potenţiali sunt: frecvenţa mare a curelor, vârsta (atât cea înaintată, cât şi vârsta foarte tânără), sexul feminin, coexistenţa altor factori de risc cardiovascular sau a unei boli cardiace preexistente, HTA, iradierea concomitentă mediastinală, asocierea altor agenţi chimioterapici. Este încă neclar dacă aceşti factori acţionează în sens aditiv cu antraciclinele sau le potenţează efectul (1, 4).

O doză cumulativă crescută de antracicline este cel mai recunoscut factor de risc pentru injuria cardiacă şi rămâne în continuare cel mai bun predictor pentru disfuncţia cardiacă subsecventă. Primele observaţii au fost ale lui Von Hoff într-o analiză retrospectivă care a arătat că toxicitatea la antracicline şi insuficienţa cardiacă au fost dependente de doză, iar incidenţa complicaţiilor a crescut paralel cu doza de doxorubicin. Procentul estimat de pacienţi care au dezvoltat insuficienţă cardiacă a fost de 3% la doza de 400 mg/m^2 şi a crescut la 7% la doza de 550 mg/m^2 şi la 18% la doza de 700 mg/m^2. Aceasta a dus la recomandarea ca doza limită cumulativă de doxorubicin de-a lungul vieţii să nu depăşească 450-550 mg/m^2. Studiile ulterioare au confirmat această observaţie cu menţiunea că stabilirea dozei cumulative trebuie să ţină cont şi de vârstă, precum şi de alţi factori de risc asociaţi cu impact pe mortalitatea de cauză cardiovasculară (1, 4).

Vârsta. Există studii care au sugerat faptul că efectul cardiotoxic al antraciclinelor este mult mai pronunţat odată cu înaintarea în vârstă. Astfel, în sprijinul acestei idei vine o analiză retrospectivă reprezentată de 3 trialuri clinice care au cuprins 630 de pacienţi trataţi cu doxorubicin, în care Swain şi colab. au arătat că pacienţii cu vârste peste 65 de ani ar fi de 2,25 ori mai predispuşi să dezvolte afectare cardiacă după terapia cu antracicline, comparativ cu cei cu vârste sub 65 ani, dar efectul este mai pronunţat la doze cumulative peste 400 mg/m^2. Numărul pacienţilor din acest subgrup este însă mic. Alte studii (*SEER – Surveillance, Epidemiology and End Results*) au raportat o creştere de 2 ori a riscului de cardiomiopatie şi cu 40% a riscului de insuficienţă cardiacă la femeile cu cancer de sân în fază precoce, tratate cu antraciclină. Aceste studii au arătat că insuficienţa cardiacă a fost asociată cu vârsta, rasa neagră, prezenţa altor comorbidităţi, a factorilor de risc cardiovascular şi a unui istoric de boală cardiacă. Riscul de insuficienţă cardiacă în aceste studii a fost mai mare decât în alte trialuri ce au utilizat altă medicaţie şi în care pacienţii au avut vârstă mai tânără (1, 2, 4).

Metode de depistare a cardiotoxicităţii la AC. Detectarea cardiomiopatiei induse de antracicline în stadiul subclinic, utilizând fracţia de ejecţie a VS în repaus şi fracţia de scurtare este o metodă suboptimală, întrucât dozele convenţionale de antracicline determină injurie miocardică permanentă şi reducerea rezervei funcţionale a miocardului. În plus, mecanismele acestei cardiotoxicităţi (deja amintite mai sus) sunt mult mai complexe la nivel celular: eliberare de radicali liberi, disfuncţie adrenergică, supraîncărcare cu calciu, eliberarea de citokine cu efect cardiotoxic. Billingham et al. au descris o metodă de

apreciere a cardiotoxicităţii la antracicline pe baza unui scor semicantitativ bazat pe metode histologice – respectiv biopsia endomiocardică- care se pare că se corelează bine cu doza cumulativă de antraciclină. Se pare că biopsia endomiocardică este cel mai fidel indicator de apreciere a cardiotoxicităţii la antracicline. Erori pot apare în cazul biopsiei la nivelul VD dacă leziunile sunt focale sau dacă injuria celulară afectează predominant VS. De asemenea limitele acestei metode derivă şi din imposibilitatea efectuării pe scară largă, a repetării ei la nevoie şi mai ales la copii (12).

Angiocardiografia cu radionuclizi este utilizată pe scară largă pentru a monitoriza cardiotoxicitatea precoce indusă de antracicline. Pe baza acestei metode, Allexander şi colab. au sugerat că scăderea fracţiei de ejecţie cu 15% sau o fracţie de ejecţie de bază sub 40% identifică pacienţii cu risc crescut de decompensare cardiacă. Metoda are însă limite legate de faptul că determinarea fracţiei de ejecţie de repaus are senzitivitate scăzută în ce priveşte detectarea cardiotoxicităţii precoce la antracicline întrucât nu există o corelaţie între modificările morfologice care pot fi semnificative şi fracţia de ejecţie care poate fi încă în limite normale. Studii cu radionuclizi la efort pot creşte şansele de depistare a cardiotoxicităţii precoce. Limita metodei este legată de imposibilitatea ca unii pacienţi să fie supuşi unui test de efort (9, 13).

Ecocardiografia bidimensională este o altă metodă noninvazivă de apreciere a cardiotoxicităţii la antracicline, mai ales la copii. Parametrii cei mai utilizaţi sunt fracţia de ejecţie şi fracţia de scurtare, cu aceleaşi limite ca şi în cazul metodei precedente (14).

Parametrii care caracterizează funcţia diastolică au fost de asemenea studiaţi în ceea ce priveşte utilitatea lor în depistarea şi aprecierea injuriei cardiace induse de antracicline. Cardiotoxicitatea cronică la antracicline determină îngroşarea importantă de tip fibros a endocardului cu impact asupra relaxării şi umplerii ventriculare ducând în final la disfuncţie diastolică (scăderea raportului E/A şi creşterea timpului de relaxare izovolumic), modificări care au apărut înaintea scăderii fracţiei de ejecţie. Conform majorităţii studiilor, disfuncţia diastolică precede disfuncţia sistolică la pacienţii trataţi cu antracicline. La copiii trataţi cu antracicline însă, se pare că fracţia de scurtare este un parametru mult mai fidel decît anomaliile de funcţie diastolică în aprecierea cardiotoxicităţii la antracicline.

Dopplerul tisular permite aprecierea velocităţilor sistolice şi diastolice ale pereţilor ventriculari şi ale inelului mitral, fiind mult mai performant în aprecierea funcţiei diastolice a VS comparativ cu ecocardiografia Doppler convenţională (1, 14). Dopplerul tisular poate evidenţia modificări ale funcţiei regionale care nu pot fi obiectivate prin simpla apreciere a fracţiei de ejecţie.

O metodă şi mai fidelă de cuantificare a deformării regionale a VS bazate pe principii de inginerie mecanică este „*strain rate*" care detectează modificările precoce ale funcţiei sistolice a VS şi care se corelează cu markerii biochimici şi inflamatori (1, 14).

Detectarea cardiotoxicităţii tardive la antracicline

Apariţia la distanţă de terapia cu antracicline a unor disfuncţii la nivel cardiac au impus necesitatea unor metode de depistare precoce a acestor modificări, mai ales la pacienţii asimptomatici. Întrucât nu există întotdeauna o corelaţie bună între fracţia de ejecţie şi cea de scurtare cu scăderea capacităţii funcţionale s-a propus utilizarea ecocar-

diografiei de stres cu dobutamină şi a angiografiei cu radionuclizi combinată cu testare de efort, mai fidele în depistarea precoce a disfuncţiei ventriculare stângi subclinice.

Disfuncţia diastolică poate fi de asemenea observată precoce la pacienţii care vor prezenta tardiv disfuncţie sistolică. În urmărirea pe termen lung a pacienţilor cu leucemie trataţi cu antracicline au fost observate cazuri de cardiomiopatie restrictivă după tratamentul chimioterapic. Au fost identificaţi câţiva parametri asociaţi cu un prognostic nefavorabil: scurtarea timpului de decelerare a undei E, creşterea velocităţii undei E (aspectul de pseudonormalizare a raportului E/A). Disfuncţia diastolică se corelează strâns cu simptomele congestive pulmonare la pacienţii cu cardiomiopatie dilatativă indusă de antracicline.

Majoritatea acestor metode amintite mai sus obiectivează anomalii ale funcţiei cardiace, însă după ce au avut loc modificări structurale şi pierderi celulare semnificative.

Din acest motiv pentru detectarea şi prevenirea cardiotoxicităţii într-un stadiu cât mai precoce, se consideră că markerii biochimici sunt mult mai fideli (1). Dintre aceştia, studiaţi au fost: peptidul natriuretic cerebral (BNP), endotelina I, troponinele (T şi I), mioglobina, creatinkinaza (CK, izoenzima MB) care sunt markeri relevanţi ai injuriei miocardice şi au fost asociaţi cu afectarea funcţiei sistolice şi diastolice a VS la pacienţii trataţi cu antracicline. Injuria miocardică sub tratamentul cu antracicline este cauzată de stresul oxidativ şi implicit speciile reactive de oxigen. Reducerea doxorubicinei de către enzimele microzomale NADPH dependente duce la generarea ROS. Celulele cardiace, prin intermediul unei dehidrogenaze NADH mitocondriale (absentă în alte tipuri celulare), generează cantităţi foarte mari de ROS în prezenţa doxorubicinei. Mai mult s-a sugerat că citokinele proinflamatorii pot contribui la apariţia cardiomiopatiei dilatative (7, 9, 10). Interleukina 6 şi receptorul său solubil (sIL-6R) sunt crescute la pacienţii cu insuficienţă cardiacă, în special la cei cu caşexie şi decompensare edematoasă (1). Nivelul citokinelor creşte paralel cu clasa NYHA. Alţi markeri studiaţi în ceea ce priveşte cardiotoxicitatea AC au fost factorul de necroză tumorală (TNF alfa) şi receptorul său solubil de tip 1 (sTNF alfa R1), care s-au dovedit markeri fideli de apreciere a injuriei miocardice cu rol predictiv pentru apariţia afectării cardiace subsecvente, anumite studii stabilind chiar o corelaţie între nivelul acestor markeri şi gradul apoptozei (1,4, 7, 9, 10).

S-a observat de asemenea că nivelul glutation peroxidazei (GPx) scade semnificativ după administrarea de AC. Acelaşi comportament îl are superoxid dismutaza (SOD) şi catalaza (1, 4).

Astfel, detectarea precoce a cardiotoxicităţii induse de antracicline prin determinarea acestor markeri s-a dovedit extrem de utilă.

Cum prevenim şi cum tratăm cardiotoxicitatea la antracicline ?

Orice metodă destinată minimalizării efectelor cardiotoxice ale antraciclinelor tebuie să menţină efectul antineoplazic. Strategiile (altele decît monitorizarea cardiacă) elaborate pentru prevenirea cardiotoxicităţii declanşate de antracicline au fost orientate în 3 direcţii principale.

În primul rând, metoda de eliberare a citostaticului poate influenţa cardiotoxicitatea acestuia. Se pare că administrarea perfuziei continui 48-96 de ore cu doxorubicin ar avea efecte cardiotoxice mai reduse comparativ cu administrarea mai rapidă

(15-20 minute). În al doilea rând, administrarea unor analogi structurali ai antraciclinelor ca mitoxantrona şi idarubicina are beneficii încă nedeterminate între cardiotoxicitate şi efectul antineoplazic. În al treilea rând, agenţii cardioprotectori sunt utilizaţi pentru a diminua efectele antraciclinelor asupra cordului.

Există studii care au utilizat inhibitori de enzimă de conversie pentru tratamentul pacienţilor care au prezentat disfuncţie contractilă după tratament cu antracicline. Se aşteaptă încă rezultate referitoare la efectul preventiv al acestor inhibitori. De asemenea, administrarea tratamentului digitalo-diuretic la pacienţii care au dezvoltat insuficienţă cardiacă pare a fi benefic (4, 16).

Dezrazoxane (ICRF-187) este un cardioprotector utilizat în unele studii pentru a reduce riscul de cardiotoxicitate la antracicline, fără a afecta răspunsul la chimioterapie şi supravieţuirea globală (15). Administrarea sa concomitent cu tratamentul chimioterapic se pare că atenuează efectele cardiotoxice. ICRF 187 este un agent chelator de fier care reduce generarea de radicali liberi produsă de antracicline, inclusiv la copii. Este aprobat şi utilizat clinic doar la femeile cu cancer de sân metastatic după o doză cumulativă de de doxorubicin de 300 mg/m^2, întrucât se pare că ar diminua efectul şi eficacitatea antitumorală a antraciclinelor în stadiile precoce de cancer.

Studii efectuate pe animale de laborator au indicat că probucolul – un hipolipemiant similar ca structură cu vitamina E furnizează cardioprotecţie faţă de injuria miocardică indusă de doxorubicin. Probucolul creşte la nivel miocardic activitatea glutation peroxidazei şi superoxid dismutazei prin aceasta având efect antioxidant. Îmbunătăţirea statusului antioxidant al miocardului are loc concomitent cu reducerea peroxidării lipidelor indusă de antracicline (8, 16). Probucolul nu interferă cu efectele antitumorale ale antraciclinelor. Sunt necesare studii pentru extrapolarea acestor rezultate la om.

Reducerea supraîncărcării celulare cu calciu produsă de antracicline constituie o altă ţintă terapeutică în cardioprotecţie. Experimentele pe animale au sugerat că antagoniştii de calciu reduc cardiotoxicitatea indusă de antracicline. Blocanţii beta-adrenergici previn supraîncărcarea cu calciu a cardiomiocitelor, indusă de antracicline, contracarând efectul cardiotoxic al catecolaminelor. Beta blocantele lipofile ca propranololul previn de asemenea peroxidarea lipidelor mediată de radicalii liberi. Totodată, metoprololul s-a dovedit eficient în cardiomiopatia indusă de antracicline, cardiomiopatia dilatativă idiopatică şi insuficienţa cardiacă ischemică. Se pare că administrarea precoce, încă din stadiile subclinice a inhibitorilor ECA şi a beta blocantelor ar putea reduce efectele cardiotoxice ale antraciclinelor şi implicit mortalitatea legată de aceasta (1, 3, 4, 11, 16).

De asemenea se pare că utilizarea formulelor lipozomale de daunorubicin şi doxorubicin par să fie mai puţin citotoxice pentru ţesutul cardiac comparativ cu formulele nonlipozomale.

În concluzie, cardiotoxicitatea la antracicline limitează efectele benefice anticanceroase ale acestora, din acest motiv se impune necesitatea unor metode cu senzitivitate crescută în ceea ce priveşte depistarea precoce a disfuncţiei cardiace. În plus, specificitatea oricărui test trebuie să permită cu acurateţe analiza raportului cost - beneficiu în ceea ce priveşte utilizarea terapiei antitumorale.

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BIOCOMPATIBILITY TESTING OF CHITOSAN HYDROGELS

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BIOCOMPATIBILITY TESTING OF CHITOSAN HYDROGELS (Abstract): Chitosan is a linear, natural cationic polysaccharide comprising β-1,4 linked glucosamine and N-acetyl-D-glucosamine. Hydrogels of chitosan were prepared by crosslinking with varying amounts of glutaraldehyde. It can be used as a bacteriostatic, fungistatic and coating agent, and the gels and suspensions may play the role of carriers for slow release or controlled delivery of drugs, as an immobilizing medium and an encapsulation material. **Material and methods:** Chitosan and glutaraldehyde were used to prepare the hydrogels and their characteristics were investigated by Fourier transform infrared (FT–IR) spectroscopy and the inhibitory effect on cellular growth was tested by chemiluminescence assay. The hemolytic activity was also determined by direct contact with human blood and the concentration of hemoglobin was spectrophotometrically measured. **Results and discussion:** Chitosan hydrogels have no inhibitory effect on cell growth, and hemolytic action below 1%, which means good blood compatibility; therefore they are promising materials. **Keywords**: CHITOSAN, HYDROGEL, CELL VIABILITY, HEMOCOMPATIBLE.

The increasing need to develop new biodegradable materials for medical purposes like skin tissue engineering, wound cover or dressings and barrier-membranes appeared with the high demand for skin replacements and skin repair treatments. An ideal material to be use as a wound dressing should associate availability with minimal storage requirements, long shelf life, versatility and biocompatible behavior.

Many polymeric materials have been investigated for the purpose of wound covering on account its importance in the treatment of burns, prevention of post surgical adhesions and cosmetic surgery. Among them the biodegradable polymers from renewable resources e.g. starch, collagen, gelatin, chitosan, alginate, represent one alternative approach, since these polymers are widely available in nature, are biodegradable and non-toxic. A proper material for wound dressing should maintain a moist environment at the wound/ dressing interface and absorb excess exudates without leakage to the surface of the dressing, to provide thermal insulation and mechanical resistance, bacterial protection, to allow gaseous and fluid exchange, absorb wound odor, to be non-adherent to the wound and easily removed without trauma, non-toxic, non-allergenic and non-sensitizing, sterile (1).

In last years, much attention has been paid to the development of hydrogels from natural, biodegradable and biocompatible polymeric materials. (2) Among other biodegradable polymers, chitosan, a linear polysaccharide, has been found to be a good chemical entity for synthesizing hydrogels because of its greater crosslinking ability due to the presence of amino ($–NH_2$) groups. Chitosan is a linear, natural cationic polysaccharide comprising β-1,4 linked glucosamine and N-acetyl-D-glucosamine and its potential applications of chitosan in medicine can only be exploited if its usable forms are properly developed and prepared, in solution and gel. It can be used as a bacteriostatic, fungistatic and coating agent (3-5) Gels and suspensions may play the role of carriers for slow release or controlled delivery of drugs, as an immobilizing medium and an encapsulation material (6-9). Film and membranes are used in dialysis, contact lenses, dressings and the encapsulation of mammal cells, including cell cultures (10-12). Chitosan sponges are used in dressings, and to stop bleeding of mucous membranes (13). Chitosan fibres there are used as resorbable sutures, non-wovens for dressings, and as drug carriers in the form of hollow fibres (14).

Wang et all (15) investigated smooth surface chitosan microspheres which were prepared by emulsion and crosslinking method. They preformed dynamic blood clotting test showing that chitosan microspheres can shorten the clotting time. By comparing the lowly hemolytic activity with the highly erythrocyte agglutination, they showed that chitosan only induce the adhesion of erythrocytes but not seriously damage the cell membrane.

Present study is dealing with the investigation of hemocompatibility and cell viability of chitosan based sponges. The sensitivity of the chemiluminescent assay was enhanced by using a metal catalyst and quinones suitable to microorganisms. The detectable cell density of viable bacteria and yeast was found to be approximately several thousand of colony forming unit (CFU)/ml. The modified chemiluminescent assay was applicable to detect single S. POmbe after 4 hours cultivation and centrifugation. The conditions for the rapid chemiluminescent assay are discussed herein.

EXPERIMENTAL

Materials

Chitosan, an Aldrich product, with a relative molecular weight of 400 kDa and a deacetylation degree of about 68 % determined by Moore and Roberts's method on the base of FT-IR spectrum was used. Glutaraldehyde used as crosslikning agent was purchased from Fluka as solution 50wt%.

Preparation method of hydrogel based chitosan

Solutions of 1 wt% Chitosan (CS) in acetic acid solution 1 wt% were prepared by continuously stirring about 4 hours in order to completely dissolution of chitosan. The samples of chitosan were crosslinked with different amounts of glutaraldehyde (GA) (CS: GA of 1:0.5; 1:3 v/v %) obtaining gels, which were repeatedly washed with twice-distilled water to remove the excess of GA.

Investigation methods

The freeze-dried hydrogels based on chitosan with different amount of crosslinking agent were spectroscopically analyzed, to evidence the structure differences between hydrogels of chitosan crosslinked with various amounts of GA and to asses

chemical modification. The ATR-FT-IR spectra were recorded using a Vertex 70 Bruker Spectrometer, through reflexion on a diamond crystal with an angle of 45 degrees.

Chemiluminescence assay

The chemiluminescence assay was used for the detection of viable cells according to Yamashoji method (16). A highly sensitive and specific assay method was developed for the detection of viable *Schizosaccharomyces pombe* wild type as an indicator organism in water, using Nucleic-Acid-Sequence-Based Amplification (NASBA) and electrochemiluminescence (ECL) analysis. (16)

This assay is composed of two steps: (A) 10 min incubation of microorganisms with quinone and (B) 2 seconds of photon counting upon the injection of luminol. Viable microorganisms can reduce quinone to produce quinol or semiquinone radicals, which generate active oxygen species during their autoxidation in culture during step A. The production rate of active oxygen species is determined by luminol-dependent chemiluminescence during step B and is proportional to the viable cell's number. The proposed mechanism for menadione - catalyzed production of active oxygen species by viable cells is represented in figure 1.

The calibration curve of control samples (*S Pombe* cultivated in absence of polymers) is presented on figure 1 where relative light units (RLU) were plotted versus cells/ ml concentration.

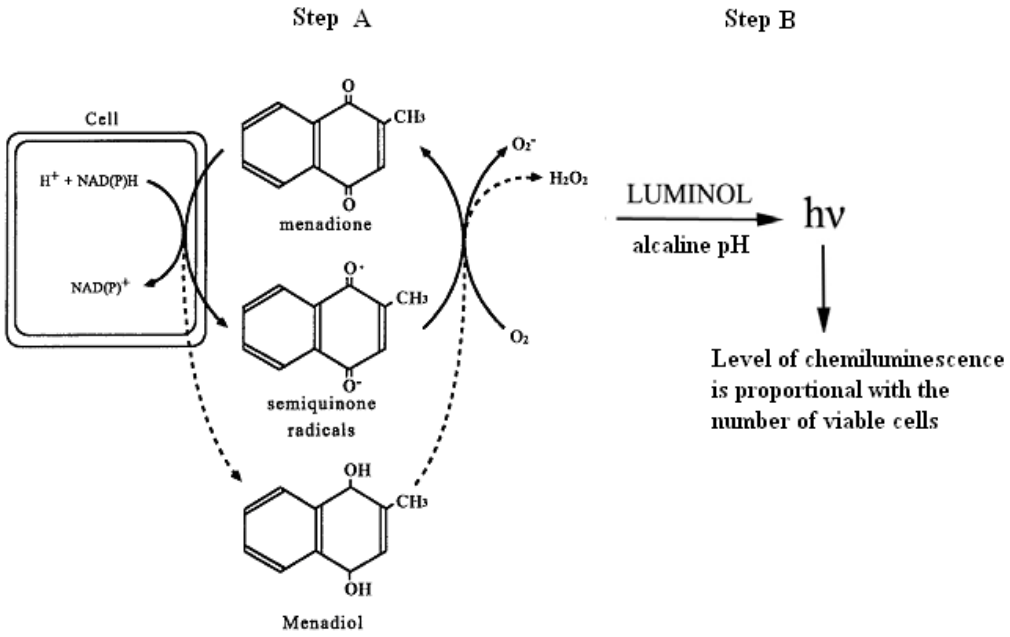


Fig. 1. Mechanism for menadione - catalyzed production of active oxygen species by viable cells (16)

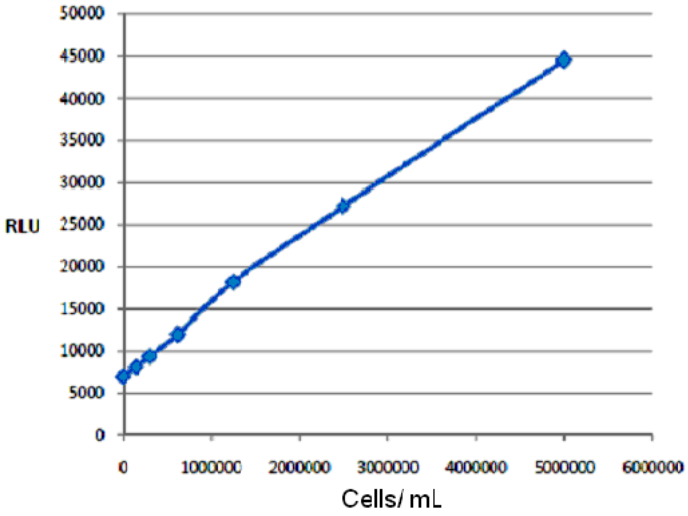


Fig. 2. Standard curve of control samples (S Pombe cultivated in absence of polymers)

Hemolysis tests

The sampling of blood was done from healthy patients and each hydrogel was tested with blood from 1 patient; the distilled water was used as positive reference and the plasma obtained from the blood sample was used as negative reference. From each recipient of blood was taken 1.5 ml of blood which are enough to ensure the contact with the hydrogel. The tested biomaterials in direct contact with blood were incubated at 37 ^{o}C during 2 hours and then centrifuged for 6 min at 5000 rpm. Prior to be spectrophotometrically measured plasma is diluted 11 times with Tris buffer adjusted with HCl to have a pH about 8 (62.5 mmol/L). The positive reference, the distilled water was prepared by blood hemolysis with distilled water in a ration of 1:11; the hemolyzed solution is incubated further during 2 hours at 37 ^{o}C and the diluted 100 times in order to be spectrophotometrically analyzed.

The used method for determination the hemoglobin concentration from plasma is the polychromatic method of Noe (17) the absorbance being measured at 380, 415 and 470 nm.

The degree of hemolysis can be calculated according the following equation:

$$Hemolysis\ (\%) = (C - C_n)/(C_p - C_n) \times 100$$

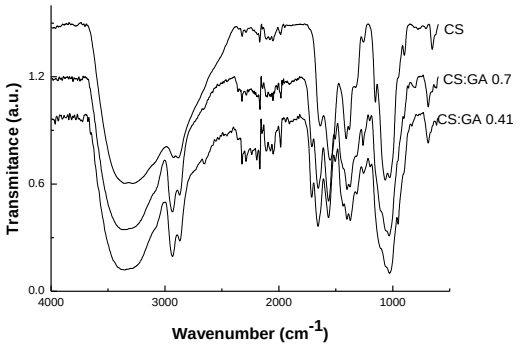
Where: C hemoglobin concentration on analyzed sample, C_n hemoglobin concentration for negative reference şi C_p hemoglobin concentration for positive reference.

RESULTS AND DISCUSSION

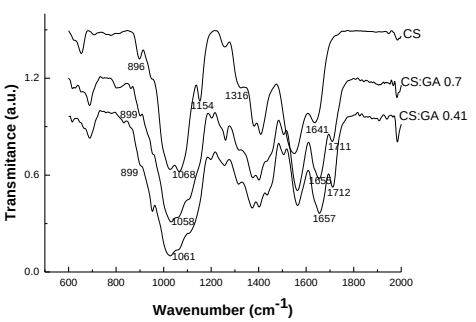
FT-IR spectra

FT-IR spectra - fig. 3 - revealed that the intensity of bands at 2900-2800 cm^{-1} increased with the increasing amount glutaraldehyde.

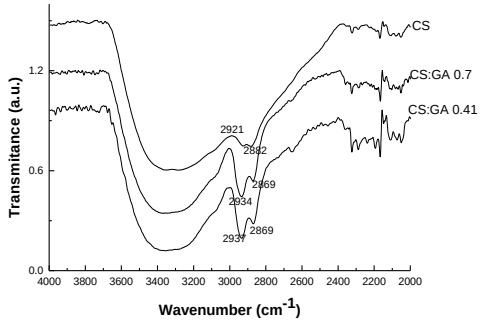
In order to evidence the structure of hydrogels and the degree of crosslinking the main bands (18) were assigned to be as: 900-1000 cm^{-1} which are characteristic bands of polysaccharide structure, while bands at 1650 cm^{-1} and 2900-2880 cm^{-1} assigned to υNH-CO-COOR and respectively C-N and C-H could appear because of crosslinking.



(a)



(a_1)



(a_2)

Fig. 3. FT-IR spectra of hydrogels in different spectral regions

Cell toxicity assays

The hydrogels based on chitosan crosslinked with glutaraldehyde exhibit potential utility for many biomedical applications due to their gel state. In figure 4 is presented the cell viability as results of chemiluminescent assays to detect the viability of S. Pombe after 4-h cultivation and centrifugation.

Cells viability was calculated by comparison with control samples (*S Pombe* cultivated in absence of polymers), considered to be 100 % viable cells. The effect of hydrogels on cell growth was evaluated by MTT test, a rapid and economical assay for measurement of cellular viability. The chitosan hydrogels exhibited cell viability comparable with the control sample which means a good biocompatibility and didn't show inhibitory effect on the cell's growth.

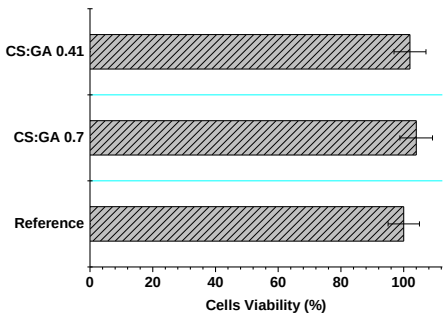


Fig. 4. *S. Pombe* viability detected by chemiluminescence assay

Cells viability was calculated by comparison with control samples (*S Pombe* cultivated in absence of polymers), considered to be 100 % viable cells. The effect of hydrogels on cell growth was evaluated by MTT test, a rapid and economical assay for measurement of cellular viability. The chitosan hydrogels exhibited cell viability comparable with the control sample which means a good biocompatibility and didn't show inhibitory effect on the cell's growth.

Hemocompatability

Table 1 represents the values of hemolysis obtained for the chitosan hydrogels. The method is based on measurement of the hemoglobin released by hemolysis. Hemolysis is regarded as a special significant screening test, once it provides quantification of small levels of plasma hemoglobin, which may not be measurable under *in vivo* conditions. As reported in literature (19), it is not possible to define a universal level of acceptable or unacceptable amounts of hemolysis. Although by definition a blood compatible material should be non-hemolytic, in practice several medical devices cause hemolysis. This means that when such hemolytic effect takes place, it is important to make sure that clinical benefits overcome the risks and that the values of hemolysis are within acceptable limits.

According to ASTM standard, (20) the materials can be classified in three different categories according to their hemolytic index (hemolysis %). Materials with percentages of hemolysis over 5% are considered hemolytic; while the ones with hemolytic index between 5% and 2% are classified as slightly hemolytic. Finally, when the material presents a hemolysis percentage below 2% it is considered as a non-hemolytic material.

The data of table I indicate a hemolysis degree less than 0.6 %therefore they are not hemolytic.

TABLE I

Hemolysis results for the chitosan hydrogels

Sample	A415	A380	A470	Biomaterial concentration	Hemolisys degree (%)
CS:GA0.7	1.0359	0.4035	0.1215	1.245285	**0.5421425**
R+	0.8816	0.2252	0.0626	1.199506	
R-	0.5131	0.1871	0.1019	0.598225	
CS:GA 0.41	0.4544	0.1785	0.1299	0.488928	**0.1138289**
R+	0.8286	0.2174	0.0591	1.121865	
R-	0.3711	0.1759	0.1193	0.361639	

R+, R-; positive and negative reference samples

CONCLUSIONS

Chitosan hydrogels were obtained by chemical croslinking with glutaraldehyde. The biomaterials didn't show inhibitory effect on the cellular growth and the biocompatibility tests were made by hemolysis (plasma hemoglobin) technique, the results obtained showed a good biocompatibility between hydrogels and blood.

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CERCETĂRI ASUPRA IMPLICĂRII UNOR FACTORI DE RISC ÎN ETIOLOGIA TUMORILOR MAMARE. OBSERVAȚII ÎN JUDEȚUL IAŞI, ÎN PERIOADA 2006-2010

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RESEARCH ON THE INVOLVEMENT OF SOME RISK FACTORS IN BREAST TUMOURS ETIOLOGY. OBSERVATIONS IN THE IASI COUNTY, BETWEEN 2007 AND 2010 (Abstract): **The aim of study** was to assess some risk factors and their involvement in female breast tumours etiology. **Material and methods**. The study was performed on a sample of 67 patients registered with breast tumours, at family phisicians' offices from Iasi, Romania. **Results**. Some risk factors were defined such as: nuliparity (20.8%) and first pregnancy after age 30 (34.3%); fibrous mastopathy in personal history (73.1% of cases with breast cancer) and breast cancer in siblings of first (8.9%) and second grade (11.9%). Relative to lifestyle, sedentary life was remarked in 59.2% of patients; modification of circadian rhythm in 28.3%, and excesive caffeine consumption in 80.9% of cases. **Conclusions**. Further prevention and educational programs on breast tumoural risk factors are needed in Romania, even at female teenage, performed by family physician. **Key words**: MAMARY DYSPLASIA, BREAST CANCER, RISK FACTORS.

Conform datelor statistice recente, boala canceroasă cauzează aproximativ 7,6 milioane decese în fiecare an, la nivel mondial, din care mai mult de 72% apar în țările cu nivel socio-economic scăzut şi mediu şi se prognozează că aceste cifre vor ajunge la 17 milioane în 2030. Cancerul mamar este cea mai frecventă cauză de deces prin cancer, la femei, în Uniunea Europeană (17,2% din total, în anul 2010) (1, 2).

În etiologia tumorilor mamare sunt recunoscuți a fi implicați numeroşi factori de risc, în relație cu diferite aspecte controlabile (stilul de viață, de nutriție, de mediu, tratamente associate pentru diverse comorbidități) sau necontrolabile (vârsta, constelația genetică ereditară), cumulându-se odată cu înaintarea în vârstă (3, 4, 5, 6).

Biroul Regional OMS pentru Europa, susține, de Ziua Mondială a Cancerului, din 4 februarie, faptul că modificări în stilul de viață şi îmbunătățirea metodelor prevenționale şi mijloacelor de screening ar putea preveni peste 40% din cazurile diagnosticate de cancer (1).

MATERIAL ŞI METODĂ

Studiul de tip descriptiv a fost realizat, în perioada 2007-2010, pe un lot de 67 paciente diagnosticate cu formaţiuni tumorale mamare şi înscrise la cabinete individuale de medicină de familie, din Iaşi. Datele au fost culese cu ajutorul unei fişe de anchetă epidemiologică, special concepută, ulterior prelucrate statistic.

REZULTATE ŞI DISCUŢII

1. Distribuţia anuală a cazurilor de tumori mamare, atât maligne (38,8% din total), cât şi benigne (61,2%), arată o tendinţă uşor crescătoare, dar nesemnificativă statistic, cu scăderea numărului de cazuri în anul 2010, fapt constatat şi la nivel mondial şi posibil explicat prin extinderea şi îmbunătăţirea educaţiei pentru sănătate, referitoare la prevenţie şi screening (3, 4, 5) (fig. 1).

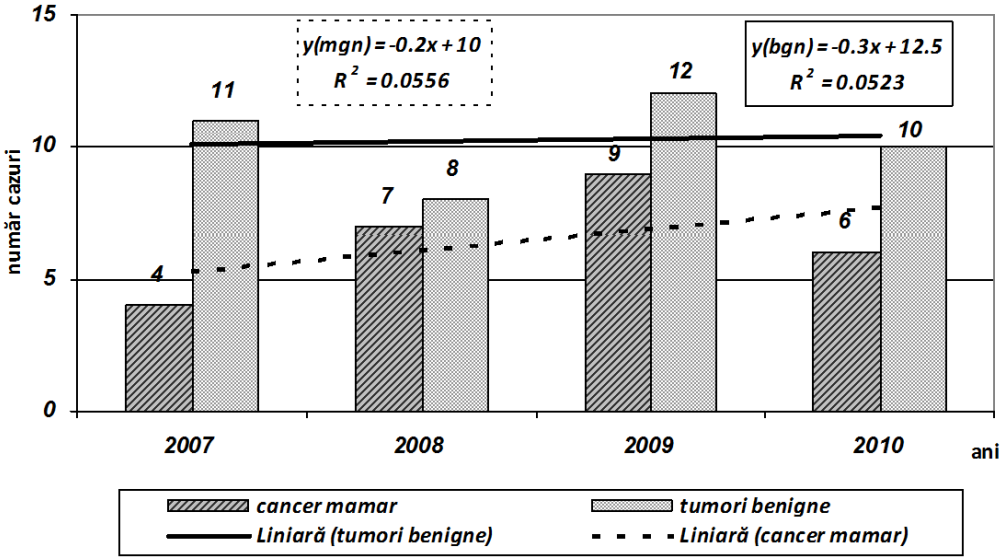

Fig. 1. Repartiţia anuală a numărului de cazuri de tumori mamare

2. Repartiţia pe grupuri de vârstă a arătat faptul că cele mai multe cazuri de tumori benigne au fost înregistrate la grupul 36-45 ani (41,4%), iar de tumori maligne la femeile între 45-65 ani (84,6%, $p>0,05$), grupuri de vârstă care cumulează factorii de risc, atât de tip hormonal, cât şi nutriţional şi comportamental (fig. 2).

3. Distribuţia cumulativă a antecedentelor personale fiziologice a arătat că, în lotul de paciente luat în studiu, menarha precoce (înainte de 12 ani) a fost constatată la 10 paciente (14,9% din total); nuliparitatea la 14 (20,8%) şi prima sarcină dusă la termen după vârsta de 30 ani la 23 femei (34,3%) (iar dintre aceste 37 cazuri, 28 având studii superioare – fapt constatat şi în literatura de specialitate) (3, 8); menopauza instalată după vârsta de 55 ani, în 6 cazuri (27,2% din cele 22 cazuri din grupul de vârstă 55 - ≥ 65 ani) (fig. 3).

4. Distribuţia cumulativă a unor antecedente personale patologice. Mastopatia fibrochistică a fost găsită la 19 paciente diagnosticate cu neoplasm de sân (73,1%); fibromul uterin în 11 cazuri (16,4%); sin-

dromul ovarelor polichistice la 9 femei (13,4%); patologie tiroidiană benignă în 7 cazuri (10,4%). La 14 paciente (20,8%) a fost constatată prezența hiperglicemiei și diabetului zaharat, iar obezitatea cu IMC > 30 kg/m^2 a fost găsită la 12 (17,9%) (fig. 4).

Datele din literatură arată faptul că acești factori de risc, prezentați mai sus, sunt întâlniți în mod frecvent în istoria patologiei tumorale a sânului, la femei cu stilul de viață caracteristic țărilor dezvoltate, în care se include și România (1, 5, 8, 9, 10).

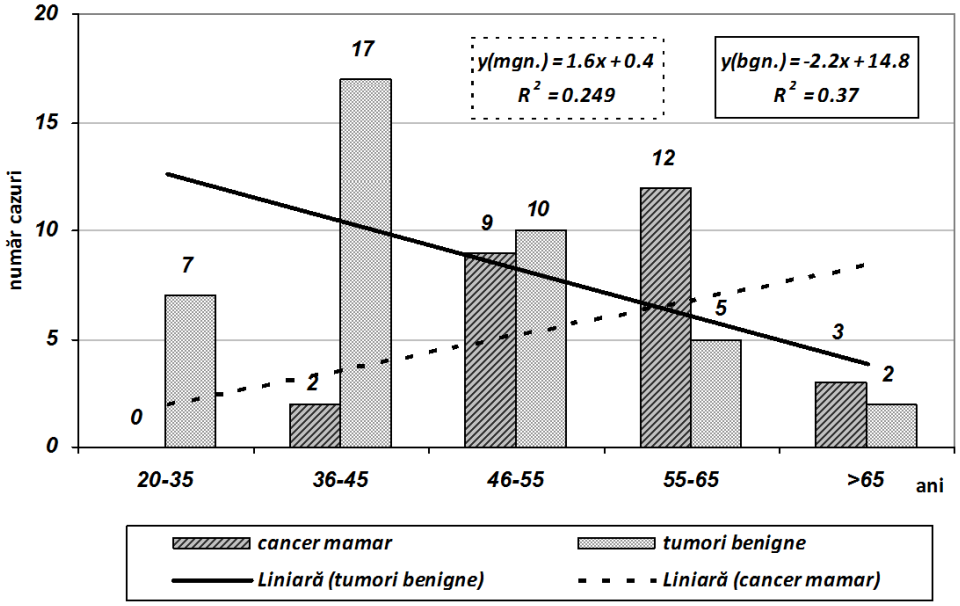

Fig. 2. Repartiția pe grupuri de vârstă

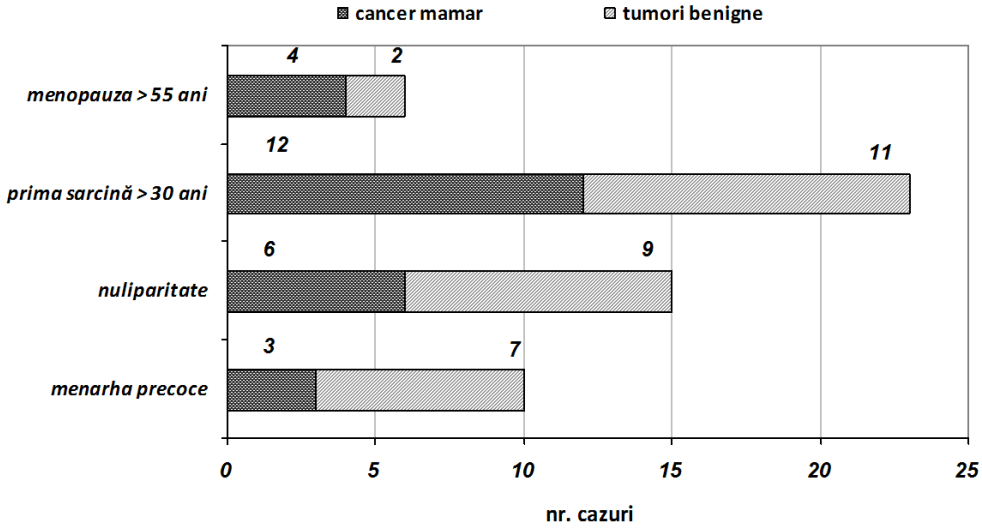

Fig. 3. Repartiția antecedentelor personale fiziologice

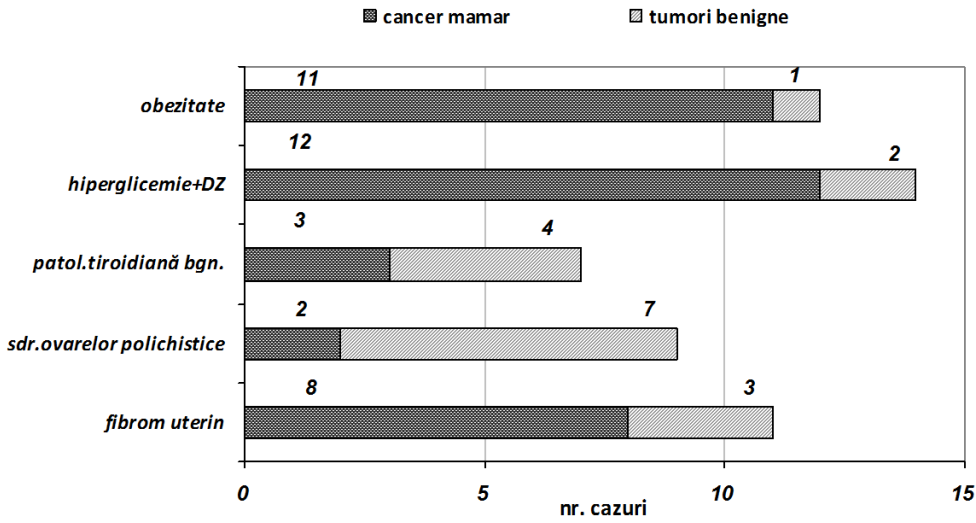

Fig. 4. Repartiția unor antecedente personale patologice

5. Distribuția cumulativă a unor antecedente familiale. Anamnestic, la rude de gradul I, a fost remarcată prezența cancerul de sân în 6 cazuri (8,9%) şi a tumorilor de prostată în 3 (4,4%); tumori cu alte localizări la 12 paciente (17,9%). La rude de gradul al II-lea, s-a constatat existența cancerului mamar în 8 cazuri (11,9%) şi a tumorilor cu alte localizări în 13 cazuri (19,4%) (fig. 5).

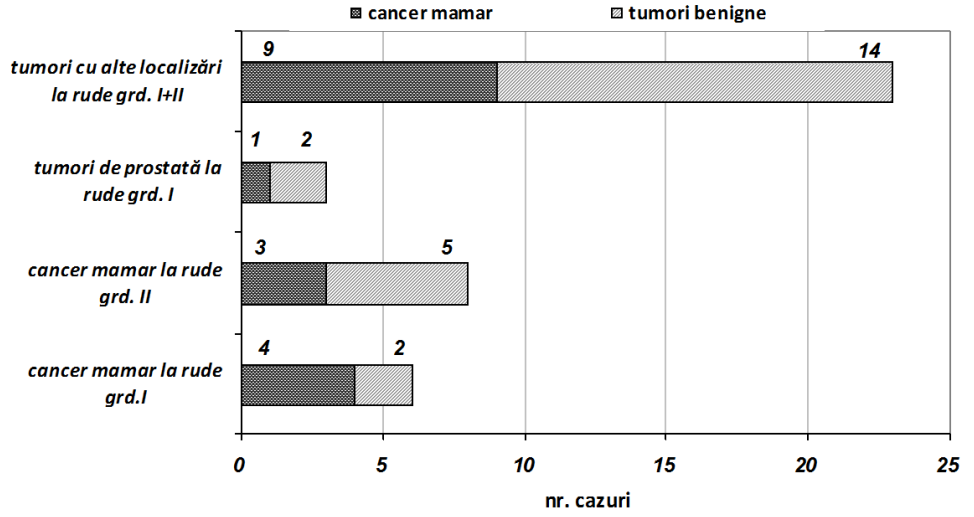

Fig. 5. Repartiția unor antecedente familiale

Aceste date, deşi în valori procentuale ridicate, nu le putem considera semnificative statistic şi de extrapolat, însă ele se calchează pe alte rezultate întâlnite în lite-

ratura de specialitate, referitoare la istoricul familial în cancerul de sân (5, 6, 7).

6. Observații referitoare la stilul de viață şi nutrițional al pacientelor. Tendința la sedentarism (4, 5, 8) a fost afirmată de 39 paciente (58,2%) (fig. 6). Am luat în studiu modificarea ritmului circadian (trezirea frecventă în timpul nopții, lucrul pe timp de noapte sau / şi insomnie), care a fost găsită la 19 femei (28,3%), date asemănătoare cu ale altor studii derulate şi publicate în ultimii ani (11). Deşi un factor de risc controversat (4, 5, 12), consumul crescut de cofeină (> 2 ceşti cafea / zi, timp de > 15 ani) a fost afirmat de 54 paciente (80,6%) (fig. 7).

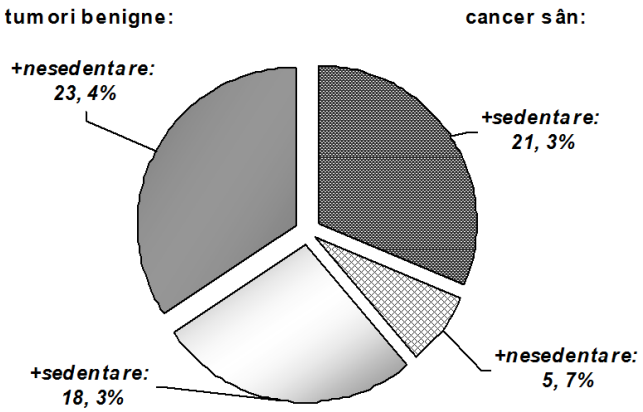

Fig. 6. Sedentare *vs* nesedentare în lotul cu tumori de sân

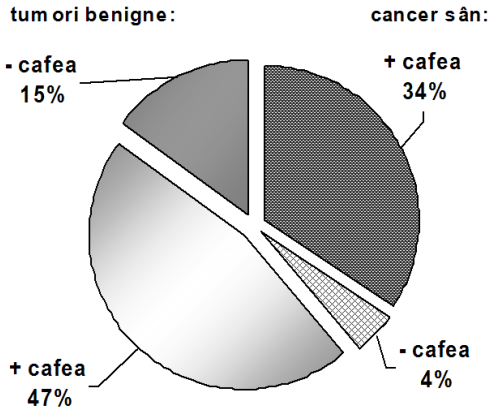

Fig. 7. Consumul de cofeină în lotul cu tumori mamare

CONCLUZII

Studiul descriptiv derulat pe un lot de 67 paciente înscrise pe lista medicului de familie şi diagnosticate cu tumori mamare, în perioada 2007-2010, a arătat faptul că cele mai multe tumori au fost diagnosticate la 45-65 ani, vârste la care se cumulează numeroşi factori de risc instrinseci şi ex-

trinseci, după cum am constatat şi noi, dintre care: nuliparitatea (20,8%) şi prima sarcină dusă la termen după vârsta de 30 ani (34,3%); mastopatia fibrochistică în antecedentele personale (73,1% din cazurile cu neoplasm mamar) şi cancerul mamar la rude de gradul I (8,9%) şi II (11,9%). Referitor la stilul de viaţă, sedentarismul a fost afirmat în 59,2% din cazuri, modificarea ritmului circadian de către 28,3% dintre femei, iar consumul în exces de cofeină, remarcat în 80,9%.

Constatăm faptul că este necesară optimizarea programelor educaționale şi pentru sănătate, referitoare la factorii de risc implicați în apariția tumorilor mamare, prin derularea acestora încă de la vârsta adolescenței feminine, prin cabinetul medicului de familie, dar şi prin cele de medicină şcolară.

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TRATAMENTUL ORTODONTIC ÎN SCOP PREPROTETIC – STUDIU STATISTIC RETROSPECTIV

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PREPROSTHETIC ORTHODONTIC TREATMENT - RETROSPECTIVE STATISTIC STUDY (Abstract): Orthodontic treatment as a help is the teeth movement made in order to facilitate the odontal,prosthetic and periodontal proceedures which are needed in the dentomaxilar and/or facial reabilitation. The aim of the study was to follow for 5 years which is the percentage of patients who asked for orthodontic treatment in the Orthodontic and Dentofacial Orthopedie Clinic from UMF 'Carol Davila'. We also followed the frequency related to the etiology of the loss of some teeth and also the relationship between the loss of the teeth and periodontal disease. In our country, the number of patients who ask for preprosthetic orthodontic treatment is lower comparing with Western and Northtern Europe, but the percentage is increasing due to the dentists calification. We can conclude by saying that the frequency of losing teeth by cavities is increased by age because all patients from the study group aged 35 and above lost their teeth from cavity etiology and the patients aged 18 and lower had genetic etiology in losing their teeth. **Key words**: ORTHODONTIC TREATMENT, TOOTH DECAY

Tratamentul ortodontic adjunct este considerat a fi acea mişcare a dinţilor realizată pentru facilitarea şi eficientizarea procedurilor odontale, protetice şi parodontale necesare pentru restabilirea funcţiilor aparatului dento-maxilar şi / sau pentru îmbunătăţirea aspectului. De cele mai multe ori, acest tip de tratament ortodontic se adresează adulţilor cu cel puţin o edentaţie parţială. După Proffit, tratamentul ortodontic solicitat de către adulţii mai în vârstă reprezintă sectorul ortodonţiei cu creşterea cea mai rapidă în zilele noastre.

Pierderea unui singur dinte comportă o serie de modificări patologice atât ale dinţilor vecini şi antagonişti, cât şi ale parodonţiului de susţinere, tratamentul aferent fiind unul complex: parodontal, ortodontic şi protetic.

Studiile din literatura de specialitate relevă faptul că un procent de 30% dintre pacienţii adulţi trataţi ortodontic necesită un management terapeutic interdisciplinar, printre care se citează: adiţii de os, inserare implante etc. Cu 18 ani în urmă, în 1988, la Congresul EOS din Londra, Perregaard şi Blixencrone-Mřller au găsit un procent de numai 9% de pacienţi adulţi care s-au prezentat pentru tratament ortodontic preprotetic şi 12% pentru boală parodontală.

Scopul studiului prezent este de a urmări pe o perioadă de 5 ani care este procentul de pacienţi, care se adresează medicului ortodont pentru tratament ortodontic adjunct în Clinica de Ortodonţie şi Ortopedie Dento-facială din cadrul Facultăţii de Medicină Dentară, UMF "Carol Davila", Bucureşti. Se urmăreşte, de asemenea, frec-

TABELUL 1

Nr. Crt	Inițiale: nume, prenume	Vârstă	Sex	Clasa de anomalie după Angle	Nr. dinților absenți	Dinții absenți	Etiol. edent.	Status parodontal
1	M.D.	26	M	III	2	26,27	C	Pmc
2	C.A.	13	F	II/2	1	21	E	Gc
3	C.M	36	F	II/1	1	46	C	Pmc
4	L.B	17	F	II/2	2	35,45	E	Gc
5	O.A.	13	F	II/1	1	35	E	Pmc
6	R.G.	18	M	III	1	46	C	Gc
7	F.T.	19	F	II/1	4	35,45,25,15	E	sănătos
8	V.V.	10	M	II/1	1	12	E	sănătos
9	E.S	24	M	III	1	22	E	Gc
10	P.I.	15	M	III	2	21,22	E	Pmc
11	F.F.	19	M	I	3	15,25,35	E	Pmc
12	M.I.	14	M	I	1	43	E	Pmc
13	S.I.	13	M	III	5	15,14,25,35,45	E	Pmc
14	S.F.	23	M	III	3	25,37,45	C	Pmc
15	F.N	36	F	I	2	36,35	C	Pmc
16	A.G.	26	F	I	4	35,36,45,46	C	Pmc
17	B.A.	27	F	II/2	4	31,32,41,42	C	Gc
18	P.G.	20	F	III	1	36	C	Pmc
19	Z.C.	20	F	I	2	26,46	C	Gc
20	P.I.	36	M	III	3	22,36,46	E+C	Pmc

venţa în strânsă relaţie cu etiologia edentaţiei anumitor dinţi, precum şi asocierea edentaţiilor cu boala parodontală.

MATERIAL ŞI METODĂ

S-a realizat un studiu retrospectiv prin analizarea a 450 de dosare ortodontice ale pacienţilor (fotografii intraorale şi extraorale, ortopantomograma şi teleradiografia de profil), care au solicitat un tratament ortodontic la Clinica de Ortodonţie şi Ortopedie Dento-facială din cadrul Facultăţii de Medicină Dentară, UMF "Carol Davila", Bucureşti în ultimii 6 ani.

Criteriul de includere în studiu a fost prezenţa cel puţin a unei edentaţii sau anodonţii protezate sau nu, care urmează a fi tratată protetic. Nu s-au luat în considerare cazurile de edentaţii sau anodonţii care au fost tratate doar ortodontic prin închiderea spaţiilor. De asemenea, s-au inclus în cercetare şi dinţii ectopici care au fost extraşi ulterior, fie din cauza poziţiei profunde în os, fie din cauza direcţiei orizontale, urmând ca spaţiul corespunzător să fie păstrat şi protezat.

Pentru fiecare subiect care corespunde criteriilor mai sus menţionate s-au reţinut următoarele date într-un tabel : iniţialele numelui, vârsta, sexul, clasa de malocluzie după Angle, numărul dinţilor absenţi, care sunt dinţii absenţi, etiologia edentaţiei ("C" – carioasă, "E" – ereditară), status parodontal ("Pmc" – parodontopatie marginală

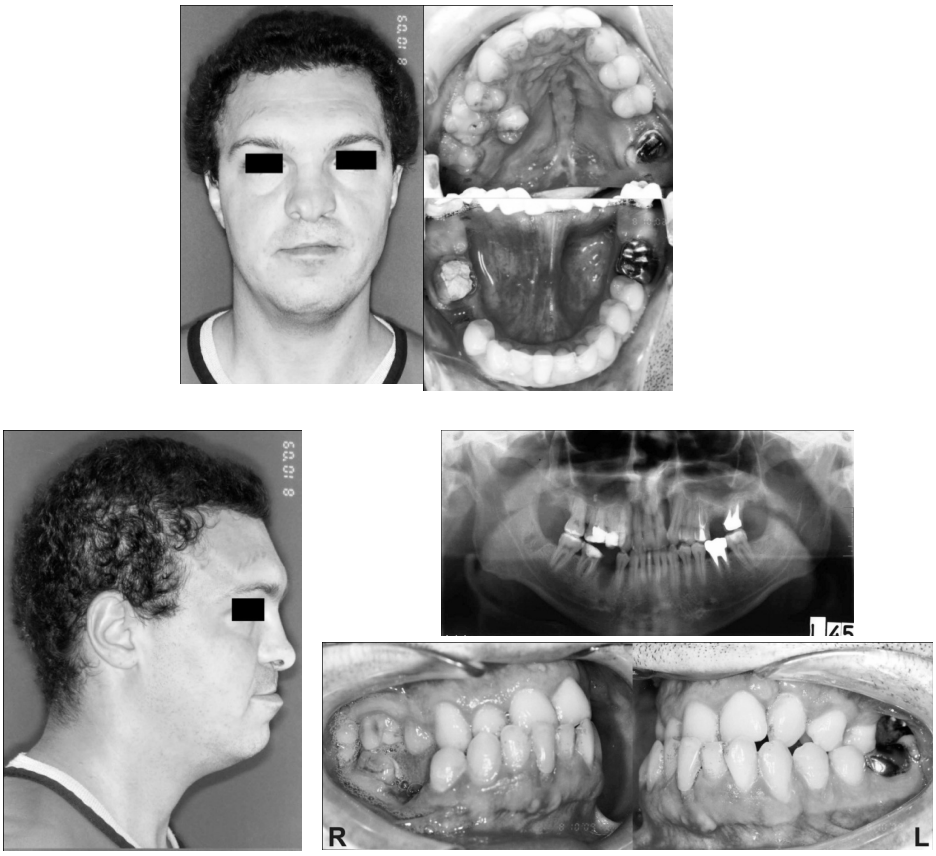

Fig. 1. P.I., 36 ani (din iconografia Dr. Anca Temelcea)

cronică, "Gc" – gingivită cronică) şi s-au găsit următoarele :

Diagnosticul de parodontopatie marginală cronică s-a pus la nivel radiologic pe ortopantomograma, indicând afectarea osoasă în boala parodontală. Diagnosticul de gingivită cronică s-a pus pe aspectul gingiei.

REZULTATE

Dintr-un lot de 450 pacienţi, cu vârsta între 10 şi 36 ani, care s-au prezentat la Clinica de Ortodonţie şi Ortopedie Dento-facială din cadrul Facultăţii de Medicină Dentară, UMF "Carol Davila", pe o perioadă de 6 ani (2005-2010), un număr de 20 de indivizi au fost consideraţi pentru urmarea unui tratament ortodontic adjunct, în vederea protezării prin implante (în mod ideal) ; Procentul corespunzător este de 4,44% pacienţi care necesită tratament ortodontic preprotetic.

Un procent de 35% dintre pacienţii luaţi în studiu au o vârstă sub 18 ani, iar dintre aceştia, toţi au o etiologie carioasă a edentaţiei / edentaţiilor prezente, prin anodonţii, ectopii sau despicături de buză şi palat.

Pacienţii cu vârstă peste 35 ani care necesită tratament ortodontic adjunct, reprezintă un procent de 15% - toţi având o etiologie carioasă a edentaţiei.

Jumătate din lotul investigat este de sex feminin şi cealaltă jumătate de sex masculin.

Împărţirea pacienţilor din lot după clasificarea lui Angle a avut rezultate uimitoare :

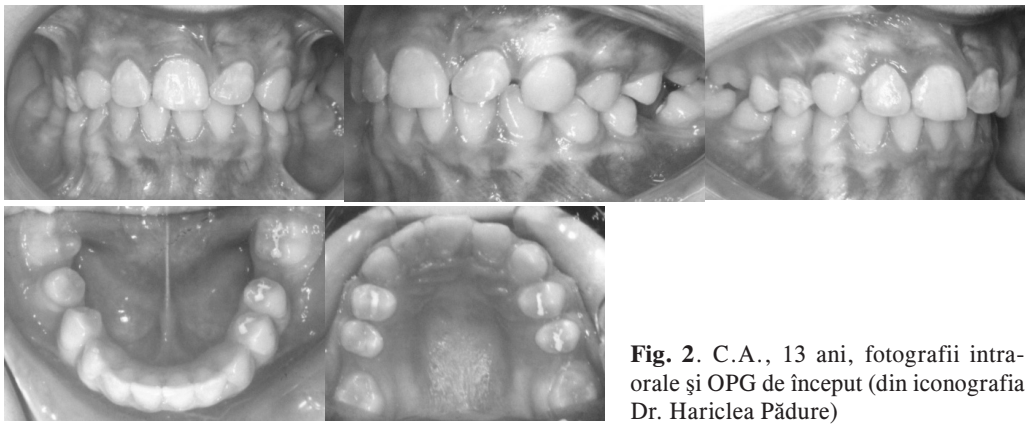

Fig. 2. C.A., 13 ani, fotografii intraorale şi OPG de început (din iconografia Dr. Hariclea Pădure)

40% clasa a III-a, 25% clasa I, 15% clasa II/2, 5% clasa II/1. Procentul crescut de pacienţi clasa a III-a Angle, care necesită tratament preprotetic este la prima vedere surprinzător, având în vedere că malocluziile de clasa a III-a sunt printre cele mai rare în populaţia ţării noastre, dar trebuie avut în vedere faptul că o astfel de anomalie dento--maxilară, netratată, generează în timp traume ocluzale şi forţe nefiziologice care pot duce la edentaţii. Pe de altă parte, trebuie menţionat şi faptul că au fost incluşi în acest studiu pacienţi cu despicături labio-maxilo--palatine cu anodonţii care necesită protezare (figura 1).

Cei mai mulţi prezintă un singur dinte absent, adică 40%, urmând ca 25% să aibă 2 dinţi absenţi, 20% - 4 dinţi absenţi (de regulă toţi cei 4 premolari secunzi), 3 dinţi absenţi – 15%.

Majoritatea dinţilor absenţi care urmează a fi protezaţi sunt premolari, respectiv 33,33%, urmaţi fiind de molarii de şase ani, cu un procent de 22,91%, 8,33% pentru incisivii laterali superiori. Acest rezultat este în concordanţă şi cu procentele apropiate care reprezintă etiologia edentaţiei: 50% pentru etiologia ereditară (anodonţii de pemolari şi incisivi laterali) şi 45% - etiologia carioasă (patologia molarului de şase ani).

În ceea ce priveşte statusul parodontal al pacienţilor care necesită tratament ortodontic preprotetic, s-a găsit un procent de 60% care prezintă o formă de parodontopatie marginală (superficială sau profundă), ceea ce înseamnă că osul alveolar este deja afectat (într-o măsură mai mare, sau mai mică). Gingivita cronică este prezentă la 30% dintre pacienţii investigaţi, iar 10% au fost consideraţi sănătoşi din punct de vedere

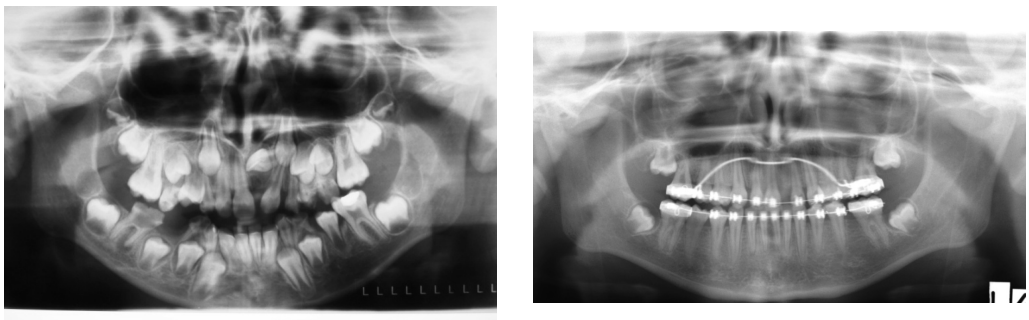

Fig. 3. C.A., OPG de etapă (din iconografia Dr. Hariclea Pădure)

parodontal. Este de remarcat că toţi pacienţii din această ultimă categorie, sunt sub vârsta de 19 ani şi prezintă o etiologie ereditară a edentaţiei.

Cele mai multe cazuri de edentaţii au primit ca indicaţie terapeutică optimă varianta protezării pe implante, în special cazurile cu edentaţii unidentare, cum este şi C.A., în vârstă de 13 ani, cu incluzie orizontală de 21, care a primit indicaţie de extracţie, urmând ca în locul său să fie poziţionat un implant după vârsta de 18 ani (figura 2). După crearea spaţiului necesar unui implant, se va conserva osul alveolar prin mişcarea succesivă a lui 22 între 23 şi 11, precum şi vice versa (figura 3).

DISCUŢII

Procentul destul de mic (4,44%) de pacienţi care necesită tratament ortodontic preprotetic, în comparaţie cu 9% găsit în 1984 în Danemarca, se poate datora atât diferenţei de nivel de trai, cât şi modului de gândire al planului de tratament de către stomatologi, în general.

Procentul egal între femei şi bărbaţi care solicită tratament ortodontic preprotetic este explicabil prin natura acestuia – atât funcţional, cât şi estetic. Astfel că majoritatea pacienţilor care se prezintă pentru tratament ortodontic este reprezentată de sexul feminin – acesta fiind cel mai adesea îngrijorat de aspectul fizic; în cazul tratamentului ortodontic preprotetic, însă, prin prezenţa edentaţiei, apar deja şi disfuncţii masticatorii, pe lângă cele estetice.

În literatura de specialitate au fost citate cazuri multiple de tratament ortodontic preprotetic la un pacient cu o anomalie de clasa a III-a, însă, pe lângă etiologia ereditară şi cea carioasă, Ive a găsit şi o etiologie traumatică pentru edentaţii, în special pentru cele extinse.

Proporţia mare de pacienţi care prezintă o formă de parodontopatie marginală cronică era de aşteptat, luând în considerare procentul ridicat de pacienţi cu anomalii dento-maxilare, întâmplător, procentul fiind identic, 60% (40% clasa a III-a, 15% clasa II/2 şi 5% II/1).

CONCLUZII

La noi în ţară, adresabilitatea pacienţilor la un tratament ortodontic preprotetic este încă scăzută, în comparaţie cu ţările Europei de Vest şi de Nord, dar procentul de astfel de pacienţi este în creştere şi la noi, datorită, în mare parte, formării profesionale a medicului stomatolog generalist.

Se poate concluziona că frecvenţa etiologiei carioase a edentaţiei creşte cu vârsta, având în vedere că toţi pacienţii de peste 35 ani din lotul de studiu au prezentat o etiologie carioasă a edentaţiilor iar cei sub 18 ani, una ereditară.

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DETECTAREA GENELOR *mecA* ŞI *pvl* LA TULPINI DE *Staphylococcus aureus* IZOLATE DIN INFECŢII ALE TRACTULUI RESPIRATOR INFERIOR

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mecA AND pvl GENES DETECTION IN *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM LOWER RESPIRATORY TRACT INFECTIONS (Abstract): Although community-acquired *Staphylococcus aureus* pneumonia with highly virulent Panton-Valentine leukocidin (PVL)-positive strains, a severe disease with significant lethality, is rare, especially in adult and adolescent patients, recent reports highlight that these infections are on the rise. On the other hand, methicillin-resistant *S. aureus* (MRSA) is one of the high-risk and potential multi-drug resistant microorganisms. **Objectives**: The aim of this study was to investigate the prevalence of *mecA* and *pvl* genes in *S. aureus* strains isolated from lower respiratory tract infections and to assess the antibiotic resistance profile of these strains. **Material and method**: The antibiotic susceptibility testing was performed by the disk diffusion method according to CLSI recommendations in 32 consecutive non-repeated *S. aureus* strains isolated from sputum specimens and endotracheal aspirates of hospitalized patients over the period from January 2005 to December 2009. Only 20 strains (2008-2009) were tested for the presence of *mecA* and *pvl* genes by real-time PCR and detection with specific fluorescence probes. **Results**: Of the 32 *S. aureus* isolates, 68.7% were MRSA. MRSA strains showed higher resistance rates to gentamicin, tetracycline, rifampicin, fluoroquinolones comparing to the methicillin susceptible ones. Only one strain produced pvl; it was isolated from a 7 year old child with lethal sepsis with pulmonary and meningeal secondary localisations. **Conclusions**: Glicopeptides and linezolid are therapeutic options indicated in the treatment of staphylococcal pneumonia produced either by MRSA strains or pvl positive *S. aureus* strains. **Key words**: *STAPHYLOCOCCUS AUREUS*, RESISTANCE, *mecA*, *pvl*

Staphylococcus aureus este un patogen uman major şi produce o gamă largă de condiţii patologice, de la infecţii cutanate localizate, benigne la infecţii care pun în pericol viaţa pacientului, cum sunt fasciita şi pneumonia necrozantă. Rezistenţa la meticilină a *S. aureus* se datorează achiziţionării genei *mecA* care codifică o proteină de legare a penicilinei cu afinitate scăzută pentru beta-lactamine, PLP2a. Prezenţa acesteia face bacteria rezistentă la toţi reprezentanţii acestei clase de antibiotice (1).

S. aureus rezistent la meticilină (SARM), una din cele mai frecvente cauze ale infecţiilor, este tradiţional recunoscut ca patogen nosocomial. Totuşi, în ultimii ani, epidemiologia sa s-a modificat radical, fiind observat din ce în ce mai frecvent în comunitate. SARM comunitar (SARM-C) poate produce infecţii la indivizi sănătoşi în prealabil, iar

TABELUL I
Primeri PCR şi sonde de hidroliză (*Biosearch Technologies*, Inc.)

Primer/sondă	Secvenţă (5'-3')	Colorant reporter 5'
mecA		
mecA For	GGCAATATTACCGCACCTCA	
mecA Rev	GTCTGCCACTTTCTCCTTGT	
Sonda mecA	AGATCTTATGCAAACTTAATTGGCAAATCC	FAM
PVL		
PVL For	ACACACTATGGCAATAGTTATTT	
PVL Rev	AAAGCAATGCAATTGATGTA	
Sonda PVL	ATTTGTAAACAGAAATTACACAGTTAAATATGA	Quasar 670

factorii de risc diferă de cei ai achiziţionării SARM nosocomial (SARM-N) (2).

Leucocidina Panton-Valentine (PVL), o leucotoxină producătoare de pori codificată de genele *lukF-PV* şi *lukS-PV*, este în momentul de faţă acceptată ca fiind cel mai important factor de virulenţă al *S. aureus*. PVL a fost asociată epidemiologic cu tulpini comunitare de *S. aureus*, virulente, transmisibile, inclusiv SARM-C (3). PVL a fost corelată cu infecţii specifice cu *S. aureus* cum ar fi infecţii cutanate sau de ţesuturi moi şi pneumonia necrozantă severă la copii şi adulţi tineri anterior sănătoşi, care adeseori urmează după o boală "flu-like". Mortalitatea pneumoniei necrozante este de aproximativ 75% (4, 5). Există mai multe dovezi pentru incriminarea PVL în patogenia pneumoniei necrozante, inclusiv corelaţia epidemiologică puternică cu izolate de *S. aureus* producătoare de PVL (4) şi imunodetecţia PVL în plămân (6), precum şi faptul că doar izolatele de *S. aureus* producătoare de PVL pot reproduce pneumonia necrozantă la modele experimentale (7). Procentul realelor infecţii virale premergătoare este necunoscut, dar coinfecţia cu virusuri respiratorii, inclusiv virusul gripal trebuie investigată.

Totuşi, pacienţii cu pneumonie comunitară cu SARM PVL-pozitive nu au o rată a mortalităţii mai mare decât pacienţii cu pneumonie comunitară cu SASM PVL-pozitive (8).

Pneumonia de ventilaţie produsă de SARM este o complicaţie frecventă în unităţile de terapie intensivă şi se manifestă ca pneumonie cu debut tardiv la pacienţi care au fost intubaţi pentru perioade lungi de timp şi/sau au suferit o bronhoscopie (9). Rezistenţa la meticilină a tulpinilor de *S. aureus* care produc pneumonie de ventilaţie este asociată cu o rată mare a mortalităţii (10, 11). Infecţiile nosocomiale cu SARM duc la creşterea costurilor economice şi de spitalizare.

Tratamentul cu antibiotice al infecţiilor cu SARM-C şi SARM-N poate să difere deoarece SARM-C rămâne adeseori sensibil la antibiotice, altele decât glicopeptide şi antibioticele noi. Mai mult, producerea de toxine, cum este PVL, ar trebui să influenţeze alegerea antibioticului în sensul utilizării unei asocieri cu agenţi antimicrobieni capabili a descreşte producerea de toxină (2).

MATERIAL ŞI METODĂ

Tulpini. Am inclus în studiu 32 de tulpini consecutive de *S. aureus* izolate de la pacienţi (22 bărbaţi, vârsta medie 57 ± 16 ani) diagnosticaţi cu pneumonie. Bacteriemia a fost pusă în evidenţă la 5 dintre aceşti pacienţi. Pacienţii au provenit din diverse localităţi din Nord-Estul Moldovei şi au fost internaţi în Spitalul Clinic de Boli Infecţioase „Sf. Parascheva" Iaşi, între 1.01.2005 şi 31.12.2009.

Testarea sensibilităţii la antibiotice a aces-

tor tulpini a fost făcută prin metoda difuzimetrică (Kirby-Bauer), pe agar Mueller-Hinton, cu discuri de antibiotice Oxoid (Marea Britanie), iar interpretarea testului a fost făcută conform standardelor CLSI (NCCLS) în vigoare la momentul testării. Pentru oxacilină şi vancomicină sensibilitatea tulpinilor a fost testată şi prin detectarea concentraţiei minime inhibitorii (CMI) folosind benzi E-test (bioMérieux, Franţa).

Detectarea genelor *mecA* şi *pvl*. A fost efectuată prin singleplex real-time PCR (RT-PCR) pentru cele 20 tulpini izolate în 2008-2009. Martori pozitivi/negativi au fost *S. aureus* ATCC 25923 (*mecA* absentă, *pvl* prezentă) şi *S. aureus* ATCC 33592 (*mecA* prezentă, *pvl* absentă). Extractele ADN au fost obţinute din cultura în bulion cord-creier a izolatelor clinice şi a martorilor, folosind *GenElute Bacterial Genomic DNA kit* (Sigma Aldrich, Germania). Amplificarea şi detecţia folosind sonde de fluorescenţă s-a făcut cu un aparat Stratagene MX 3005P. Primerii şi sondele utilizate (12) sunt descrise în tabelul I.

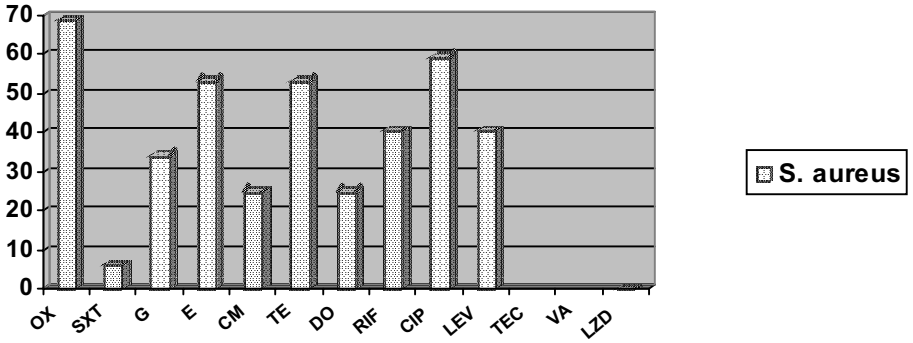
Amestecul de reacţie (20μL) a conţinut : 10 μl 2×*HotStar Taq Master Mix* (Qiagen, SUA), 4 μl amestec de primeri specifici, 0,2 μL sondă de fluorescenţă specifică, 2 μL ADN ţintă şi 3,8 μL apă PCR. Reacţiile au fost efectuate în triplicat, cu următorul profil termic : 7 minute la 95°C, urmate de 40 cicluri de câte 15 secunde la 95°C şi apoi 60 secunde la 60°C.

REZULTATE

Între 1.01.2005 şi 31.12.2009 am izolat 32 tulpini de *S. aureus* de la pacienţi internaţi cu infecţie a tractului respirator inferior în Spitalul Clinic de Boli Infecţioase „Sf. Parascheva" Iaşi, dintre care 22 (68,7%) au fost SARM. 20 dintre tulpinile SARM au fost izolate din infecţii nosocomiale şi 2 din infecţii comunitare.

În figura 1 este prezentată rata rezistenţei la antibiotice a celor 32 tulpini de *S. aureus*. Remarcăm rata înaltă (>50%) de rezistenţă la oxacilină, eritromicină, tetraciclină şi ciprofloxacin. Toate tulpinile rezistente la clindamicină au fost cu rezistenţă inductibilă la acest antibiotic.

Valorile CMI oxacilină au variat între 0,38 μg/mL şi >256 μg/mL, cu CMI(50) = 64 μg/mL şi CMI(90) >256 μg/mL. Pentru cele 20 tulpini izolate între 2008-2009, rezistenţa la oxacilină a fost evaluată şi prin detectarea genei *mecA*. Ea a fost depistată în toate tulpinile cu CMI ≥ 4 μg/mL, stabilind o concordanţă de 100% între cele 2 metode.



OX = oxacilină, SXT = sulfametoxazol&trimetoprim, G = gentamicină, E = eritromicină, CM = clindamicină, TE = tetraciclină, DO = doxiciclină, RIF = rifampicină, CIP = ciprofloxacin, LEV = levofloxacin, TEC = teicoplanină, VA = vancomicină, LZD = linezolid

Fig. 1. Rezistenţa la antibiotice a tulpinilor de *S. aureus* izolate din probe de spută, 2005-2009

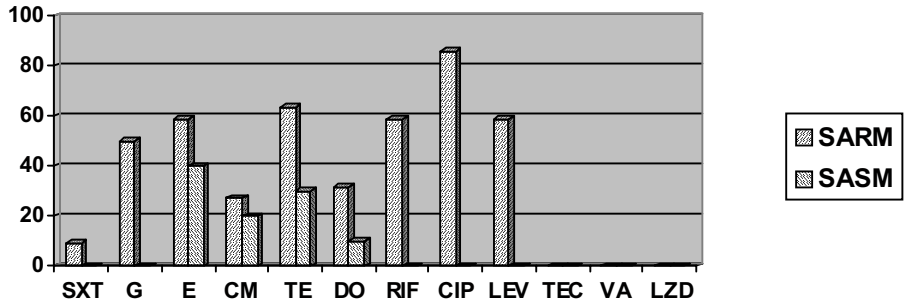


Fig. 2. Rezistenţa la antibiotice a SARM vs. SASM izolate din probe de spută, 2005-2009

Valorile CMI vancomicină au variat între 1-2 μg/mL, cu CMI(50) = 1,5 μg/mL şi CMI(90) = 2 μg/mL.

Remarcăm diferenţe statistic semnificative ($p < 0,05$) între SARM şi SASM privind rata rezistenţei la gentamicină, tetracicline, rifampicină, fluorochinolone (fig. 2).

Am investigat prezenţa genelor *pvl* la cele 20 tulpini *S. aureus* izolate între 2008--2009. Doar una din cele 20 tulpini (5%) a fost producătoare de PVL (fig. 3).

Am remarcat 2 pacienţi între cele 20 cazuri diagnosticate între 2008-2009.

1. Pacienta L.A., 7 ani, fără antecedente patologice sau spitalizări anterioare, care se prezintă în urgenţă cu sindrom meningean apărut la 2 zile după instalarea unui sindrom gripal. Se instituie tratament injectabil cu ampicilină după recoltarea probelor biologice pentru examene microbiologice. Radiografia pulmonară relevă opacităţi diseminate pe ambele arii pulmonare. În sângele periferic s-a obiectivat leucocitoză (11.200/mmc), cu predominenţa PMN, în special elemente imature, cu granulaţii toxice şi trombocitopenie. Din lichidul cefalo-rahidian (LCR), hemocultura şi sputa pacientei se izolează aceeaşi tulpină de *S. aureus*, cu sensibilitate păstrată faţă de toate antibioticele cu excepţia penicilinei; CMI oxacilină

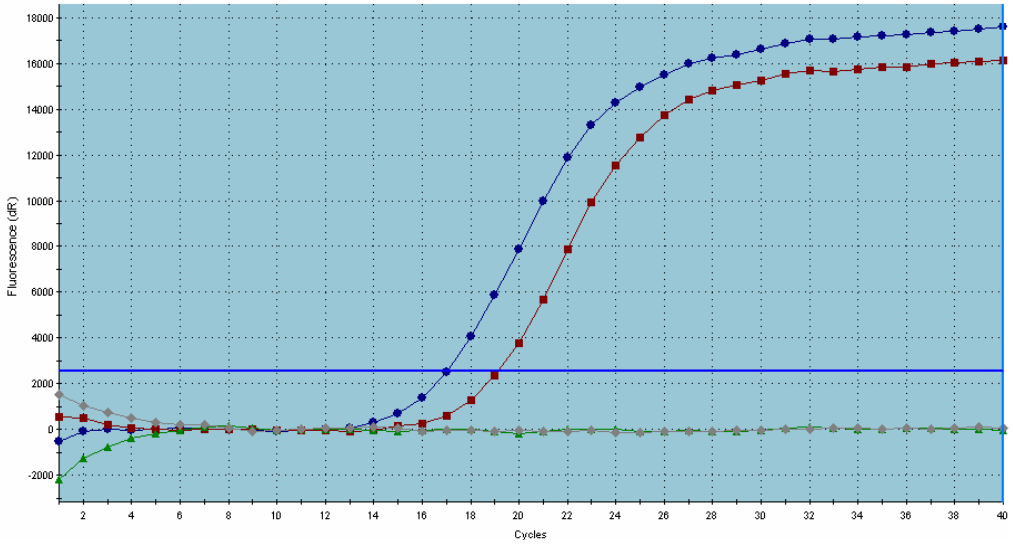


Fig. 3. RT-PCR pentru detectarea genei *pvl* la tulpina 8 (pacient LA), martor pozitiv (*S. aureus* ATCC 25923), martor negativ (*S. aureus* ATCC 33592) şi NTC (*non-template control*)

= 0,125 μg/mL, CMI vancomicină = 1 μg/mL. Retrospectiv, tulpina izolată s-a dovedit a fi producătoare de PVL. Starea generală a pacientei s-a deteriorat rapid, aceasta decedând la mai puţin de 24 ore de la internare prin şoc toxico-septic şi coagulare intravasculară diseminată.

2. Pacienta M.M., 76 ani, imundeprimată (leucemie limfatică cronică), se internează în stare gravă, cu febră şi sindrom meningean. Se instituie terapie cu ceftriaxonă şi ciprofloxacin. Din hemoculturi şi LCR se izolează *S. aureus* cu acelaşi antibiotip – tulpina este rezistentă doar la penicilină şi gentamicină; CMI oxacilină = 0,75 μg/mL, CMI vancomicină = 1,5 μg/mL. Evoluţia pacientei este nefavorabilă, necesitând ventilaţie mecanică. După 5 zile, se izolează din aspiratul traheo-bronşic o tulpină de SARM cu rezistenţă multiplă la antibiotice: gentamicină, eritromicină, clindamicină (rezistenţă inductibilă), teraciclină, rifampicină, ciprofloxacin, levofloxacin; CMI oxacilină >256 μg/ml, CMI vancomicina = 1,5 μg/mL. Pacienta decedează prin insuficienţă multiplă de organ în cadrul sepsisului.

DISCUŢII

Comparativ cu pacienţii cu pneumonie comunitară, cei cu pneumonie nosocomială au fost mai în vârstă (60 ani vârsta medie a pacienţilor cu pneumonie nosocomială vs. 28 ani vârsta medie a pacienţilor cu pneumonie comunitară). Rata mortalităţii a fost similară pentru cele 2 categorii de infecţii (mortalitate 27,2% pentru pneumonii nosocomiale şi, respectiv, 22,2%). SARM este mai frecvent izolat de la pacienţi cu pneumonie nosocomială (100%) decât de la cei cu pneumonie comunitară (25%).

Pneumonia comunitară cu tulpini de *S. aureus* PVL-pozitive este o boală severă. De obicei afectează copilul sau adultul tânăr (vârsta medie 15 ani). Infecţiile cu tulpini PVL-pozitive se asociază mai frecvent cu: temperatură peste 39°C, puls peste 140/minut, hemoptizie, apariţia de exsudat pleural în cursul spitalizării. De regulă leucopenia, simptomele pseudogripale sau gripa cu confirmare de laborator şi hemoptizia sunt factori asociaţi cu decesul (13). În cazul descris de noi infecţia a fost letală, a avut o durată scurtă de la instalarea simptomelor până la deces (48 ore), diseminare sistemică (hemoculturi pozitive), localizări secundare meningeene şi pulmonare, leucocitoză moderată cu polinucleoză cu predominanţa elementelor imature şi granulaţii toxice în sângele periferic. Terapia de primo-intenţie instituită în acest caz nu a fost susţinută de antibiograma tulpinii izolate.

Antibioticele care inhibă producerea PVL ar putea fi mai adecvate pentru tratamentul pneumoniei severe necrozante, prin analogie cu utilizarea lor în sindromul şocului toxic streptococic sau stafilococic (14,15, 16). Într-un studiu al lui Dumitrescu et al. (17), examinând capacitatea *S. aureus* de a elibera PVL în prezenţa antibioticelor, nu a fost detectată PVL dacă *S. aureus* a fost incubat în prezenţa unor concentraţii inhibitoare de antibiotic, pe când concentraţii subinhibitorii de oxacilină au crescut nivelul PVL. Clindamicina, linezolidul şi acidul fusidic au fost inhibitorii, iar vancomicina nu a avut aproape niciun efect. Linezolidul este eficient clinic împotriva *S. aureus* PVL-pozitive (18).

Tulpinile de *S. aureus* izolate din pneumonii în studiul nostru se remarcă prin procentul mare de rezistenţă la oxacilină (68,7%), mult peste rata rezistenţei la oxacilină a tulpinilor de *S. aureus* izolate din infecţiile cu alte localizări (45,7%). 33% dintre tulpinile testate au avut CMI oxacilină >256 μg/ml. De asemenea remarcam procentele mari de rezistenţă la gentamicină, tetracicline, rifampicină, fluorochinolone la tulpinile SARM comparativ cu SASM.

Niciuna din tulpinile testate nu a fost rezistentă la glicopeptide sau linezolid, indicând aceste antibiotice ca alternative excelente

pentru tratamentul infecţiilor cu SARM.

Cazul de pneumonie nosocomială cu SARM descris de noi se remarcă prin achiziţionarea tulpinii infectante în cursul terapiei intraspitaliceşti pentru sepsis cu SASM. Tulpina nosocomială a fost multiplu-rezistentă.

Vârsta înaintată, sexul masculin, dificultăţi la deglutiţie şi inabilitatea de a ingera medicaţia orală sunt factori de risc semnificativi pentru pneumonia asociată îngrijirilor medicale, produsă cel mai frecvent de *S. aureus*, inclusiv SARM, *Pseudomonas aeruginosa* şi bacili gram-negativi intestinali (19).

Deşi tratamentul standard al pneumoniei cu SARM sunt glicopeptidele, linezolidul este considerat o alternativă în special în pneumonia nosocomială. Comparativ cu glicopeptidele, linezolidul atinge niveluri pulmonare mai ridicate, care se pot corela cu eficacitate sporită în tratamentul pneumoniei nosocomiale. Studiile clinice nu susţin însă superioritatea linezolidului faţă de glicopeptide în tratamentul pneumoniei nosocomiale. Deşi a fost obţinută o eradicare microbiologică mai rapidă prin terapie cu linezolid a pneumoniei produse de SARM în secţii de terapie intensivă, totuşi eficacitatea clinică şi rata supravieţuirii au fost similare cu cele obţinute prin terapia cu teicoplanină. Linezolidul are un risc de două ori mai mare de trombocitopenie şi efecte secundare gastrointestinale. Vancomicina şi teicoplanina nu sunt asociate cu un risc mai mare de disfuncţie renală decât linezolidul (20).

CONCLUZII

Tulpinile de *S. aureus* izolate din pneumonii în studiul nostru se remarcă prin procentul mare de rezistenţă la oxacilină (68,7%), mai mare decât procentul SARM izolate din infecţiile cu alte localizări (45,7%).

Pneumonia nosocomială cu *S. aureus* apare mai frecvent la bătrâni, iar pneumonia comunitară cu *S. aureus* producător de PVL apare mai frecvent la tineri anterior sănătoşi.

Glicopeptidele şi linezolidul sunt opţiunile terapeutice indicate în cazul pneumoniei stafilococice, atât cu SARM, cât şi cu *S. aureus* PVL pozitiv.

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MODIFICĂRILE HISTOPATOLOGICE ŞI IMUNOHISTOCHIMICE DIN MUCOASA GASTRICĂ COLONIZATĂ DE *HELICOBACTER PYLORI*

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HISTOPATOLOGIC AND IMMUNOHISTOCHEMIC CHANGES IN *HELICOBACTER PYLORI* COLONISED GASTRIC MUCOSA (Abstract): *Helicobacter pylori* (*H. pylori*) colonize gastric mucosa causing both inflammatory changes, premalignant lesions and malignant tumors, including gastric lymphoma and carcinoma. In this study, our propose was to evaluate the histopatological changes corellated with immunohistochemical results demonstrating the types of cellular infiltration and proliferative activity of gastric mucosa infected with *H. pylori*. **Material and method**: Gastric endoscopic examinations was performed in 468 patients with anti-*H. pylori* antibodies and dispeptic phenomena. Snippets harvested endobiopsic stomach were fixed in formalin and processed by paraffine inclusion. Histological sections were stained with hematoxylin-eosine and Giemsa. In 65 cases of endobiopsic fragments (36 deep chronic gastritis with intestinal metaplasia, glandular atrophy and intraepithelial neoplasia and 29 carcinomas) immunohistochemical reactions were performed by applying reagents for evidence of *H. pylori* colonies, of T lymphocytes (CD3) and macrophages (CD68) and Ki-67 reagent for proliferating nuclear antigen labelling. **Results**: Endobiopsic specimen found in all *H. pylori* or by Giemsa staining or by anti-*H. pylori* antibodies when they were in small numbers. Histologically, were diagnosed: 463 superficial and deep chronic gastritis associated with premalignant lesions, 29 carcinomas, 2 non-Hodgkin's lymphoma and an ademathous polyp. Immunohistochemically, inflammatory infiltrate consisted of numerous T lymphocytes, macrophages and lymphoid follicles. Foveolar cell nuclei, in areas of intraepithelial neoplasia and carcinomatous cells were intensely stained with Ki-67, demonstranting increased proliferation. **Conclusions**: In gastric infection with *H. pylori,* inflammatory infiltrat is composed of abundant macrophages and T lymphocytes. Ki-67 was absent or minimal in chronic gastritis, while in areas of intraepithelial neoplasia was positive in both foveolar and coating epithelium. Anti-*H. pylori* antibodies in human serum remains one of the simplest methods to detect *H. pylori,* therefore it plays an important role in practice. Medical eradication of bacteria may cancel inflammatory changes, metaplasia and proliferation of gastric mucosa and thus it prevents the cascade of carcinogenesis. **Key words**: HELICOBACTER PYLORI, CHRONIC GASTRITIS, PROLIFERATION, Ki-67, CARCINOGENESIS

Helicobacter pylori (*H. pylori*) colonizează mucoasa gastrică cu predilecţie în aria antrală (1), determinând leziuni de intensitate variabilă, atât cu caracter inflamator asociat cu leziuni premaligne, cât şi, în timp, apariţia de tumori maligne, ca:

limfomul şi carcinomul gastric (2,3). Luând în considerare prevalenţa mare a infecţiei cu *H. pylori* (peste 80% din populaţie, în ţările în curs de dezvoltare, 50% din persoanele infectate fiind asimptomatice) (4,5, 6), devine importantă investigarea populaţiei în vederea detectării anticorpilor anti-*H. pylori*. În acest studiu ne-am propus evaluarea modificărilor histopatologice în corelaţie cu rezultatele imunohistochimice ce demonstrează tipurile celulare din infiltratele inflamatorii şi activitatea proliferativă ale mucoasei gastrice infectate cu *H. pylori,* ce pregătesc condiţiile de transformare malignă.

MATERIAL ŞI METODĂ

Lotul de studiu a fost constituit din 468 pacienţi cu anticorpi serici anti-*H. pylori,* examinaţi endoscopic cu obţinerea de fragmente endobiopsice multiple din corpul gastric, antru şi din leziunile constatate la acest examen. Fragmentele endobiopsice au fost prelucrate prin metoda de includere la parafină, apoi secţiunile histologice de 5 μm au fost colorate prin tehnici curente (hematoxilin-eozină) şi histochimice (coloraţia Giemsa pentru aprecierea coloniilor de *H. pylori* şi albastru alcian pH 2,5 pentru evidenţierea mucusului acid). Un grup selectat de 65 de pacienţi cu anticorpi serici anti-*H. pylori*, cu gastrită cronică profundă asociată cu leziuni premaligne şi carcinoame gastrice, decelate histopatologic, a fost investigat imunohistochimic (IHC) prin aplicarea de anticorpi monoclonali. Tehnica de colorare imunohistochimică: prelucrarea lamelor s-a efectuat după metoda Complexului Steptavidin-Biotină (sABC) / Horse Radish Peroxidase (HRP). S-au utilizat următorii anticorpi primari:

- anticorpul monoclonal anti CD3 reacţionează cu limfocitele T;
- anticorpul monoclonal anti CD68 reacţionează cu macrofagele şi monocitele; în acest studiu am utilizat clona PG-M1, DAKO a anticorpului anti-CD68, RTU, pentru a evidenţia macrofagele;
- pentru evidenţierea *H. pylori* am folosit anticorpul policlonal anti-*H. pylori* cu diluţie de 1: 20; epiteliul gastric infectat cu *H. pylori* marcat cu anticorpul policlonal anti-*H. pylori* prezintă colorare apicală a membranelor celulare precum şi reacţie pozitivă în lumenul gastric, deasupra epiteliului;
- anticorpul monoclonal anti Ki67 permite monitorizarea directă a proliferării celulare. Antigenul Ki-67 este o proteină nucleară mare (395 kDa) exprimată preponderent în cursul fazelor active ale ciclului celular (G1, S, G2, M) şi absentă în faza G0; în acest studiu am utilizat clona MIB-1, cu diluţie 1:75.

Pentru controlul reacţiilor imunohistochimice am folosit evaluarea controlului pozitiv extern pe secţiuni realizate din ţesuturi în care pozitivitatea pentru un anumit marker era certă cu scopul de a verifica eficienţa reactivilor folosiţi şi corectitudinea tehnicii folosite. Imunoexpresia a fost gradată în raport cu intensitatea reacţiei (0-negativ; 1-slab; 2-moderat; 3-intens) şi extensia colonizării, cu gradele: 1≤10% din celule; 2-25%; 3-25-50%; 4-50-75%; 5≥75%). Evaluarea a fost apreciată în raport cu localizarea în mucoasa gastrică: epiteliul superficial, foveolar, glandular, tumoral.

REZULTATE

Lotul de studiu a cuprins 468 pacienţi cu anticorpi serici anti-*H. pylori şi* examen endoscopic asociat cu endobiopsii gastrice. Lotul a fost constituit din 270 femei şi 198 bărbaţi, cu vârsta medie de 54±5 ani.

Histopatologic, la examenul standard, s-au diagnosticat 436 gastrite cronice, 29 carcinoame gastrice, 2 limfoame non-Hodgkin gastrice şi 1 polip adenomatos cu neoplazie intraepitelială uşoară. Din cele 436 gastrite cronice, s-au constatat 19 gastrite cronice superficiale localizate mai ales în zona corpului gastric, 417 au fost gastrite cronice profunde antrale şi pangastrite. În

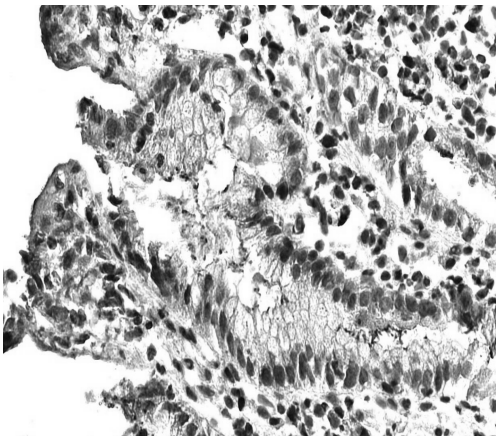

Fig. 1. Evidenţierea *H. pylori* (x20, Ac monoclonali anti-*H. pylori*)

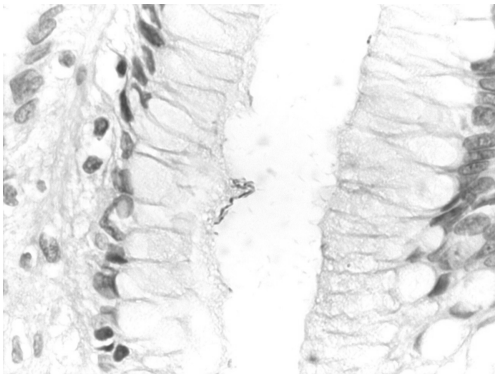

Fig. 2. Evidenţierea *H. pylori* în cazurile cu număr redus de colonii (x40, Ac monoclonali anti-*H. pylori*)

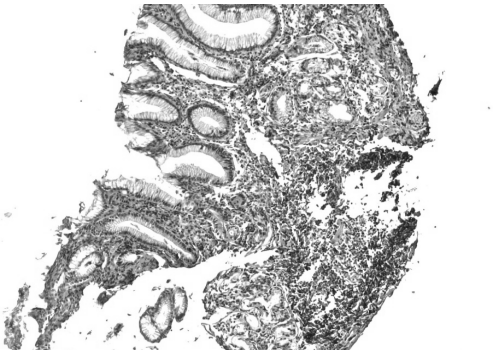

Fig. 3. Foliculi limfoizi în mucoasa gastrică antrală (x10, CD3 pozitiv)

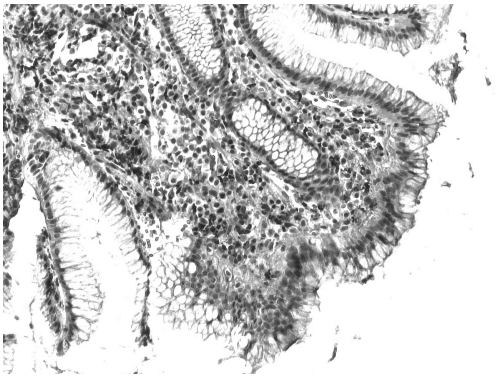

Fig. 4. Macrofage în lamina propria (x40, CD68 pozitiv)

32 cazuri de gastrite cronice profunde antrale, s-a observat asocierea cu leziuni premaligne severe, ca : atrofie glandulară, metaplazie intestinală, neoplazie intraepitelială de grad redus şi de grad înalt. Imunohistochimic, s-a evidenţiat în toate cazurile investigate cu anticorpi anti-*H. pylori,* prezenţa bacteriei, mai ales în ariile cu număr redus, greu decelabil prin coloraţia Giemsa (fig. 1, 2).

În cazurile studiate s-a observat creşterea numărului de limfocite T, asociate cu prezenţa de foliculi limfoizi (fig. 3) şi de macrofage (fig. 4).

Prin aplicarea reactivului Ki-67 s-a constatat colorarea nucleară intensă în celulele

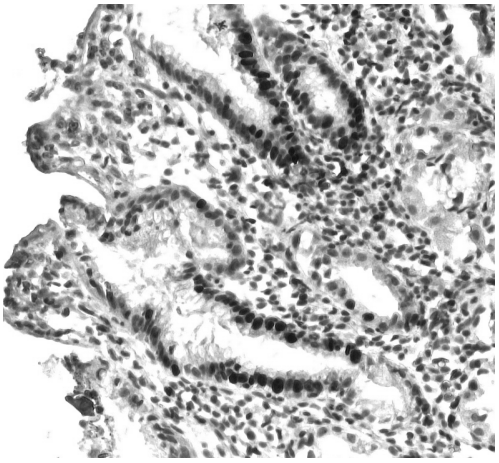

Fig. 5. Gastrită cronică profundă, Ki-67 + în foveole (x10)

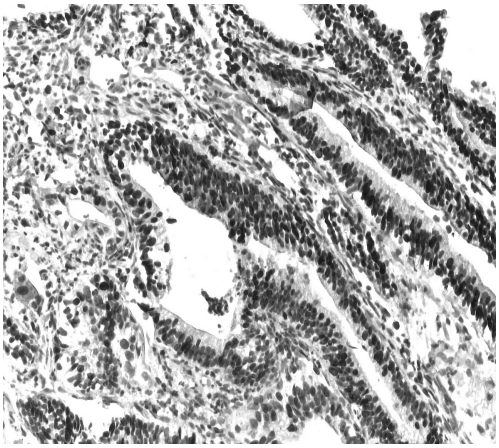

Fig. 6. Gastrită cronică cu displazie severă foveolară, Ki-67+(x20)

epiteliale din foveole (fig. 5) şi din ariile de neoplazie intraepitelială de grad înalt şi în celulele tumorale carcinomatoase (fig. 6).

DISCUŢII

Este acceptat că infecţia *H. pylori* reprezintă un factor de risc pentru apariţia tumorilor maligne gastrice (7,8). Efectele directe ale *H. pylori* sunt relevante pentru inducerea modificărilor inflamatorii (9) ce ulterior determină leziuni premaligne, ca: gastrita atrofică, metaplazia intestinală şi neoplazia intraepitelială de grad redus sau înalt (10,11). Rezultatele studiului nostru demonstrează IHC că inflamaţia determinată de *H. pylori* se manifestă prin creşterea numerică a limfocitelor T, a limfocitelor B, sub formă de foliculi limfoizi şi numeroase macrofage (11,12,13). Astfel, infecţia cu *H. pylori* este asociată cu infiltrat inflamator abundent, cu intensificarea apoptozei şi proliferării celulare (14,15,16).

CONCLUZII

Rezultatele studiului arată că infecţia cu *H. pylori* se manifestă mai ales în antru, sub formă de gastrită cronică profundă, cu hiperplazie micropolipoasă a epiteliului de înveliş şi foveolar, atrofie glandulară asociată cu metaplazie intestinală şi neoplazie intraepitelială (displazie).

O altă concluzie a studiului este că în endobiopsiile gastrice negative pentru *H. pylori* în coloraţia Giemsa este necesară efectuarea reacţiei IHC în scopul evidenţierii bacteriei.

Prezenţa anticorpilor anti-*H. pylori* din serul uman rămâne una din cele mai simple metode de detectare a *H. pylori*; în acest fel, metodele serologice de detectare a *H. pylori* joacă un rol important în practică.

Eradicarea medicală a bacteriei poate să anihileze modificările inflamatorii, metaplazice şi proliferative din mucoasa gastrică şi, astfel, să împiedice cascada carcinogenezei.

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NOUTĂŢI

IMPLICAREA GENETICII IN BOLILE AFECTIVE ŞI SUICID

Comportamentul suicidal arată o evidentă concentrare familială şi o concordanţă la gemeni ceea ce sugerează existenţa unei heritabilităţi moderate. Factorii genetici implicaţi în comportamentul suicidal par să se suprapună peste cei implicaţi în bolile afective. Există dovezi incontestabile că atât boala bipolară cât şi cea unipolară sunt influentaţe de genotipul persoanei. Recent, atenţia cercetătorilor a fost atrasă de genele candidate la suicid la pacienţii aflaţi sub tratatment cu antidepresive. Studiile genomice au relevat câteva posibile gene candidat : genele pentru transportorii serotoninei (5-HTT sau SLC6A4), gena pentru triptofan hidroxilaza 1 (TPH1), genele ce codifică factori neurotrofici (BDNF) şi receptorii lor, receptorul pentru neurotrofic tirozin-kinaza tip 2 (NTRK2) sau receptorul adrenergic 2A- (ADRA2A). Tratamentele cu inhibitori selectivi ai recaptării serotoninei sau cu antidepresive triciclice par a creşte, la unii pacienţi, riscul suicidar. La aceşti pacienţi s-a descris existenţa unui polimorfism de tip SNP în gena BDNF. La pacienţii cu risc crescut suicidal în cursul tratamentului cu nortriptyline s-a descris existenţa unui polimorfism SNP în gena pentru receptorul ADRA2A. Aceste date sugerează implicarea sistemului neurotrofic în vulnerabilitatea la suicid. De asemenea s-a observant şi legătura între boala bipolară şi două noi gene : CACNA1C si ANK3. CACNA1C codifică subunitatea alpha-1 a unui canal ionic de calciu voltaj-dependent, în timp ce ANK3 codifică proteina ce leagă acest canal de citoschelet. Polimorfismul de tip SNP în CACNA1C este semnificativ asociat cu boala bipolară, dar şi cu susceptibilitatea crescută la schizofrenie sau depresie unipolară (McGuffin P, Perrouda N, Uhera R. The genetics next term of affective disorder and suicide. *Eur Psych* 2010, 25 (5) : 275-277).

Elena Braha

PERFORMANŢELE UNUI MEDIU CROMOGEN ÎN DETECTAREA ENTEROBACTERIILOR PRODUCĂTOARE DE BETA-LACTAMAZE CU SPECTRU EXTINS DIN NORD-ESTUL ROMÂNIEI

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CHROMOGENIC MEDIUM PERFORMACES FOR DETECTION ENTEROBACTERIA PRODUCING EXTENDED-SPECTRUM BETA-LACTAMASES FROM NORTH--EASTERN ROMANIA (Abstract). We evaluated the performances and analyzed practicability of an ESBL-detecting chromogenic agar medium (chromID ESBL, bioMerieux) for the screening of ESBL production. **Material and methods**: We have tested 112 enterobacteria strains, isolated in 2009 year, in North Eastern region of Romania. Each bacterial strain was cultured on a prepared plate chromogenic medium and the results were recorded after incubation at 37°C for 18 to 24 h or, if necessary, for 48 h. We calculated the sensitivities, specificities, and positive and negative predictive values for this medium, using as gold standard ceftzidime or cefotaxime and clavulanate combination disks on Iso-Sensitest agar. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as the positive and negative control for ESBL production, respectively. **Results**: For all tested strains, the chromID ESBL agar showed a high sensitivity (97%), but a low specificity (66%). The positive and negative predictive values were 98% and 57%, respectively. The chromID ESBL had false-positive results for 2 confirmed ESBL-positive strains. **Conclusion**: The very good sensitivity of the chromID ESBL agar ESBL detection represent a convenient method for the recovery and identification of ESBL-producing enterobacteria. **Key words**: CHROMOGENIC MEDIUM, ENTEROBACTERIA, EXTENDED-SPECTRUM BETA-LACTAMASES

OPTIMIZATION OF THE SYNTHESIS OF SOME NEW THIAZOLIDINE-4-ONE DERIVATIVES WITH XANTHINE SCAFFOLD

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OPTIMIZATION OF THE SYNTHESIS OF SOME NEW THIAZOLIDINE-4-ONE DERIVATIVES WITH XANTHINE SCAFFOLD (Abstract): In the last time many researchers were focused on synthesis of new compounds with thiazolidine-4-one scaffold based on the important biological effects of this heterocycle. **Aim:** The aim of this study was to optimize the synthesis of some new thiazolidine-4-one derivatives with xanthine scaffold in order to obtain high yields and advanced purity. **Material and methods:** The synthesis was based on a cyclization reaction between various hydrazones and thioglycolic acid. The optimization was performed for 2-(4-methylphenyl)-3-[(1,3-dimethylxanthin-7-yl)acetamido]thiazolidine-4-one (**b**) by varying different parameters such as molar ratio between reagents, solvent, catalyst, temperature, time of reaction and type of method. There were used ten chemistry procedures for detecting the desired compound and other ones for establishing the optimal conditions. The optimized method was also applied for other thiazolidine-4-one derivatives with xanthine structure. **Results:** The most favorable parameters for optimal synthesis were established as follow: an excess of 20 equivalents of thioglycolic acid to 1 equivalent of hydrazone, freshly distilled toluene as solvent, temperature of 120 °C and reflux for 18 hours. The time and temperature of reaction proved to have a key role for increased yield. **Conclusions:** The optimal conditions of reaction were established in order to obtain new thiazolidine-4-one derivatives in high yields and increased purity. **Keywords:** THIAZOLIDINE-4-ONE, XANTHINE, OPTIMIZATION, SYNTHESIS.

In the last period, the interest of many researchers was directed towards the study of thiazolidine-4-one derivatives due to the intense and important biological activity proved by this heterocycle (1). The studies proved for this scaffold many biological effects, such as antidiabetic, antioxidant, anti-inflammatory, anticancer, antimicrobial, antiviral, anticonvulsant or antihypertensive (2).

This heterocycle is usually found in the structure of synthetic compounds, but also it was identified in natural compounds, such as actithiazic acid (acidomycin) which was isolated from *Streptomyces* strains and proved an intense and specific action on

Mycobacterium tuberculosis (3).

In the literature (1) different methods for the synthesis of thiazolidine-4-one derivatives are described. The most used pathway involves three components: an amine, a carbonylic compound (aldehyde or cetone) and thioglycolic acid and it can be performed in one pot (fig. 1) or two steps (fig. 2).

When the synthesis is performed in two steps, the first reaction is based on the condensation between the amine and carbonyl compounds, resulting the corresponding imine, which, in the next step, it will be cyclized in the presence of thioglycolic acid (fig. 2). In order to apply this method to different thiazolidine-4-one scaffolds, different parameters were varied by the researchers, such as: time and temperature of reaction, solvent (dry toluene, benzene, dioxane, dry tetrahydrofuran, ethanol, dimethylformamide, polypropylene glycol (4)), catalyst (silica oxychloride (5), zinc chloride, N,N'-dicyclohexyl carbodiimide (6), 1-ethyl-3-(3-dimethylamino propyl) carbodiimide,N,N-di-isopropyl-ethylamine (7), N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uranium-hexafluoro-phosphate(8)), water extraction system (Dean Stark apparatus), pH (sodium acetate/acetic acid buffer (9)), methods based on "green chemistry" (ultrasounds, molecular sieves).

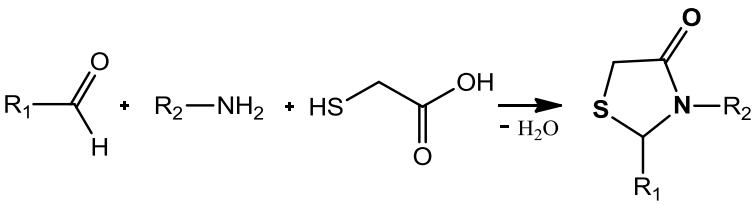

Fig. 1. One pot synthesis for thiazolidine-4-one derivatives.

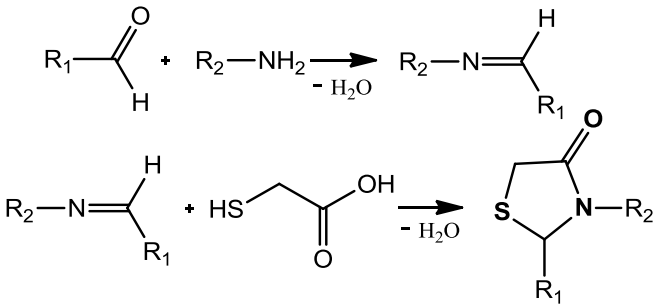

Fig. 2. Synthesis in two steps for thiazolidine-4-one derivatives.

The main objective of this study was the optimization of the synthesis of some new thiazolidine-4-one derivatives with xanthine scaffold, in view of identifying the optimal conditions of reaction (molecular ratio between reagents, time, temperature, solvent, catalyst etc.).

MATERIAL AND METHODS

Reagents. N-(4-Methylbenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide synthetized in our previous paper (10), thioglycolic acid, organic solvents (freshly distilled toluene and tetrahydrofuran, dimethylformamide - *p.a.* quality), zinc chlo-

ride, 1-ethyl-3-(3-dimethylamino propyl) carbodiimide and molecular sieves were purchased from Sigma Aldrich Company. All reagents and solvents were used without previous purification. Thin Layer Chromatography (TLC) Silica gel 60 F_{254} plates produced by Merck Company and Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra were used for monitoring the reactions and detecting the resulted compounds. The spectra were obtained in dimethyl sulfoxide deuterated (DMSO-d_6) and chloroform deuterated ($CDCl_3$), both produced by Sigma Aldrich Company. Microwave irradiation was carried out in sealed 2–20 mL vessels placed in a Biotage Initiator system, using a standard absorbance level (300 W maximum power).

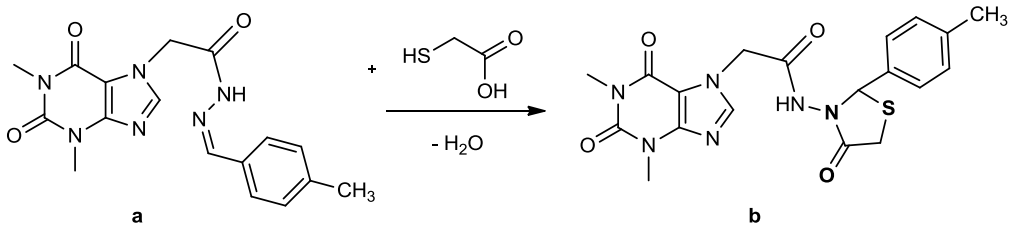

Fig. 3. Synthesis of 2-(4-methylphenyl)-3-[(1,3-dimethylxanthin-7-yl) acetamido]thiazolidine-4-one (**b**).

Chemistry. 2-(4-Methylphenyl)-3-[(1,3-dimethylxanthin-7-yl)acetamido]thiazolidine-4-one (**b**) was obtained based on reaction between N-(4-methylbenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**a**) and thioglycolic acid (fig. 3). The synthesis was performed by optimization of different procedures and methods described in the literature for cyclization between various hydrazones and thioglycolic acid.

For the optimization of the chemistry procedure different parameters were varied: molar ratio between the reagents (1:1; 1:1.55; 1:2; 1:10; 1:15.2; 1:20; 1:24; 1:25.75; 1:27.3), solvent (without solvent (11), dry toluene, dimethylformamide - DMFA, dry tetrahydrofuran - THF), temperature (room temperature - 20ºC, 70ºC, 100ºC, 120ºC, 130ºC, 140ºC, 153ºC, 160ºC, 180ºC), time (between 30 min and 62 hours), catalyst (zinc chloride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), type of method (classic or microwaves).

The reactions were monitored by TLC using different eluent systems: dichloromethane: methanol (9:1; 9.6:0.4; 9.8:0.2), dichloromethane: acetone (7:3), ethyl acetate, ethyl acetate: acetone (7:3). The structure of the compound **b** was proved by NMR spectra.

RESULTS AND DISCUSSION

Chemistry

By varying different synthesis parameters: ratio between reagents, catalyst, solvent, temperature and time of reaction and the applied method (classic or microwave), 10 chemistry procedures were developed (tab. I). Freshly distilled toluene proved to be the most favorable solvent in synthesis of compound **b**. Using this solvent, associated with a ratio between compound **a** and thioglycolic acid of 1 eq.:1.55 eq., in microwave for 40 min, at a temperature of 160 ºC, the condensation reaction was carried out successfully and compound **b** was

detected by NMR spectra.

In the next step the procedure established for synthesis of compound **b** was optimized in order to obtain the compound in high yields and high-grade purity. The experimented conditions are presented in tab II. It was observed that, as described in our previous paper (10), the highest yield (52 %) was obtained after the reaction between 1 eq. of hydrazone **a** and 20 eq. of thioglycolic acid, at a temperature of 120 ºC, for 18 hours, using freshly distilled toluene as solvent.

TABLE I

Chemistry procedures applied for synthesis of compound b

No.	Reagents (eq.)		Catalyst (eq.)	Solvent	T (ºC)	Time (min/h)	Type of method	b
	a	AT						
1.	1 eq.	24 eq.	-	-	100	30 min	MW	nd
2.	1 eq.	27.3 eq.	-	-	130	42 min	MW	nd
3.	1 eq.	25.75 eq.	-	-	180	1 h	MW	nd
4.	1 eq.	20 eq.	-	dry toluene	140	40 h	classic + dean stark (12,13)	nd
5.	1 eq.	1 eq.	$ZnCl_2$ 0.5 eq.	DMFA	100	30 min	MW (14)	nd
6.	1 eq.	1 eq.	$ZnCl_2$ 0.5 eq.	DMFA	153	28 h, 48 h	classic (15,16)	nd
7.	1 eq.	1 eq.; 15.2 eq.	$ZnCl_2$ (a pinch)	dry THF	70	20 h	classic (17)	nd
8.	1 eq.	1 eq.	$ZnCl_2$ (a pinch)	dry THF	20	20h, 40h	classic, molecular sives (18)	nd
9.	1 eq.	2 eq.	EDCI 1.2 eq.	THF	20	20 h, 60 h	classic (19)	nd
10.	1 eq.	1.55 eq.	-	dry toluene	160	40 min	MW	d

AT = thioglycolic acid; T (ºC) = temperature; MW = microwave; nd = nondetected; d = detected

TABLE II

Optimization of the synthesis procedure of compound b

No.	Reagents (eq.)		T (ºC)	Time (min/h)	Type of method	η (%)
	a	AT				
1.	1 eq.	1.55 eq.	160	40 min	MW	< 5%
2.	1 eq.	20 eq.	160	156 min	MW	< 5%
3.	1 eq.	20 eq.	160	2 h	MW	10,8%
4.	1 eq.	20 eq.	140	45 h	classic + dean stark	nd
5.	1 eq.	10 eq.	120	18 h	classic	< 5%
6.	1 eq.	20 eq.	120	62 h	classic	< 5%
7.	1 eq.	20 eq.	120	37 h	classic	47,2%
8.	1 eq.	20 eq.	120	13 h	classic	31,4%
9.	1 eq.	20 eq.	120	18 h	classic	52%
10.	1 eq.	25 eq.	120	18 h	classic	50%

AT= thioglycolic acid; T (ºC) = temperature; η (%) = yield; MW = microwave; nd = nondetected

The results showed also that the microwave method was not favorable for the synthesis of derivative **b**, the calculated yield being less than 11%. On the other hand for classic method was observed that the increasing of the temperature was associated with the degradation of the compound, the yield being less than 5%. Referring to the time of reaction, the results proved that a lower time than 18 hours was not enough for a total synthesis.

The developed procedure was applied for the synthesis of other thiazolidine-4-one derivatives with xanthine scaffold as described in our previous paper (10).

CONCLUSIONS

In this study, the synthesis of some new thiazolidine-4-one derivatives with xanthine scaffold was developed by optimization of the reaction between N-(4-methylbenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**a**) and thioglycolic acid in order to obtain 2-(4-methylphenyl)-3-[(1,3-dimethylxanthin-7-yl) acetamido]thiazolidine-4-one (**b**) in high yield and an increased purity. The optimization was performed by varying different reaction parameters such as: molar ratio between reagents, time and temperature of reaction, solvent, catalyst and type of method.

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NEWS

THE DIAGNOSTIC CONCORDANCE OF WHOLE SLIDE IMAGING AND LIGHT MICROSCOPY A SYSTEMATIC REVIEW

Traditionally, light microscopy (LM) has been the reference method used in anatomic pathology in the diagnosis of many diseases. However, advances in digital imaging hardware and software have led to the development of whole slide imaging (WSI) devices. These devices allow the digital capture, analysis, storage, sharing, and viewing of whole slide pathology images. Digital pathology evolved as a practical technology in the 1980s with the development of ‘‘telepathology’’ technology. Initially, telepathology existed in 2 forms: static-image telepathology and dynamic robotic telepathology. Static-image telepathology involved the transmission of preselected regions of microscopy images. Dynamic robotic telepathology enabled realtime image alterations by granting a remote user control of the microscope. Since these 2 initial telepathology technologies, more than 12 types of telepathology systems have evolved. Whole slide imaging is the latest technology used in digital pathology (Goacher E, Randell R, Williams B, Treanor D. The Diagnostic Concordance of Whole Slide Imaging and Light Microscopy a Systematic Review. *Arch Pathol Lab Med.* 2017; 141:151–161).

Doina Butcovan

MOTIVATION, STRESS AND SATISFACTION AMONG MEDICAL STUDENTS

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MOTIVATION, STRESS AND SATISFACTION AMONG MEDICAL STUDENTS (Abstract): **The aim** of the study was to identify the relationship between the strength of motivation of medical students, the symptoms they experience, the thoughts of dropping out and the satisfaction with the academic results obtained during their last exam session. The relationship between depression and physical symptoms was also explored. **Material and methods**: A number of 91 students from 4th year of study were included in the survey. Depression and the strength of motivation were measured using *Beck Depression Inventory* and *Strength of Motivation for Medical School.* The obtained data have been processed using SPSS 14 statistical software. **Results**: A positive correlation between the strength of motivation and physical symptoms was identified, meaning that a higher motivation is associated with a higher number of symptoms experienced by students in stressful moments ($r=0.33$, $p=0.01$). Also, women present a higher level of the strength of motivation compared to men [$t(89)=0.47$; $p=0.004$, $p < 0.05$]. A statistically significant difference [$t(89)=0.98$; $p=0.009$, $p < 0.05$] between the average strength of motivation in students who thought about dropping out ($M=53.03$) and in students who never thought about leaving medical school ($M=58.45$) was also identified. Perceived depression was also found to predict the number of symptoms felt by students during stressful times. **Conclusions**: Students that are mostly motivated in medical school also get stressed the most because they want to have good results. **Keywords:** MEDICAL STUDENTS, STRESS, DEPRESSION, MOTIVATION, ACADEMIC SATISFACTION.

The stress experienced by medical students during their academic years was subject of many researches (1, 2, 3). Medical studies doubled by clinical practice and working with human being determine a high level of stress during academic years (4). These factors may affect the psychological and somatic well-being and their academic performance (5, 6).

The strength of motivation could be described as the amount of effort, invested time and sacrifices people would make in order to learn something (7, 8, 9). When battling against stress, the strength of motivation could be an important factor that would help medical students to find a way to cope with it. It has been found that intrinsic motivation leads to better academic performance than extrinsic motivation (10). Luo Lu has also proven in a study that the

interaction between stressors such as work demands and extrinsic motivation could predict physical symptoms and anxiety (11). Not having a high strength of motivation was also shown to be linked to higher levels of stress and a lower academic performance (12, 13). Also, literature states that there is a gender difference regarding the strength of motivation, meaning that women have a higher strength of motivation than men (14).

Due to the increased stress and demands that are placed on medical students, their life satisfaction is affected. It has been found that life satisfaction decreased during medical school, even though students start with the same level of satisfaction in the first year as the students from other universities (15). Another study shows that students of first years were more optimistic regarding their future profession compared to the students from later years of study, thinking that they will work in the medical field (16).

The fact that life satisfaction is decreasing overtime could also be a warning sign for student dropout. Studies show that dropout may be associated with motivation to study medicine, commitment and resilience, besides the institution factors, gender or age (17). In this way, without the incentives of having a high strength of motivation, being resilient in the face of stressors and finding ways to cope with stress, student dropout could increase significantly.

Stress has also many times been linked to depression (1, 18, 19). Depression affects medical students more than it affects the general population (1, 20, 21), possibly because of high demands in medical school, stressors and few coping mechanisms that students find available. The depressive symptoms may have serious consequences on the way students perceive their competencies and their professional future, causing them to dropout or even have suicidal thoughts (1), especially during their 4th year of study (22) or causing somatic problems (23).

The aim of the study is to identify the relationship between the strength of motivation, the symptoms medical students experience during stressful times, the thoughts of dropping out and the satisfaction with the academic results obtained during their last session of exams. Also, we explored the link between depression and physical symptoms, trying to see whether the mental and somatic health is connected during stressful times in medical students. Through observing the relationship between these variables we could begin to have a better understanding of the medical students' lives and try to find strategies in order to improve their health and well-being.

MATERIAL AND METHODS

The lot of participants to our study consists of 91 students from 4th year of study from the University of Medicine and Pharmacy "Grigore T. Popa" Iasi" (74 women and 17 men). The age was 23.16 ±1.157 years (with a minimum of 22 and a maximum of 32 years)

The level of depression was measured using *Beck Depression Inventory* (24) which has 21 questions and the answers are given on a four-point scale. The Cronbach alpha coefficient was 0.93 in college student samples in literature and the Cronbach alpha was 0.87 in our study, showing good internal consistency.

The motivation was evaluated using the *Strength of Motivation for Medical School* (SMMS) (7). The Cronbach alpha in the literature for this scale is 0.79 and, in our study, the internal consistency was 0.83.

Socio-demographic data like age, sex, illness, the type of family of origin, the presence of a chronic disease among the members of the family, the living conditions and academic results (the number of credits obtained during the previous year) were also considered.

To evaluate the physical and psychological symptoms that students feel during stressful times we created a multiple answer question that consists of a list with 35 common symptoms. One of the items referred to the tendency to leave medical school. Contentment with the results obtained in the previous session of finals was also assessed on a five point Likert scale.

The obtained data have been processed using SPSS 14 statistical software.

RESULTS

Socio-demographic and academic data

A number of 19 students come from a rural area, and 72 students come from urban areas. Concerning their current accommodation, 25 students state they are living in the dorm, 31 (34.06%) are living in a rented apartment, 29 (31.86%) are living with their parents and 6 students (6.59%) are living alone. Regarding the relationship between parents, a number of 8 students said that their parents are divorced, the rest of the subjects declaring that their parents are married.

A number of 8 students (8.79%) declared they are chronically ill. Also, concerning the presence of a chronic disease within the family of origin, 30 students (32.96 %) said that they have family members suffering from a chronic disease, while 61 students (77.04%) said they don't have chronic disease among the members of their families.

Concerning the number of academic credits students had in their previous year of study, 2 (2.19%) scored lower than 400, 26 (28.57%) scored between 400 and 500, 43 (47.25%) scored between 500 and 550 and 9 (9.89%) scored between 550 and 600, while 11 (12.08%) didn't state the number of credits obtained, leaving the question unanswered.

Correlational statistics

Strength of motivation

The research explored the relationship between the strength of motivation and physical and psychological symptoms in times of stress, thoughts of dropping out and satisfaction with the academic results obtained.

We wanted to see whether the strength of the motivation of medical students is associated with the number of symptoms checked by the students as being present during stressful times. We found that the motivation correlates with the number of symptoms ($r=0.33$, $p=0.01$). The correlation coefficient demonstrates a moderate strength of the relationship and the correlation is positive, showing that a higher motivation is associated with a higher number of symptoms experienced by students in stressful moments.

Regarding the relationship between the strength of motivation and the contentment with results obtained during the last finals session, we found that the level of motivation correlates with the satisfaction with results ($r=0.22$, $p < 0.05$). The correlation coefficient demonstrates a weak, but significant relationship between the two variables, showing that a higher motivation is associated with a greater satisfaction with results obtained.

Stressors and depression

In the second part of the study we wanted to see whether the perceived depression would be able to predict the number of

symptoms checked by students during stressful times. A significant regression equation was found [F (1.89) =18.75, $p < 0.01$], with an R square=0.174.

Comparative statistics

We compared the strength of motivation considering the existence of thoughts regarding interrupting or abandoning the medical studies. A statistically significant difference was obtained [t(89)=0.98; p=0.009, $p < 0.05$] between the average strength of motivation in students who thought about dropping out (M 53.03) and in students who never thought about leaving medical school (M=58.45).

The sex difference on the strength of motivation was also measured. We found a statistically significant difference [t(89) =0.47; p=0.004, $p < 0.05$] between the average strength of motivation of men (M=50.47) and of women (M=57.86), meaning that women present a higher strength of motivation compared to men.

DISCUSSION

Stress is affecting daily activities and has a great impact on the psychological and physical well-being. It also determines how people are reacting in common situations and is closely linked to the level of performance (1, 25). Researches proved that in some jobs, especially in the medical profession, a high rate of burnout is associated with a high level of depression and suicidal thoughts (22).

We found that the strength of motivation is correlated with the number of physical and psychological symptoms felt, meaning that the higher the students' motivation, the greater the number of symptoms shown. When the students act without being satisfied about their work, they are more stressed comparing to the colleagues who are more pleased about their academic work. Our results show that students with a high strength of motivation are more stressed because they want to be overachievers, train well for their future profession and, when they feel they aren't at their best compared to the image they created for themselves, they could feel emotionally and physically exhausted, thus experiencing more symptoms than a student with a lesser motivation, one that isn't so preoccupied with the studies and with results.

The correlation between motivation and the contentment with results from the previous exam session shows that students with a higher motivation are also the ones that are more satisfied with their results, demonstrating that motivation is connected to greater academic performance, finding that is consistent with other researches (26, 27).

The strength of motivation varied significantly in students who have thought about dropping out and in students who haven't had such thoughts, meaning that the students that didn't see quitting medical school as an option had a higher motivation than the others. Literature has stated that one important risk factor for student dropout is motivation, besides scores obtained in high school and parents' education (28). Stress is very high and constant in medical school and, if the motivation of students isn't strong enough in order to help them efficiently deal with problems, thoughts about dropping out appear.

A difference in the strength of motivation was also found in men and women, showing that women have a significantly higher motivation than men. The fact that female medical students seem to have a higher strength of motivation and motivation quality than men has also been stated in literature (29).

Depression and stress have been linked

in numerous times in literature (30, 31, 32). We found that the level of depression in medical students was able to predict a higher level of somatic symptoms during stressful times. This is an issue that needs to be addressed in order to contribute to the health and well-being of future doctors, given the fact that the stress in medical school is constant and is followed by stress after graduation, during their entire profession. Also, the medical profession is associated with a higher rate of burnout, especially for professionals working in emergency departments, surgery, obstetrics and gynecology and anesthesiology clinics (33) so students must have strong knowledge about coping strategies.

CONCLUSIONS

Medical students have to get through the university and to endure lots of pressure and stress until they become doctors. Our study proves that depression, the strength of motivation and stressors are affecting the somatic and psychological health and that they also have an impact on academic activity and satisfaction. The results are important for students and for the academic staff. In order to help them prevent or diminish the level of stress experienced during their academic years we need to have lectures in the curricula information about medical psychology and coping strategies.

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EPIDEMIOLOGIC RESEARCH REGARDING THE IMPACT OF RISK FACTORS ON HEALTH-RELEATED QUALITY OF LIFE IN CANCER PATIENTS

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EPIDEMIOLOGIC RESEARCH REGARDING THE IMPACT OF RISK FACTORS ON HEALTH-RELEATED QUALITY LIFE IN CANCER PATIENTS (Abstract): **Aim:** To assess the health-related quality of life in cancer patients and the relative and attributable risks for the degree of dissatisfaction related to intrinsic and extrinsic factors. **Material and methods**: Our research included 128 cancer patients treated at the Surgical Clinic II of the Iasi Regional Cancer Institute between December 2014 and June 2015. Thus, data were collected by using an inquiry sheet derived from the SF-36 questionnaire, which included closed and open questions about the quality of life, socio-demographic data and such risk factors as smoking, alcohol use, related diseases and risk behaviours. **Results:** The patient sample had a mean age of 60.85 years, 51.6% of them lived in urban areas, were high school graduates, and of low economic status. **Discussion:** As to the behavioural risk factors we found that over 80% of the patients were non-smokers, did not use alcohol, and were normal weighted. Pain was experienced by 70.7% of the cancer patients and depression affected 74% of the study cases. Patients were asked to self-assess their own health status on a scale of 0 (worst) to 100 (the best health status). **Conclusions:** Our study showed a poor health status (mean score 65) in the study cancer patients. The relative and attributable risks for the degree of dissatisfaction related to intrinsic and extrinsic factors were calculated and ranked factors in decreasing order: smoking, depression, pain, alcohol use, male gender, obesity, low income. We found that prolonged suffering affects the psyche and causes depression and that the patients of low financial status, most of them living in villages, have difficulty in accessing medical care. The obtained data about the quality of life in relation with risk factors are in agreement with the data in the literature. **Keywords:** QUALITY OF LIFE, DISSATISFACTION, QUESTIONNAIRE, ONCOLOGY.

The World Health Organization (WHO) defines quality of life as an individuals' perception of their position in life, in relation to their goals and value system, accepted from the perspective of the decisions they make. Quality of life in medicine is the physical, mental and social well-being, and also the ability of patients to perform their daily activities (1, 2).

By assessing symptom progression pa-

tient's quality of life cannot be adequately quantified because, besides measuring the results in terms of symptoms and functioning, patient's view on treatment and its way of administration is also very important (3, 4).

Besides the evaluation of quality of life by objective clinical criteria, an epidemiological approach became necessary as it analyses the efficiency of medical interventions in chronic and incurable diseases in which only a temporary relief can be obtained and treatment is aimed at prolonging patient's life and making it more comfortable. Epidemiological studies are focused on the impact of disease and its treatment on patient's physical and emotional state and lifestyle (5, 6). Health-related quality of life (HRQL) is a complex concept that includes positive and negative aspects of physical, mental and social health, subjective health status (patient's perspective of his health), the impact of this perception on his life and the influence of health and medical care on quality of life (7, 8, 9).

MATERIAL AND METHODS

Our researche included 128 cancer patients treated at the Surgical Clinic II of the Regional Oncology Institute Iaşi between December 2014 and June 2015. Data were obtained in cooperation with the medical personnel and patients. After obtaining the informed consent, all study subjects completed the questionnaire.

We used a self-administered questionnaire derived from SF-36 quality of life questionnaire, which included closed (precoded) questions (the respondent could choose an answer from a preset list of answer choices, quantified by using a scale) and open (postcoded) questions. The questionnaire had a general information module, including identifying and sociodemographic data and questions about way of life outside hospital and risk behaviour (smoking, alcohol use and related diseases). Control questions were used to check the constancy of the expressed opinion (9).

The impact of risk factors on patient satisfaction was evaluated and interpreted by calculating the relative risk, attributable risk, p value and chi – square.

RESULTS

Our epidemiological questionnaire was completed by 128 cancer patients treated at the Surgical Clinic II of the Regional Oncology Institute Iaşi (mean age 60.85, range 18-85 years). Of the 126 patients who answered the question about gender, 51.6% were male and 48.4% female; 51.2% of the patients lived in urban and 48.8% in rural areas.

A low percentage of our study patients were employed (25%), which correlates with a high level of cancer-related disability and a higher frequency of this disease in the elderly.

Most of the patients had a high school diploma (48.4%), 25.4% a secondary school diploma, 14.8% higher education, and 11.5% had only a primary school diploma. Most patients had a stable social status - 72.5% were married and over 70% had a low financial status (monthly personal or household income per person under 1000 lei).

Evaluating the behaviour risk factors, we had found that the vast majority of patients were non-smokers (106 - 82.8%), did not use alcohol (107 of the 125 patients who answered, 85.6%), ate a healthy diet (84 of 127 - 66%) and were normal weighted (83 – 64.8%).

Most of the patients (70.7 %) had experienced pain occasionally, 13% had constant

pain and only 16.3% had no pain (tab. I).

Because cancer is an invalidating chronic disease, depression had affected most of our patients (74% - 88 of the 119 patients who answered); only 26% of the cases were in a good mood (tab. II).

TABLE I

Pain frequency in our lot of patients

Pain	Frequency	Percent	Cum Percent		95% Conf Limits		
No pain	20	16.3%	16.3%		1	10.2%	24.0%
Sometimes	87	70.7%	87.0%		2	61.9%	78.6%
Permanently	16	13.0%	100.0%		3	7.6%	20.3%
Total	123	100.0%	100.0%				

TABLE II

Depression frequency in our patient sample

Depression	Frequency	Percent	Cum Percent		95% Conf Limits		
No depression	31	26%	26.1%		1	18.4%	34.9%
Sometimes	74	62.2%	88.2%		2	52.8%	70.9%
Very often	14	11.8%	100.0%		3	6.6%	19.0%
Total	119	100.0%	100.0%				

We asked the patients to self-assess their own health status on a scale from 0 (the worst imaginable health) to 100 (the best health state). A score of 80-100 indicates a good health status and the mean score obtained in our study patients (65 points) indicated a poor health state (tab. III).

TABLE III

Mean score of life satisfaction in our patient sample

Obs	Total	Mean	Variance	Std Dev
119	7747.0000	65.1008	415.6677	20.3879

Minimum	25%	Median	75%	Maximum	Mode
0.0000	50.0000	70.0000	80.0000	100.0000	50.0000

DISCUSSION

Epidemiological analysis of the impact of risk factors on the level of life satisfaction

Smoking was proved to be a risk factor associated with lower levels of satisfaction with relative risk (RR) 4.82 (2.69<RR< 8.6). Chi-square was 27.11. P value was 0.0001, a statistically significant result. The attributable risk (AR) was 50.42, meaning that the chance for smokers to have poor self-perceived health status is higher with 50.4 than for non-smokers.

Excessive alcohol use proved to con-

tribute to a decrease in the level of satisfaction with RR 1.19 (0.9<RR< 1.52). Chi-square was 1.36, P value = 0.24. This is not a statistically significant result. The attributable risk was 12.72, meaning that the chance for alcohol users to have a poor self-perceived health status is higher with 12.72 than for non-alcohol users.

Unhealthy diet contributes to a decrease in the level of satisfaction RR 1.16 (0.93<RR< 1.44), chi-square = 1.56, P value 0.21; this is not a statistically significant result. The attributable risk was 10.60, meaning that the chance for the people who eat unhealthy food to have a poor self-perceived health status is higher with 10.60 than for people who eat healthy food.

Obesity can affect the level of satisfaction with RR 1.15 (0.92<RR< 1.43); chi-square was 1.4; P value = 0.23, statistically insignificant result. The attributable risk was 9.92, meaning that the chance for the obese patients to have a poor self-perceived health status is higher with 9.92 than for normal weighted patients.

The male gender proved to be a risk factor for a decreased level of satisfaction with RR 1.19 (0.94<RR< 1.54), chi-square 2.27, and P value 0.13. This is a statistically insignificant result. The attributable risk was 12.18, meaning that the chance for men to have a poor self-perceived health status is higher with 12.18 than for women. Other authors point out that the age-related incidence and mortality are higher in males than in females (1, 9).

Low economic status can contribute to a decrease in the level of satisfaction with RR 1.1 (0.84<RR< 1.45), chi-square 0.49, P value 0.48, statistically insignificant result. The attributable risk of 6.32 means that the chance for people with low income to have a poor self-perceived health status is higher with 6.32 than for people with an income over 1000 lei/month.

TABLE IV

Risk factors which can contribute to health status assessment

Risk factors	Share	Score		
		< 80	> 80	*Total*
Smoking	Smokers	14	8	22
	Non smokers	14	92	106
	Total	28	100	128
Alcohol use	Present	17	4	21
	Absent	73	34	107
	Total	90	38	128
Nutrition	Unhealthy	34	10	44
	Healthy	56	28	84
	Total	90	38	128
Obesity	Present	36	11	47
	Absent	54	27	81
	Total	90	38	128
Gender	Male	51	16	67
	Female	39	22	61
	Total	90	38	128
Financial status (income per month)	< 1000 lei	67	26	93
	> 1000 lei	23	12	35
	Total	90	38	128
Pain	Present	80	28	108
	Absent	10	10	20
	Total	90	38	128
Depression	Present	13	1	14
	Absent	69	45	114
	Total	82	46	128

Pain lowers the level of dissatisfaction with RR 1.48 (0.92<RR< 2.33); chi-square was 4.69, P value = 0.03, statistically significant result. The attributable risk was 24.07, meaning that the chance for people who ex-

perience pain to have a poor self-perceived health status is higher with 24.07 than for people who do not experience pain, as also mentioned by other authors (2, 4, 5, 10).

TABLE V

Relative and attributable risks for the level of dissatisfaction related to intrinsic and extrinsic factors, in descending order

Factors	RR	AR
Smoking	4.48	50.42
Depression	1.53	32.33
Pain	1.48	24.07
Alcohol use	1.19	12.72
Male	1.19	12.18
Unhealthy diet	1.16	10.6
Obesity	1.15	9.92
Low income	1.1	6.32

Depression is an important risk factor for a lower level of satisfaction with RR 1.53 (1.25<RR< 1.89); chi-square was 5.66, P value = 0.001, statistically significant result. The attributable risk was 32.33, meaning that the chance for the patients who experience depression to have a poor self-perceived health status is higher with 32.33 than for patients without depression.

In tables IV and V we had analysed the importance of risk factors affecting the quality of life in cancer patients.

CONCLUSIONS

Our study showed a poor health status (mean score 65) for the cancer patients. We calculated the relative and attributable risks for the degree of dissatisfaction related to intrinsic and extrinsic factors and ranked these factors in decreasing order: smoking, depression, pain, alcohol use, male gender, obesity, low income. We found that prolonged suffering affects the psyche and results in depression, and that the patients of low financial status, most of them living in rural areas have difficulty in accessing medical care. The obtained data about the quality of life in relation with risk factors are in agreement with the data in the literature.

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A NEW SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF LISINOPRIL

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A NEW SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF LISINOPRIL (Abstract) **Aim:** A new spectrophotometric method for the assay of lisinopril using 2,4-dinitrophenol as reagent is described. **Material and methods:** The method involved the addition of 2,4-dinitrophenol to lisinopril in methanol followed by absorbance measurement at 400 nm. Experimental conditions that provide wide linear range, maximum sensitivity, selectivity, accuracy and precisions were optimized. **Results and discussions:** Beer's law was obeyed in the concentration range 2.0-14.0 µg/mL. Linear regression equation of calibration graph was A = 0.005 + 0.045 · concentration, with a regression coefficient (*r*) of 0.9995 (*n* = 8). The limits of detection (LOD) and quantification (LOQ) calculated according to the ICH guidelines were 0.42 and 1.42 µg/mL, respectively. Accuracy and precision of the assays were determined by computing the intra-day and inter-day variations at three different lisinopril concentrations; the intra-day and inter-day RSD was < 1.43% and accuracy was better than 1.72 %. **Conclusions:** The proposed method is simple, easy to perform, sensitive, linear, precise, accurate and robust. **Keywords:** LISINOPRIL, ASSAY, SPECTROPHOTOMETRY, 2,4-DINITROPHENOL.

Lisinopril (fig. 1) is chemically known as N2-[(1S)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline. It is a drug of the angiotensin converting enzyme inhibitor class that is primarily used in the treatment of hypertension, congestive heart failure, heart attacks and also in preventing renal and retinal complications of diabetes. Historically, lisinopril was the third angiotensin converting enzyme inhibitor, after captopril and enalapril, and it was introduced into therapy in the early 1990s. Lisinopril has a number of properties that distinguish it from other angiotensin converting enzyme inhibitors: it is hydrophilic, has a long half-life and high tissue penetration and it is not metabolized by the liver enzymes (1).

This paper presents a UV-Vis spectrophotometric method for the assay of lisinopril using 2,4-dinitrophenol. The developed method was validated using the following criteria: linearity, detection and quantification limits, precision, accuracy, and robustness (2-5).

MATERIAL AND METHODS

The reagents used in the development of the method were: lisinopril - 100.03% reference substance (Lupin, India), 2,4-dinitrophenol (Fluka) and methanol

(Merck).

The following solutions were used:

- 1 mg/mL lisinopril stock solution: 100 mg lisinopril were dissolved in methanol in a 100 mL volumetric flask;

- lisinopril working solutions with concentrations between 2 and 100 µg/mL lisinopril were obtained by diluting the stock solution with methanol;

- 0.5% solution of 2,4-dinitrophenol: 500 mg 2,4-dinitrophenol were dissolved in methanol in a 100 mL volumetric flask.

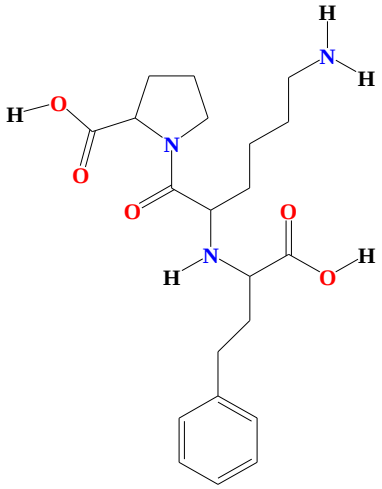

Fig. 1. The chemical structure of lisinopril

A Hewlett Packard 8453 UV-Vis Spectrophotometer, a Kern 770 analytical balance and an Ultrasonic 08849-02 sonicator were used.

Principle of the method: lisinopril forms with 2,4-dinitrophenol in methanol a yellow compound which can be spectrophotometrically measured at 400 nm.

In order to establish the optimum detection wavelength 2 mL of the 50 µg/mL lisinopril working solution were mixed with 1 mL 0.5% 2,4-dinitrophenol solution and 2 mL methanol. The UV-Vis absorption spectrum was recorded with 1 cm cell, after 15 minutes.

In order to establish the optimum concentration for the reagent solution, five solutions of 2,4-dinitrophenol (0.1%, 0.25%, 0.5%, 0.75% and 1%) were used, while the parameters of the method were unchanged.

Procedure: 1 mL of lisinopril working solutions with concentrations in the range 10-70 µg/mL were mixed with 1 mL 0.5% 2,4-dinitrophenol solution and 3 mL methanol. The absorbance was measured after 15 minutes at 400 nm versus a blank solution prepared in similar conditions.

RESULTS AND DISCUSSIONS

From the analysis of absorption spectra (fig. 2), we observed a maximum absorbance for the reaction product at 400 nm. That wavelength was used for all measurements.

The best concentration of 2,4-dinitrophenol reagent solution was established to be 0.5%, because when using that particular solution the maximum absorbance measured at 400 nm had the greatest value (tab. I). After studying the stability of the samples it was established that the chemical reaction between lisinopril and 2,4-dinitrophenol was final 15 minutes after the reagent was added (tab. II). Also, it was proved that the absorbance remained almost the same for at least another 20 minutes, time sufficient enough for the analysis to be performed.

Linearity was studied in the 0.4 - 20 µg/mL concentration range (fig. 3). The obtained data were statistically evaluated (tab. III) and the calibration curve was obtained (fig. 4).

According to the experimental data, the developed method was linear in 2 – 14 µg/mL concentration range. When this

concentration range was compared with that of other published methods we found that it was very similar, but this new method had the following advantage: it did not require the use of rare or complex reagents. It is simple and easy to perform (6-10).

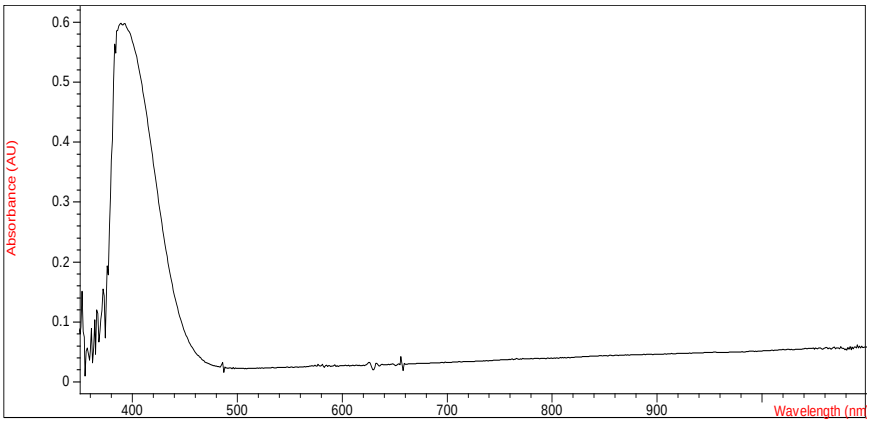

Fig. 2. The spectrum of the reaction product for 20 µg/mL lisinopril

TABLE I

Study of reagent concentration

2,4-dinitrophenol %	Lisinopril	
	2.0 µg/mL	14.0 µg/mL
	Absorbance (400 nm)	
0.10	0.01222	0.35587
0.25	0.04415	0.56442
0.50	0.09014	0.64712
0.75	0.08708	0.62522
1.00	0.08294	0.60233

TABLE II

Stability study

Time (minutes)	Lisinopril	
	2.0 µg/mL	14.0 µg/mL
	Absorbance (400 nm)	
5	0.08051	0.62298
10	0.09118	0.63274
15	0.09098	0.64022
20	0.09054	0.64152
25	0.08941	0.64191
30	0.08947	0.63145
35	0.08832	0.60203
40	0.08702	0.59157

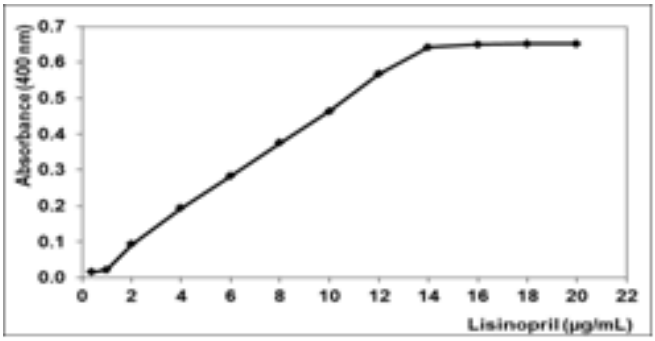

Fig. 3. Study of method linearity

TABLE III

Linearity study of the method

Lisinopril (µg/mL)	Absorbance (400 nm)				
	Ist Series	IInd Series	IIIrd Series	IVth Series	Average
0.4	0.01452	0.02440	0.00945	0.01147	0.01496
1.0	0.02245	0.01942	0.03144	0.01478	0.02202
2.0	0.08954	0.09314	0.09545	0.09291	0.09276
4.0	0.19056	0.20411	0.19412	0.18457	0.19334
6.0	0.28252	0.28147	0.27455	0.28990	0.28211
8.0	0.37046	0.37147	0.38412	0.37512	0.37529
10.0	0.46574	0.45892	0.47014	0.46025	0.46376
12.0	0.56559	0.57421	0.56142	0.56842	0.56741
14.0	0.63934	0.63412	0.64721	0.64455	0.64131
16.0	0.65435	0.64125	0.66120	0.64125	0.64951
18.0	0.64560	0.65744	0.64855	0.65470	0.65157
20.0	0.63547	0.65122	0.64177	0.67444	0.65073
Correlation and regression coefficients r = 0.9995, r^2 = 0.9991; Standard error = 0.0065; Intercept = 0.005; Slope = 0.046					

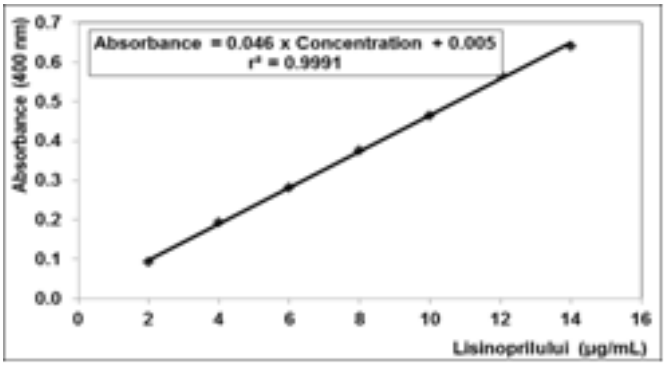

Fig. 4. Calibration curve

The calibration curve equation was established:

- Absorbance = 0.046 x Concentration – 0.005.

- Detection and quantification limits were calculated using the following formulas (2, 3):

- LD=3 x Standard error / Slope = 0.42 µg/mL

- LQ=10 x Standard error / Slope = 1.41 µg/mL.

Precision: three solutions of 6, 8 and 10 µg/mL lisinopril were used. Three assays were performed for each concentration. Two sets of assays were performed in different days in order to evaluate the intermediary precision.

The sample concentrations were calculated using the calibration curve equation (tab. VI). We observed that the relative standard deviation for each data set and for both data sets together was less than 2% (at most RSD = 1.43%) (4), proving that the proposed method was precise.

In order to establish the accuracy of the method, lisinopril solutions of 6, 8 and 10 µg/mL were analyzed. For each concentration, three determinations were performed (5).

The concentration of the samples was calculated from the experimental values of absorbance, using the regression curve equation (tab. IV). For the studied concentration range recovery was 100.59%, the mean was between 98.51% - 102.39%, and the relative standard deviation was lower than 2% (RSD = 1.42 %). Those values proved that the proposed method was accurate.

The evaluation of robustness was performed for system suitability to ensure the validity of analytical procedure. That was done by varying the instrument, analyst, and time of study. The analysis was performed also on a 1700 Shimadzu UV-Visible spectrophotometer. Interday and intraday analyses were performed by various analysts. Reproducibility of the results confirmed the robustness of the method.

TABLE IV

Study of the precision and the accuracy of the method

Concentration (µg/mL)	Precision		Intermediate precision		Accuracy	
	Real concentration (µg/mL)	RSD %	Real concentration (µg/mL)	RSD %	Absorbance	Recovery (%)
6	5.9650	1.768	5.965	1.708	0.28411	100.86
	5.8428		6.0606		0.27455	97.40
	6.0524		5.8571		0.28954	102.83
8	8.0089	1.640	7.9223	1.639	0.36841	98.56
	7.9073		8.1393		0.37455	100.23
	7.9506		8.1622		0.37276	99.74
10	10.0963	1.440	10.0815	1.647	0.47018	100.98
	10.1894		9.9141		0.47855	102.80
	9.9034		10.2463		0.46625	100.12
					Mean	100.39

CONCLUSIONS

A UV-VIS spectrophotometric method was developed for the assay of lisinopril using 2,4-dinitrophenol. The reaction product showed a maximum absorbance at 400 nm.

The analytical method was validated by establishing the linearity domain in the range of 2.0 – 14 μg/mL, with a correlation coefficient of r = 0.9995, the detection limit was 0.42 μg/mL, the quantification limit was 1.41 μg/mL lower than the lowest concentration from the linearity domain, method precision RSD% = 1.61% and the accuracy evaluated through recovery was R = 100.39%.

In conclusion, the proposed method is simple, easy to perform, sensitive, linear, precise, accurate and robust.

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ASSESSMENT OF PERSONAL QUALITIES OF HEALTHCARE PROFESSIONALS – DETERMINING FACTOR FOR THE IMPROVEMENT OF HEALTHCARE SERVICES IN THE ROMANIAN HEALTH SYSTEM

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ASSESSMENT OF PERSONAL QUALITIES OF HEALTHCARE PROFESSIONALS– DETERMINING FACTOR FOR THE IMPROVEMENT OF HEALTHCARE SERVICES IN THE ROMANIAN HEALTH (Abstract). **Aim:** A quality health care system has been a perpetual desideratum. The increased importance of assessing quality of health care is lately emphasized by the alternation between the level, role and place of technology in healthcare and especially by the need for a balance between the values of Hippocratic medicine and the valences of technologized medicine of today society. **Material and methods**: The study is based on the evaluation of responses to an online questionnaire from 1013 health professionals in different regions of Romania, questionnaire hosted by SurveyMonkey. **Results**: Sacrifice, humanity, dedication, knowledge, respect and rigor were the top key traits according to our responders. The qualities of health professionals and how they are assessed are undoubtedly an important source for determining the qualities to be formed during education, and which are the basis for a healthcare system based on quality and competence and increasing patient satisfaction. **Keywords**: HEALTHCARE SYSTEM, PROFESSIONAL QUALITIES, SATISFACTION OF HEALTHCARE PROFESSIONALS.

The safety and quality of healthcare services are worldwide concerns. Many problems arise from human and health system errors, but also from the dissatisfaction and lack of motivation of healthcare personnel. If initially the concept of patient satisfaction seemed to be an element of analysis relevant only for medical practice, increasingly more voices believe that both human components of the health system: healthcare professionals and patient, have be taken into account realizing that negative events, including the low level of medical staff satisfaction is an important factor that can influence the quality of care. If deaths from specific disease are not difficult to quantify, those produced as a result of poor care are more difficult to identify, although according to some studies they are the 8th leading cause of death in the USA

(5), mostly of them avoidable (3).

The qualities of healthcare professionals are the least studied factors in the equation quality of care - patient satisfaction, and should not be seen as individual factors but as interconnected causes that may intervene both subjectively and individually, but leading to a phenomenon at the level of the whole system. Factors such as negative stress can influence both the health status of health professionals and how they act during professional activity. If scales and standards to measure occupational stress are available, few studies were dealing with moral stress, although the crisis of moral models is quite widespread throughout society (9).

Each society and field area is under the sign of one of the most difficult to distinguish human quality: the ethical-moral qualities of the person who provides health care, especially when the ethical dilemmas the healthcare professionals are faced with may generate tensions and thus diminish their ability to focus solely on the quality of care. In this context it is important to understand that: "If true collaboration is to be achieved, it is important to understand the perspectives of others" (6).

Ensuring quality healthcare services in Romania has been a significant concern since 1989 and especially in the perspective of our country's integration in the European Union (EU) and the need to harmonize the national healthcare system with EU rules. Quality of healthcare services is influenced by several factors, of which the more frequent are the wide variations in medical practice performance, unequal access to healthcare or longer waiting time to access certain services (1). In recent years, in Romania, the main criterion analyzed by opinion polls to measure the quality of healthcare was patient satisfaction, often using own tools or instruments internationally validated and adapted to the conditions in our country (2).

Internationally, there are validated studies that evaluated performance in healthcare; however, we should not forget that healthcare employee satisfaction is of utmost importance when it comes to quality of care, in Romania, as in other countries in the world, they being faced with many problems related to personnel cut, less funding allocated to health care and the increased demand for health services (2, 4). Moreover, it has already been proven that the performance of the health system and the level of care are directly influenced healthcare employee satisfaction (7).

The basis for providing healthcare services in health management and medical practice is represented by the *nine* dimensions for quality assessment: professional competence, accessibility, effectiveness - the applied procedures and treatment; efficiency; interpersonal relations, continuity, security, physical infrastructure and comfort, choice of supplier, type of insurance or treatment (8, 10).

The aim of this study was to assess healthcare employee satisfaction by the study of 19 characteristics of significance in performing healthcare activities, related to competence, self-sacrifice, self-control, responsibility etc.

MATERIAL AND METHODS

A poll/survey was conducted with the help of an online questionnaire using the SurveyMonkey platform. The study included a total of 1013 health professionals working in Bucharest, Iasi, Cluj Napoca, Tg. Mures, Timisoara and Craiova university hospitals. The questionnaire included questions related to the 19 characteristics that define the medical profession. The analyzed

characteristics were divided into three distinct categories: 1) included competence, dedication, sacrifice, literacy and humanity; 2) included dedication, knowledge, skills, perfection, responsibility, "cold blood", self-control and tenacity; and 3) creativity, experience, intelligence, accuracy, respect, rigor and speed. A correlation analysis was performed to identify the main issues that may be associated with healthcare employee satisfaction. The results were considered statistically significant at a confidence interval of 95%.

RESULTS

Following the statistical analysis of study group composition we found that according to age variable the group of responders had a homogeneous distribution. Distribution of the study group according to the variable "Age" showed that most subjects (28.3%) were in the age range 45-50 years, followed by those in the age range 35-45 years (21.5%) and 30-35 years (20.4%). Very close percentages were recorded for the subjects below 30 years (14.7%) and those over 50 years (15%) (tab. I).

The variable "level of education" showed a quasi-uniform distribution, the study group being composed of people with higher education, of which one with postgraduate studies (tab. II).

TABLE I

Distribution of participants by "Age"

Age (years)	Frequency	Percentage	Cumulative percentage
Under 30	149	14.7	14.7
30-35	207	20.4	35.1
35-45	218	21.5	56.7
45-50	287	28.3	85.0
Over 50	152	15.0	100.0
Total	*1013*	*100.0*	

TABLE II

Distribution of participants by "Level of education"

Level of education		Frequency	Percentage	Cumulative percentage
Valid	Higher education	1012	99.9	99.9
	Postgraduate	1	0.1	100.0
	Total	*1013*	*100.0*	

The variable "Length of service in the health system" showed that most participants have worked in this field for over 20 years old (37.8%), followed by those with a length of service of 10 to20 years (25.3%) and 5 to 10 years (22.2%), the fewest being those with a length of service less than 5 years (14.7%) (tab. III). As 63% of the respondents had over 10 year experience in healthcare it gave us the opportunity to get information from people who are familiar with the health system and managed to gather enough experience, including in communication with the patient and under-

standing their objective needs.

Ranking of competence, commitment, sacrifice, literacy, humanity, characteristics that define the medical profession showed that the surveyed people made a clear distinction between competence and the other features. When asked to name three characteristics that define the medical profession, an equal number of doctors, 338 (33.4%) responded that sacrifice and humanity, respectively were the defining characteristics of their profession and a total of 335 physicians (33.1%) indicated competence (tab. IV).

TABLE III

Distribution of participants by "Length of service in the health system"

Length of service in healthcare system (years)	Frequency	Percentage	Valid percentage	Cumulative percentage
Under 5	149	14.7	14.7	14.7
5-10	225	22.2	22.2	36.9
10-20	256	25.3	25.3	62.2
Over 20	383	37.8	37.8	100.0
Total	*1013*	*100.0*	*100.0*	

TABLE IV

Distribution of participants by characteristics that define medical profession (competence, devotion, sacrifice, literacy, humanity)

Traits	Frequency	Percentage	Valid percentage	Cumulative percentage
Competence	335	33.1	33.1	33.1
Devotion	1	0.1	0.1	33.2
Sacrifice	338	33.4	33.4	66.5
Literacy	1	0.1	0.1	66.6
Humanity	338	33.4	33.4	100.0
Total	*1013*	*100.0*	*100.0*	

A second category of individual characteristics that define the medical profession included the following items: dedication, knowledge, gifted, perfection, responsibility, cold-blooded/self-control, determination. Very close percentages (16.5 to 16.8%) were obtained for most characteristics, except for determination which was considered important by a single physician who participated in the survey (0.1%) (tab. V).

The third category of individual characteristics that define the medical profession was composed of the following items: creativity, experience, intelligence, accuracy, respect, rigor, general knowledge, and speed. Six of the 8 items had approximately equal percentages, 16.3% to 16.8%, the remaining two, creativity and general knowledge, with a percentage of only 1% each, surprisingly ranked last; this can be explained by the fact that medical profes-

sion being considered a very rigorous profession which requires a thorough analysis, creativity and general knowledge, the more so as medicine is associated with the need for continuous learning are not seen as valuable attributes (tab. VI).

TABLE V

Ranking of characteristics that define medical professions (dedication, knowledge, gifted, perfection, responsibility, composure, determination)

Traits	Frequency	Percentage	Valid percentage	Cumulative percentage
Dedication	170	16.8	16.8	16.8
Knowledge	170	16.8	16.8	33.6
Gifted	169	16.7	16.7	50.2
Perfection	169	16.7	16.7	66.9
Responsibility	167	16.5	16.5	83.4
Cold blood	167	16.5	16.5	99.9
Determination	1	0.1	0.1	100.0
Total	*1013*	*100.0*	*100.0*	

TABLE VI

Ranking of the characteristics that define medical profession (creativity, experience, intelligence, accuracy, respect, rigor, general knowledge and speed)

Traits	Frequency	Percentage	Valid percentage	Cumulative percentage
Creativity	1	0.1	0.1	0.1
Experience	169	16.7	16.7	16.8
Intelligence	169	16.7	16.7	33.5
Accuracy	165	16.3	16.3	49.8
Respect	170	16.8	16.8	66.5
Rigor	170	16.8	16.8	83.3
General knowledge	1	0.1	0.1	83.4
Speed	168	16.6	16.6	100.0
Total	*1013*	*100.0*	*100.0*	

The analysis of all responses revealed that according to our subjects the medical profession is defined by three fundamental characteristics: humanity, sacrifice and competence.

DISCUSSION

All subjects had higher education, and only one postgraduate studies. Most of the subjects were older than 45 years, and only few under the age of 30 years. The obtained results allowed us to hierarchize the characteristics considered by healthcare professionals important in the practice of medicine.

Considering the top two in each category, the most important attributes were: sacrifice, humanity, dedication, knowledge, respect and rigor. The same characteristics

were selected by healthcare professionals regardless of length of service in the health system (5, 6).

CONCLUSIONS

In our study, human resources management in medical institutions should also be based on the assessment of qualities required to healthcare professionals, and ideally staff recruitment and selection should not be based solely on knowledge but also on the most important qualities that those who wish to practice medicine should have. To meet the challenges of the health system is absolutely necessary that these characteristics to be formed during medical education and "maintained" by forms of continuing education.

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QUALITY OF LIFE WITH MASTECTOMY FOR BREAST CANCER, IN TERMS OF PATIENTS' RESPONSES OF SF-36 QUESTIONNAIRE

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QUALITY OF LIFE WITH MASTECTOMY FOR BREAST CANCER, IN TERMS OF PATIENTS' RESPONSES OF SF-36 QUESTIONNAIRE (Abstract): **The aim** of the study was to describe the quality of life in female patients with mastectomy performed for breast cancer. **Material and methods**: The study was carried out in Iaşi, between October 2014 - January 2015, on a sample of 23 patients with mastectomy, using the SF-36 Questionnaire (Short Form-36) in a single visit, with direct questioning of patients; a secondary instrument was a general anamnestic questionnaire referring on age group, area of residence, type of surgery, associated diseases, family history, psycho-emotional traumas. **Results**: The overall profile of the studied case was: woman of 40-49 years (47.8%), from urban area (82.6%), with a family history of neoplastic pathology (43.5%), with psycho-emotional traumas (73.9%) and a self-estimated general health as "moderate" (26.1%), with "a lot of energy" only "some of the time" (43.5%), "feeling worn out" (43.5%), and "cutting down the amount of time spent on work or other activities" (69.6%), with "moderate" (34.8%) or even "intense" (21.7%) "bodily pain", "some of the time" feeling "very nervous" (43.5%) and "so down in the dumps that nothing could cheer her up" (39.1%). The strongest correlation was found between the variable "cut down the amount of time spent on work and other activities" as a result of "physical health" (Role-physical items) and "limited in kind of work or other activities" (r=0.8981). **Conclusions**: Application of SF-36 Questionnaire in female patients with mastectomy for breast cancer highlighted that strenuous and moderate activities were limited than about half of the surveyed cases, somatic pain affecting their work and normal activities and physical and emotional health influenced the social activity of patients for more than half of the lot. It is necessary to extend the study in order to bring relevant data on the quality of life of these patients, in the social, economic and cultural center of Romania. **Keywords**: QUALITY OF LIFE, SF-36 QUESTIONNAIRE, MASTECTOMY, BREAST CANCER.

According to the World Happiness Report for 2012, Romania was ranked 90 in the world, with a score of 5.033, between Kyrgyzstan and Zambia (first was Denmark). Comparing the term of *happiness*, therefore, the *quality of life*, Romania has lost 0.186 points during 2005-2007 (1). The first survey on quality of life in the population of European countries was performed in 2006 (2). At that moment, Romania, as a candidate country for EU, was below the continent average, with a score of 6.2. According to the third survey conducted in 2012, Romania has very low scores on several indica-

tors of quality of life, compared to other countries, and, on the perception of quality of life in terms of health services, ranked the next to last position (3).

The notions of *subjective well-being* and *happiness* have a long tradition as core elements of the concept of *quality of life*. However, until recently, these ideas were not too often considered beyond the purpose of statistics. In recent years, this view has changed, especially after the publication of the report of the Commission on the Measurement of Economic Performance and Social Progress, which recommended that national statistical agencies collect and make public aspects of these concepts. According to the researchers from the United Nations (4), the quality of life is an intersection of notions of professional status, health level, balance between work and life, education and skills, social connections, civic engagement of the individual, environment quality and personal safety.

There are numerous questionnaires to assess the quality of life, validated on various healthy population groups and also on groups with different pathologies. *SF-36* (Short-Form 36-Item Health Survey) uses eight scales with questions regarding physical functioning (first 10 items), diseases caused by physical problems or role-physical (the next 4 items), bodily pain (2 items), general health (5 items), vitality (4 items), social functioning (2 items), emotional problems caused by social dysfunctions or role-emotional (3 items), and mental health (5 items) (4).

In 2012, in Romania was recorded breast cancer incidence of 50.04 cases per 100,000 inhabitants, an average value (which ranks 33 among European countries) compared to the top three worldwide, those in Belgium (111.98%ooo), Denmark (105.0%ooo), and France (104.46%ooo) (5). Approximately 12.3% of women will be diagnosed with breast cancer at some point in their lives, in the coming years, as stated in a prognosis made by the National Cancer Institute, USA, based on data collected between 2009 and 2011 (6). In recent decades, the five-year survival of female patients with breast cancer increased from 75.2% in 1975 to 86.5% in 1994 and 90.6% in 2006 (6). Therefore, it is necessary to take into account the quality of life of those women who received successful anti-cancer therapies, having, as the main target, the increase of survival.

The aim of our study was to describe the physical and emotional impact on quality of life lived by women with mastectomy performed for clinical and paraclinical diagnosis of breast cancer, using the SF-36 questionnaire.

MATERIAL AND METHODS

The study was conducted between October 2014 - January 2015 on a sample of 23 female patients treated surgically at Regional Institute of Oncology Iaşi (19 patients) and "St. Spiridon" Emergency Hospital Iaşi (4 patients). Inclusion criteria for the study were: clinical and paraclinical diagnosis of breast cancer; total or partial mastectomy. Exclusion criteria: patients who refused the participation in the study.

Informed consent of research participants received the Ethical Approval from the Research Ethics Committee of the University of Medicine and Pharmacy, of Regional Institute of Oncology, and "St. Spiridon" Hospital. Every patient participating in the study became aware of its contents and signed two copies of it, one of which remained in the project file and the other to the participant.

The study was conducted in a single visit with direct questioning of patients as the method. The main tool used was SF-36 questionnaire validated for healthy population of Romania in a project conducted by the "Romanian Association for Public Health and Health Management" and "Centre for Urban and Regional Sociology", Bucharest (7).

The second instrument used was a general anamnesis questionnaire with items regarding age group, area of residence, type of surgery, associated diseases, family history, and psycho-emotional traumas 3-5 years before breast cancer diagnosis.

Statistical processing was performed using MS Excel 2010 and EPI/INFO 7 software.

RESULTS

Statistical descriptive data for the study group. Distribution by age group showed a preponderance of cases at 40-49 years (47.8%), followed by group 50-59 (21.7%), 30-39 and 60-69 (13.05%), then 70-79 (4.4% of cases), with a mean of 50.59 (standard deviation = 11.07). Age histogram tended to be within normal limits, describing Gaussian distribution. Patients were mostly from urban areas (82.6%), mentioning cities in the North-East region of Romania. All patients were hospitalized for clinical and paraclinical diagnosis of breast cancer; the surgical treatment was total mastectomy in 86.9% of the patients and partial mastectomy / lumpectomy (sector, quadrant) in 13.1%; axillary lymph node dissection was performed in 100% of cases.

Patients mentioned tumors and depression as associated diseases in 3 cases (13.05%) respectively, but 2 patients indicated the presence of autoimmune diseases in personal history. Other conditions were cardiovascular diseases (ischemic heart disease, arterial hypertension) and diabetes mellitus. Family history of neoplastic pathologies was positive in 10 cases (43.5%); 7 patients (30.4% of the total group) mentioned malignant and benign tumors of the breast, ovary, prostate and colorectal cancer in first and second degree relatives. Regarding the psycho-emotional traumas of the past 3-5 years, 17 patients (73.9%) noticed their presence (divorce - in 2 cases, 8.7%; deaths in family - in 6 cases, 26.1%; other emotional traumas considered important by patients - 11 cases, 47.8%).

Results of SF-36 questionnaire. The questionnaire is divided into two concepts: physical health and mental health. We described statistically each scale of the SF-36 questionnaire and the following results were obtained:

I. *General health scale* included 5 items estimating the own health; patients considered that it was "good" in 52.2% of cases and 26.1% assessed it as "tolerable". One patient (4.3%) mentioned an "excellent" condition and another one, a "bad" state of health. 30.4% of total indicated that it was "definitely true" and "mostly true" they "get sick easier than other people", and 43.5% were denied "absolutely" and "mostly" this fact. 39.1% indicated that it is "definitely" and "mostly true" that were healthy like anybody know, and 34.7% denied this fact. 21.7% of patients expected ("definitely" + "mostly") that "their condition to get worse", 52.2% denied this fact, and 26.1% did not know how to respond. If "definitely and mostly true" that "health is excellent" said 52.2% of patients and that is false, 21.7%.

II. *Vitality scale*: "full of life" felt "much of the time" 30.4% of patients and

43.5% “had a lot of energy” only “some of the time”. No patient chose the answer “none of the time” at this item. Most patients felt “some of the time” “worn out” (34.7%) and “tired” (47.8%), but “all of the time” and “most of the time” only 8.7% and 17.4%, respectively.

III. *Physical functioning scale*. “Vigorous” activities “limited a lot” 43.5% of patients, but “not limited at all” one of them (4.3%); those “moderate” “limited a lot” 26.1% of total cases and “not limited at all” 8 patients (34.8%). "Lifting or carrying groceries” “limited a lot” 47.8% of all cases and “not limited at all” 21.7% of them. About “climbing several flights of stairs”, 39.1% of patients said they were “not limited at all”, but 30.4% indicated that they were “limited a lot” or "limited a little”, respectively; “climbing one flight of stairs” was “not limited at all” for 60.9% of patients. “Walking more than a mile” “limited a lot” and “a little” 56.5% of all cases, but “not at all” 43.5%; “walking several hundred yards” “limited a lot” only 26.1% of patients, and “walking one hundred yards” “limited not at all” in 86.9%. Activities such as “bathing or dressing oneself” “limited a lot and a little” only 43.5% of cases. It is noted that physical functional limitation increased with the increasing of degree of activities difficulty.

IV. *Role-physical scale*. It was found that 69.6% of women “counted down the amount of time spent on work or other activities”, 65.2% “accomplished less than they would like” and 73.9% of patients were limited “in kind of work and other activities” and “had difficulties performing work or other activities”, respectively.

V. *Bodily pain scale* contains items about changes in normal work. Patients experienced bodily pain “moderate” in 34.8% cases and “severe” and “very severe” in 21.7%, which “interfered with normal work” “moderate” in 30.4% of patients and “severe” and “very severe” in 21.7%.

VI. *Social functioning scale* showed how physical health or emotional problems interfered with social activities currently carried out with family and friends. Patients indicated that these activities changed “moderately” in 30.4% of cases and “extremely” in only 8.9%. During the last four weeks, there were times when physical or emotional health influenced social activity of patients, “most of the time” for 17.4% of them and “some of the time” for 34.8%. However, no patient chose the answer “all of the time” at this item.

VII. *Role-emotional scale* highlighted difficulties occurred when performing work or other regular daily activities as a result of any emotional problem such as “feeling depressed or anxious”; those have been reported in more than half of the cases, respectively 52.2% reduced “the amount of time spent on work or other activities” and “accomplished less activities”; 56.5% of patients stated difficulties of achieving them “with the same care as usual”.

VIII. *Mental health scale*. Patients mentioned that “some of the time” felt “very nervous” (43.5%) and “felt so down in the dumps that nothing could cheer them up” (39.1%), but “most of the time” “calm and peaceful” (43.5%). "Downhearted and depressed” were "all of the time” 2 patients (8.7%), others only “some of the time” or “a little of the time” (30.4%). The next item about if she was deemed a “happy person”, 17.4% of the cases mentioned that “some of the time” 4.3% “ a little of the time” or “none of the time”; however, more

than half of sample chose answers such as "all of the time" (26.1%), "most of the time" (17.4%), and "a good bit of the time" (30.4%).

Correlations analysis. The patients' age was positively correlated with the difficulty of carrying out "moderate activities" (r=0.2184), with the "cut down the amount of time spent on work" and the occurrence of difficulties in "performing the work and other activities" (r=0.3530). Also, the variable "age" was positively correlated with variables "been very nervous" (r=0.4298) and "felt so down in the dumps that nothing could cheer them up" (r=0.3938).

The strongest correlations were found between the variable "cut down the amount of time spent on work or other activities" as a result of physical health and "been limited in kind of work or other activities" (r=0.8981), followed by correlation between "intensity of bodily pain" and "extend pain interfered with normal work" (r=0.8711). The next correlation was between the variable "cut down the amount of time spent on work and other activities as a result of emotional problems" and "accomplished less than they would like due to emotional problems" and also "frequency health problems interfered with social activities"(item no. 10) (r=0.8360 in both cases).

It is necessary to notice that there was no correlation (r=0) between the variable "healthy as anybody they know" and "cut down the amount of time spent on work or other activities as a result of physical health", "cut down the amount of time spent on work or other activities as a result of emotional problems", "accomplished less than they would like to due to emotional problems" and "extend pain interfered with normal work" (item no. 8).

Concerning the question of the existence of psycho-emotional traumas, this variable, from the second questionnaire, was positively correlated with items "been very nervous" (r=0.5985), "intensity of bodily pain" (r=0.5456), and "feel downhearted and depressed" (r=0.3887).

DISCUSSION

There are numerous valid instruments used to measure quality of life in breast cancer patients. EORTIQ-C30 questionnaire (European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core Cancer) and that specific for breast cancer (EORTC QLQ-BR23), FACIT-G (Functional Assessment Chronic Illness Therapy-General Questionnaire) and the variant for cancer breast (FACIT-B) were considered the most useful tools and best developed to measure the quality of life in those patients. The various surgical procedures have led to similar results on quality of life assessments, although patients with mastectomy compared with those who underwent conservative treatment have been reported frequently a body image issues and sexual dysfunction.

Regarding systemic therapies, almost all studies have indicated that patients receiving chemotherapy for breast cancer suffered from certain side effects and symptoms that adversely affected their quality of life, as well as adjuvant hormone therapy, although they were associated usually with an increase in survival rates. In patients with metastases, data on quality of life represented prognostic and predictive factors of survival. Psychological distress, anxiety, and depression were common in studies on groups of breast cancer patients, even after years from the date of diagnosis and treatment. Psychological factors were

used, also, as predictors of subsequent quality of life or overall survival of patients with breast cancer.

Sexual functioning of patients with breast cancer, especially those younger, is being reduced, having a negative impact on their quality of life. Antiemetic supportive treatment, for example, or interventions such as counseling, social support, and exercise can improve the quality of life of these patients. Pain, fatigue, arm disability and postmenopausal symptoms are common in patients with breast cancer. Therefore, it is recommended their recognition and management as important aim until they do not start to disrupt their health-related quality of life (8).

Health-related quality of life is now considered an important aim in oncology trials. Studies conducted over the years have shown that the assessment of the quality of life in patients with cancer could improve the treatment and even was considered a prognostic factor indicating the future directions useful for the implementation of effective therapies (9, 10).

Among studies on quality of life, breast cancer received the most attention, for certain reasons, primarily because of the increasing incidence of the disease (over half a million women worldwide are diagnosed with breast cancer yearly – 1.67 million in 2012, 25% of all cancers - and over half a million die from this disease - 522,000 in 2012) (11); secondly, because techniques for early detection and treatment of breast cancer are currently at high standards, increasing significantly the survival time, compared to previous decades; therefore, to study the quality of life in this context should become a priority (9).

Another reason is that the location of the breast neoplastic process affects women at all levels of their identity, thus, to study the quality of life of these patients who have lost breast or breasts should become vital. In addition, women play important roles as life partner, wives, mothers, in every family. When a woman develops breast cancer, all family members are about to develop various types of pathological conditions. Thus, psycho-emotionally speaking, breast cancer becomes a disease of the whole family.

So, a multitude of other reasons could be added. Therefore, the question formulated by many researchers becomes evident: to what extent the quality of life studies have improved therapeutic outcomes and, ultimately, improve the quality of life of women with breast cancer? (10).

CONCLUSIONS

The results of the SF-36 questionnaire used to assess quality of life in female patients with mastectomy for breast cancer highlighted that strenuous and moderate activities limited almost half of the surveyed cases, bodily pain affecting their work and normal activities. Also, physical or emotional health influenced social activity of patients, most of the time and some of the time for more than half of them. The existence of psycho-emotional traumas in patients' history was positively correlated with current physical health or emotional issues.

Analysis of a larger group of patients, including those assisted in hospitals from other university centers, will bring more relevant data concerning the mastectomy performed for breast cancer and quality of life lived in the social, economic and cultural space of Romania.

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RESPONSIBILITY AND EXPECTATIONS IN ANTIRETROVIRAL THERAPY – PATIENTS' VERSUS DOCTORS' PERSPECTIVE

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RESPONSIBILITY AND EXPECTATIONS IN ANTIRETROVIRAL THERAPY – PATIENTS' VERSUS DOCTORS' PERSPECTIVE (Abstract). **Aim:** This paper aims to uncover what patients really expect form ART, and also what infectious diseases doctors expect from a patient's ART regime, thus exploring an important side of adherence to ART. **Material and Methods:** From July to November 2014 we have conducted a qualitative study regarding both patients' and doctors' expectations form the ART. We interviewed 30 patients and 4 doctors. We used semi-structured interviews that were conducted in the Psychosocial Compartment of the HIV/AIDS Regional Center in Iasi. **Results:** The patients we interviewed came from all 6 counties in the Moldova area. Age varied from 16 years to 59 years; 55% were female and 45% male. 30% came from a rural area. The most common expectations that patients have regarding ART are: "to help me live", "not to make me feel sick", "to be easy to take (not to big, not a lot)", "not to show on the outside what I have on the inside". The infectious diseases doctors that we interviewed work in the HIV/AIDS Regional Center in Iasi. Their expectations regarding an ART regimen for patients were: "to reduce HIV viral load", "to increase CD4 cell count" and "to have minimal impact on the proper functioning of other organs". Patients consider themselves the only factors responsible for their own ART adherence in 56.6% of cases; 20% consider the doctor to be responsible for their adherence, 16.6% feel that their family, friends, and spouse are responsible, and 6.6% (2 patients) couldn't answer. Infectious diseases doctors considered that patients are 100% responsible for adhering to the antiretroviral therapy. **Conclusions:** In order to assure adherence to the ART it is important to explore both the doctor and the patient's perspective and to find ways to find a common ground in building a healthy relationship. **Keywords**: HIV/AIDS, ANTIRETROVIRAL THERAPY, ADHERENCE, PATIENTS' EXPECTATIONS, DOCTORS' EXPECTATIONS, RESPONSIBILITY.

Antiretroviral therapy (ART) is essential in maintaining a low HIV viral load, which allows the immune system to function properly and assures a qualitative life style for HIV positive patients (1). However, low rates of adherence to ART are still registered, in spite of all efforts.

In Romania, around 90% of all HIV infected individuals are part of the "pediatric cohort" – young people born in 1986-1989 and infected in the hospital. They were diagnosed at the age of 3-5 years and have lived with HIV/AIDS for more than 20 years. They have a history of up to 10 ex-

perimental drug regimens, having passed from mono – to bi-therapy to HAART (highly active antiretroviral therapy) (2).

The rest of HIV-positive patients are adults infected through unprotected sexual contact or through the use of unsterilized syringes in the case of injecting drug users. They have a shorter history of living with HIV/AIDS and their numbers are continuously increasing.

A highly researched issue regarding people living with HIV/AIDS (PLWHA) is adherence to antiretroviral therapy.

Studies identified a number of factors associated with adherence to ART (3,4):

- Medical aspects and characteristics of the therapy - number of pills/day, ART side effects;
- Social aspects – stigma and discrimination, lack of knowledge and understanding of HIV, the doctor-patient relationship, peer support and family support;
- Psychological aspects – treatment fatigue, IQ, psychopathology, treatment fatigue, disclosure of the diagnosis, responsibility, expectations from ART.

This paper aims to uncover what patients really expect form ART, and also what infectious diseases doctors expect from a patient's ART regime, thus exploring an important side of adherence to ART.

MATERIAL AND METHODS

We realized a qualitative study for a period of 5 months, from July to November 2014. We interviewed 30 patients and 4 doctors. The patients came from all 6 counties in the Moldova area – Iasi, Vaslui, Bacau, Botosani, Suceava, Neamt. The infectious diseases doctors that we interviewed work in the HIV/AIDS Regional Center in Iasi.

We used semi-structured interviews as a method of gathering data. The interviews were conducted in the Psychosocial Compartment of the HIV/AIDS Regional Center in Iasi.

The demographics revealed 55% were female and 45% male (fig. 1); 30% came from a rural area (fig. 2). Age varied from 16 years to 59 years, in concordance with the requirements of qualitative research.

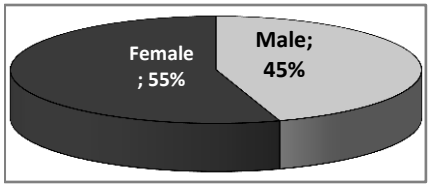

Fig 1. Gender distribution

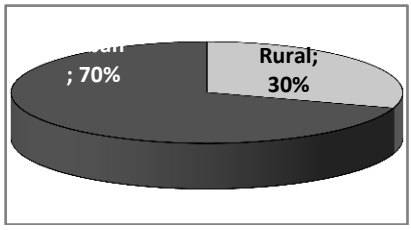

Fig 2. Environment distribution

RESULTS

Patients' expectations revolved around general health and a healthy appearance.

The most common expectations that patients have regarding ART were:

- "to help me live" – Patients fear death, especially due to myths about HIV/AIDS. One of the first questions patients ask after diagnosis is how long they have to live;
- "not to make me feel sick" – Patients fear antiretroviral drug side effects, such as nausea, diarrhea, headaches, skin rash, etc. They expect a therapy that makes them feel better instead of worse;
- "to be easy to take (not to big, not a lot)" – Some patients have difficulty swallowing large pills. Having to take too many

pills a day is an often evoked reason for non-adherence;

- "not to show on the outside what I have on the inside" – The stigma associated with the HIV infection is something that patients often fear. They expect that ART would make have minimal impact on their exterior appearance. This offers a sense of security, because society cannot tell they suffer from this disease.

Patients' expectations from ART show the importance they give to the impact that the therapy has on their overall quality of life, especially on their body's reaction to medication.

The infectious diseases doctors that we interviewed work in the HIV/AIDS Regional Center in Iasi. Their expectations regarding an ART regimen for patients were: "to reduce HIV viral load", "to increase CD4 cell count" and "to have minimal impact on the proper functioning of other organs". Doctors expect pragmatic results from the ART, focusing on the medical aspects.

Patients consider themselves the only factors responsible for their own ART adherence in 56.6% of cases; 20% consider the doctor to be responsible for their adherence, 16.6% feel that their family, friends, and spouse are responsible, and 6.6% (2 patients) couldn't answer (fig. 3).

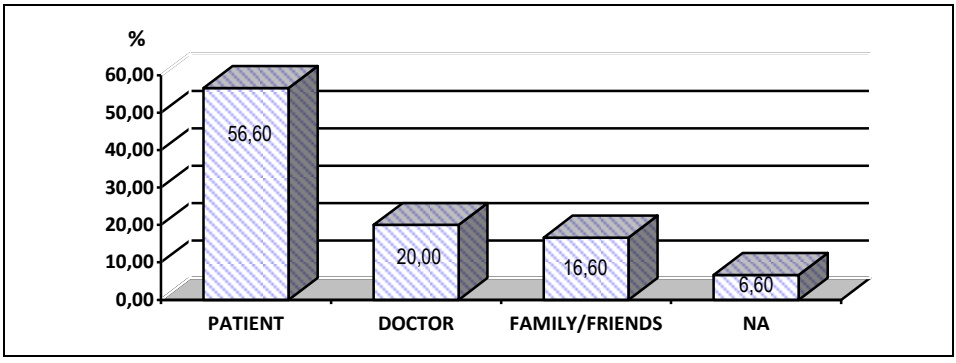

Fig. 3. Patients' perception of responsibility for adherence to antiretroviral therapy

Infectious diseases doctors considered that patients are 100% responsible for adhering to the antiretroviral therapy.

DISCUSSION

Exploring patients' expectations from ART reveals the importance they give to the impact that the therapy has on their overall quality of life, especially on their body's reaction to medication (5). Multiple side effects, such as nausea, diarrhea, headaches, skin rash, etc or a large number of pills to take are, for patients, signs that the therapy did not meet their expectations and therefore a reason to stop taking it (6). This is an important aspect in working towards adherence to antiretroviral therapy.

Doctors expect pragmatic results from the ART. Their perspective is centered on medical aspects.

Even though both doctor and patient aim for a higher quality of life, the meaning of this concept is often different. This is why often the doctor-patient relationship has to suffer.

Also, doctors consider patients to be fully responsible for their adherence. Patients, however, attribute responsibility to

outside factors in a high percent, considering that adherence is controlled by other people as well as themselves. It is important to understand this aspect that is essential in working towards an optimal adherence (7,8).

The role of a psychologist or counselor is crucial in dealing with this adherence. It is essential that the patient is evaluated from a psychological point of view and that every aspect of his/her expectations about ART is explored.

In order to assure adherence to the ART it is very useful to find common ground between the patient and the doctor. Building a healthy relationship is essential in monitoring the HIV positive patient and in encouraging adherence.

Education is important in every moment of the patient's life, in order to assure full assumption of responsibility, a key factor in adherence to ART.

CONCLUSIONS

In our research, we realized that, in order to assure adherence to the ART it is important to explore both the doctor and the patient's perspective and to find a common ground in building a healthy relationship. Both parties have the same main objective – to improve the patient's health status and quality of life. However, doctors see this objective reflected in the viral and immunological status, where as patients see it in psychological aspects and social integration. It is essential for both to realize the common direction their actions and expectations have, and to work together in achieving goals. The differences in perspective need to be addressed by both parties and it is essential to have support from a psychologist.

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SPATTER CONTAMINATION IN DENTAL PRACTICES – HOW CAN IT BE PREVENTED?

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SPATTER CONTAMINATION IN DENTAL PRACTICES – HOW CAN IT BE PREVENTED? (Abstract): Infectious diseases endanger all dental personnel during treatment, especially when spatter and aerosols are produced. Therefore, there is a strong need for better infection control principles during all treatments. The purpose of this in-vitro pilot study was to measure the environmental spatter contamination through a fluorescence technique. Scaling was performed using different power-driven devices and high-volume evacuation combined with a newly developed cannula (PS), standard suction cannulas (STS) and saliva ejectors (CDS). **Material and methods:** One sonic (AIR) and two ultrasonic devices (TIG, VEC) were utilized to remove biofilm from 168 artificial teeth in a manikin head. Teeth were scaled for 120s supra- or subgingivally. The spatter contamination of an area of 1.5m2 around the manikin head was assessed. **Results and conclusions:** The contaminated area (%) was significantly different for the AIR (median [25th; 75th percentiles]: 2.5 [1.16; 6.05]) versus TIG (0.25 [0.18; 0.88]) and VEC (0.08 [0.06; 0.1]) ($p < 0.001$). Irrespective of the instrument, subgingival scaling led to a less contaminated area (0.18 [0.07; 1.05]) than supragingival scaling (0.34 [0.1; 2.24]) ($p < 0.001$). High-volume evacuation combined with STS (0.17 [0.07; 1.04]) and PS (0.18 [0.07; 1.14]) reduced the contamination similarly ($p=0.302$) and was more effective compared to CDS (1.01 [0.12-5.78]) ($p<0.001$; $p=0.002$). Beside the limitation of an in-vitro investigation, it can be conclude that only high-volume evacuation with an adequately calibrated cannula is capable of significantly reducing the amount of spatter contamination produced during power-driven scaling. **Keywords:** SPATTER, CONTAMINATION, DENTAL PRACTICES, SCALERS

A central part of the initial and supportive periodontal therapy (SPT) is the mechanical removal of supra- and subgingival plaque and calculus (1, 2). Suitable oscillating scaler systems can be divided into sonic and ultrasonic scalers. Such scalers are the major source of potential aerosol and spatter contamination in dental practices besides high-speed hand pieces (3-7), however with some differences between sonic or ultrasonic scalers (8). The reported resurgence of bacterial diseases such as tuberculosis (9) and the presence of other pathogenic organisms with the potential for airborne transmission causes increasing concern about aerosol contamination and minor air quality in dental offices (10-12). Capturing as much cooling spray as possible seems important since there is evidence for blood contamination in spatter produced by power-driven devices (13). Therefore, recent investigations analyzed

the infection risk through spatter in order to create a prediction model of the total number of bacteria. Thus a positive correlation between the number of decayed teeth and calculus as well as a negative correlation to preoperative rinsing with chlorhexidine was found (14). In addition to that, this pilot study looked at the use of a special new cannula combined with a high-flow evacuation system for the reduction of spatter during scaling with different sonic and ultrasonic scalers to give further recommendations for working safely.

MATERIAL AND METHODS

Manikin head and teeth test

The manikin head was equipped with periodontitis models (A-PB, Frasaco, Tettnang, Germany). The jaw model of upper and lower jaw, full dentition with permanent teeth (28 teeth without wisdom teeth) and the elastic gingiva mask in pink color (A-PB WOK/WUK, Frasaco, Tettnang, Germany) simulated periodontitis with advanced horizontal bone loss and isolated, deep vertical pockets. During the investigation, the root surfaces of eight test teeth (all first incisors and all first molars in both jaws; a total of 168 teeth resulting in 672 root surfaces) had to be cleaned either with a sonic or one of two ultrasonic devices. The test teeth show no furcation involvement or concavity of the root surface and the probing depths were 4-6 mm. No calculus was applied locally considering the fact that the primary aim of this study was to imitate the patient's treatment with power-driven devices during SPT to investigate the formation of liquid deposit and droplets. Therefore a maximum of two minutes was allowed for the instrumentation of one tooth (15). Badersten et al. (15) and our pre-trials had shown that this time span is sufficient for biofilm removal with instruments during SPT and did not restrict the work of a trained operator (16).

Instrumentation procedure

The three power-driven devices for sub-/supragingival scaling were randomly assigned to the three suction devices using a computer-generated table of random numbers. One sonic scaler AIR (Synea, W&H, Bürmoos, Austria) and two ultrasonic hand-pieces TIG (Tigon+, W&H, Bürmoos, Austria) and VEC (Vector, Dürr, Bietigheim-Bissingen, Germany) were used with slimline tips (P60 and P1, W&H, Bürmoos, Austria; P1, Dürr, Bietigheim-Bissingen, Germany). One experienced operator (C.G.) performed all study procedures during all 18 trial series; six trials for each of the two high-volume evacuation cannulas with a bore diameter of 12 mm (STS: standard suction cannula and PS: prophylaxis cannula; Dürr, Bietigheim-Bissingen, Germany) and six trials for a saliva ejector (Pluradent, Offenbach, Germany) with a bore diameter of three mm (CDS) (fig. 1). The operator wore black, nonabsorbent clothes and gloves. The camera was placed at a distance of 1.93 m from the ground above the manikin head from where a baseline picture was taken. For demonstrative purposes see Figure 2a: experimental setup under normal light conditions. Scaling was performed with the assigned sonic or ultrasonic unit for a standardized period of time of two minutes. During instrumentation the two different suction cannula as well the saliva ejector were used with a high-speed evacuation system (VAS 300 S, Dürr, Bietigheim-Bissingen, Germany) with a standardized suction volume of 300 ml/min and a depression of 180 mbar.

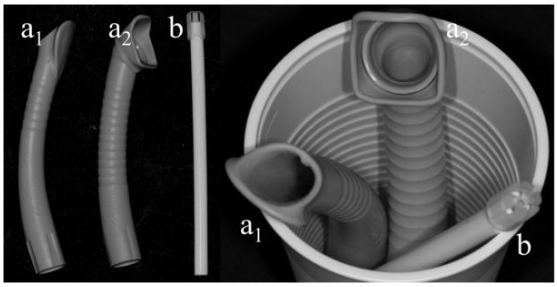

Fig. 1. The three tested cannulas; (a1) STS and (a2) PS as high-volume evacuation devices with a bore diameter of 12 mm and (b) a saliva ejector with a bore diameter of 3 mm (CDS). All of them were combined with a high-flow evacuation system.

Treatment room and visualization of spatter contamination

For the current investigation a treatment room at the Department of Conservative Dentistry and Periodontology in Kiel without any ventilation system was darkened and all surfaces 1.5m2 around the manikin head (see Figure 2a, red rectangle measuring 1.11m x 1.35m) were colored with matt black lacquer (Plasti Dip Deutschland GmbH, Aschaffenburg, Germany). To visualize the spatter, droplets and deposit during treatment, 50 mg/l fluorescein (Uranin, Niepötter Labortechnik, Bürstadt, Germany) was added to the water supply, which would fluoresce with bright orange color when exposed to ultraviolet light (UV-A, 350-370 nm). Therefore not only deposited fluorescing material on all black surfaces but also non-deposited airborne particles floating between the floor and the camera were visible in the photographs (fig. 2b).

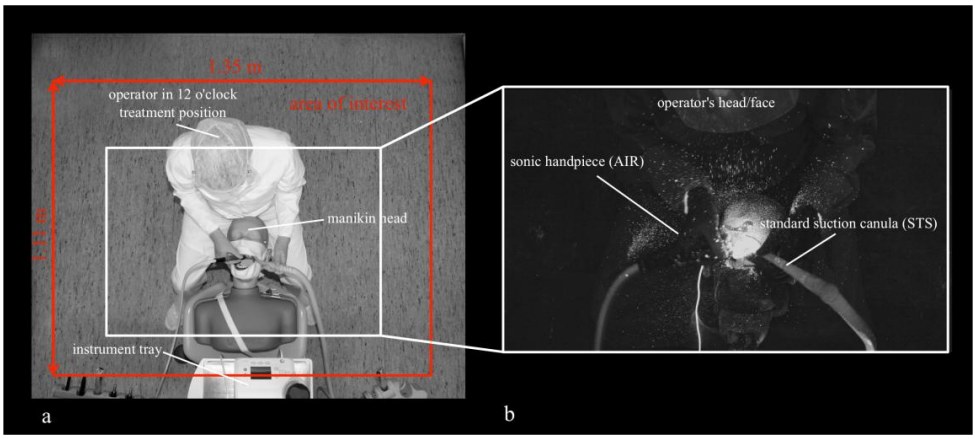

Fig. 2. The treatment room with the dental unit and the operator in 12 o´clock treatment position. (a) For demonstration purposes: experimental setup under normal light conditions and no black lacquer coverage of all surfaces/black clothes. The assessed area of interest of 1.5 m2 is marked by red frame. (b) Photo taken under experimental conditions: Enlargement of framed area after 120 s of scaling the first incisor in the right maxilla with sonic instrument AIR and standard suction cannula STS.

Plan metric evaluation of spatter contamination

The evaluation of the contaminated area with spatter and droplets was plan metrically assessed. To enable standardized evaluation through the camera, the manikin head was placed over fixed marks on the ground in the treatment room for a reproducible position (fig. 2). Due to the downward facing camera position, the surface directly underneath the manikin head wasn't measurable (fig. 2). Using a camera (Canon, EOS D30, Tokyo, Japan) with a 18 – 55 mm zoom (EFS, Canon, Tokyo, Japan), eight photographs of the work area were recorded (every 15 seconds during two minutes of instrumentation for each tooth). Focus and position were checked on a monitor (HP Compaq LA2205wg, Hewlett-Packard GmbH, Böblingen, Germany) and the photographs then transferred to the evaluation program by means of digitizer software (NIH Image, Version 1.41, National Institutes of Health, Bethesda, USA). To evaluate the individual aerosol and spatter distribution at every point of time during scaling, the program (Excel 2013, Microsoft Corporation, Redmond, USA) was set up to draw standardized data masks with quadrangle arrays of 12 x 12 pixel (4.6 mm x 4.6 mm), which assisted in including the determined area in the evaluation. The percentage of the spatter distribution area was calculated via selective marking of the contaminated areas from the base area of 69984 quadrangle arrays through simple scoring of positive or negative areas, in which the bright orange color of the marker was seen. Due to the absence of measurement of the fluorescence intensity, only a quantitative analysis of the deposit distribution was possible.

In order to determine the amount of water routinely used, the flow of water during one minute was measured using a graduated cylinder. Prior to each trial, the operator adjusted the coolant flow for the sonic and ultrasonic scaler to the clinically ideal level of 25-30 ml per minute as recommended in literature (17). The actual amount of cooling water was measured during all trials.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS Statistics 20, IBM, Chicago, IL, USA). The assumption of a normal distribution of data was checked by the Shapiro-Wilk test. Differences between the operator's localization, suction devices and instruments were analyzed using the Kruskal-Wallis non parametric ANOVA. Post hoc tests were afterwards performed by the Mann-Whitney-U test, whereby the significance level was adjusted after the Bonferroni Method (α divided by the number of performed tests). Deviation within teeth was calculated by the Wilcoxon test. Differences in coolant flow measurements were assessed using the t-test. All statistical tests were two-sided and used a significance level of $p=0.05$.

RESULTS

High-volume evacuation with PS and STS versus saliva ejector CDS

In regard to the suction devices the Kruskal-Wallis test showed significant results ($p<0.001$). The high-volume evacuation systems combined with the two cannulas STS (0.17 [0.07; 1.04]) and PS (0.18 [0.07; 1.14]) showed a similar reduction of the aerosol ($p=0.302$). Compared to the saliva ejector CDS (1.01 [0.12-5.78]) they were more effective ($p<0.001$ and $p=0.002$, respectively) (fig. 3). After subdivision in supra- and subgingival scaling we saw a favourable trend for subgingival scaling, due to the Bonferroni adaption STS and PS now differing significantly for subgingival (0.07 [0.03; 0.9] and 0.18

[0.07; 1.06]; p=0.029). Nevertheless, when performing supragingival scaling there existed no significant differences in spatter formation (tab. I).

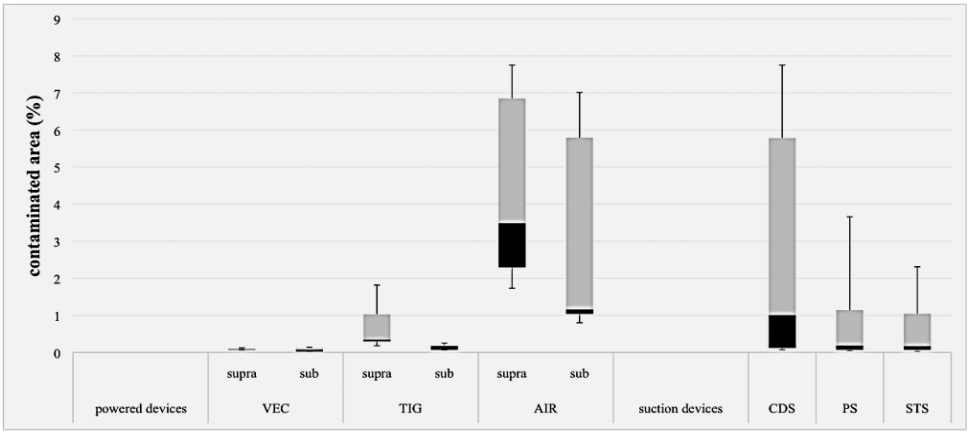

Fig. 3. Illustration of the contamination area (whiskers show minimum and maximum data) divided into the different power devices AIR, TIG and VEC as well as into the different evacuation methods STS, PS and DTS.

TABLE I

Area of contamination (median [25th; 75th percentiles]) divided by the different power-driven devices: sonic scaler (AIR) and two ultrasonic scalers (TIG and VEC) as well as by the high-volume evacuation systems combined with the standard cannula (STS) and a newly developed cannula (PS) and the conventional saliva ejector (DTS).

Power-driven device		Contaminated area (in total)	Contaminated area (supragingival scaling)		Contaminated area (subgingival scaling)	
	AIR	2.5 [1.16; 6.05]	3.49 [2.29; 6.85]	p<0.001	1.16 [1.04; 5.79]	p<0.001
	TIG	0.25 [0.18; 0.88]	0.34 [0.3; 1.03]	p<0.001	0.18 [0.07; 0.19]	p<0.001
	VEC	0.08 [0.06; 0.1]	0.09 [0.07; 0.09]	p<0.001	0.07 [0.03; 0.11]	p<0.001
Suction device						
	STS	0.17 [0.07; 1.04]	0.31 [0.09; 2,01]	p=0.993	0.07 [0.03; 0.9]	p=0.029
	PS	0.18 [0.07; 1.14]	0.29 [0.09; 3.22]	p=0.155	0.18 [0.07; 1.06]	p<0.001
	CDS	1.01 [0.12-5.78]	1.04 [0.1; 5.93]	p=0.096	0.23 [0.12; 5.79]	p<0.001
Localization						
	Maxilla		0.44 [0.1; 2.36]	p=0.874	0.23 [0.06; 0.62]	p=0.155
	Mandible		0.26 [0.12; 1.98]		0.11 [0.07; 1.1]	
	Incisors		0.47 [0.11; 2.73]	p<0.001	0.15 [0.06; 1.13]	p=0.926
	Molars		0.27 [0.11; 1.64]		0.14 [0.07; 1.02]	
Maxilla	Incisors		0.57 [0.1; 2.67]	p=0.604	0.22 [0.03; 0.59]	p=0.008
	Molars		0.38 [0.08; 1.84]		0.21 [0.05; 0.41]	
Mandible	Incisors		0.35 [0.12; 2.59]	p=0.339	0.11 [0.07; 1.21]	p=0.217
	Molars		0.24 [0.14; 1.43]		0.11 [0.07; 0.99]	
Treatment position 8-9 o'clock			0.25 [0.09; 1.68]	p<0.001	0.11 [0.04; 0.63]	p<0.001
Treatment position 10-12 o'clock			0.49 [0.12; 2.68]		0.21 [0.1; 1.32]	

Statistical significance was verified with the Kruskal-Wallis test and followed by post hoc Mann-Whitney-U test (operator's localization, suction devices and instruments) and by the Wilcoxon test (teeth). For better understanding we left out the results from the Kruskal-Wallis ANOVA.

Power-driven devices

The size of the contaminated area was significantly different during supragingival scaling for the three different scalers (all groups $p < 0.001$): AIR, TIG, VEC (fig. 3). The same results were found while performing subgingival scaling (all groups $p<0.001$) (fig. 3). Irrespective of the power-driven device used, subgingival scaling led to a lower contaminated area in percent (median [25th; 75th percentiles]: 0.18 [0.07; 1.05]) compared to supragingival scaling (0.34 [0.1; 2.24]) ($p < 0.001$).

Spatter contamination and treatment position

A significant difference in the contaminated area was found concerning treatment positions regardless of the used instrument or the suction device (all groups $p<0.05$). The contamination in 10-12 o'clock treatment position was higher than in other treatment positions. The highest contamination was generated when scaling the upper incisors supragingivally (0.57 [0.1; 2.67]). During supragingival instrumentation and evacuation there were significant differences between incisors and molars to be found ($p<0.001$). For subgingival scaling the results were not significant ($p=0.926$). There were no significant differences seen for neither mandible vs. maxilla nor for supra- vs. subgingival scaling ($p=0.874$).

Coolant flow

The mean coolant flow measured during all six trials varied from 27.7±2.7 ml (TIG), 30.1±3.5 ml (AIR) to 30.5±1.9 ml (VEC) per minute. During these trials the loss of coolant medium, potential for generating spatter and aerosols, was without significance ($p=0.378$) for TIG (2.7±1.4 ml/min) and VEC (2.4±1.5 ml/min) except for sonic scaler (AIR). The use of sonic scaler AIR resulted in an increased loss of 4.7±2.4 ml/min ($p=0.054$). The percentage of evacuated coolant (amount of evacuated coolant medium x 100/amount of used coolant medium) was 47.0±14.8 for the STS and 44.3±16.1 for PS ($p = 0.873$) and was significantly less by 6.3±7.8 for the CDS ($p = 0.001$).

DISCUSSION

Previous research has shown that ultrasonic and sonic scalers, are routinely established and used in modern prophylaxis and periodontal treatment (18, 19), but with the disadvantages to generate spatter and aerosol (8, 20-22). Both can contain airborne or blood-borne pathogens (23) and may cause various infections such as legionellosis, tuberculosis or hepatitis (24-26) or other more insidious problems like watery eyes, running noses, headaches or itchy skin (27). Several authors strongly suggest that dental staff using power-driven devices for scaling may be at a greater risk than other healthcare workers (7, 8, 27). Nevertheless, no clinical study has so far shown a direct cause-effect relationship between bacteria-contaminated spatter or aerosols and infections. It seems indisputable that spatter produced by ultrasonic or sonic scalers is greatest in the immediate vicinity of the patient. But an alteration of bacterial contamination in a distance of up to three meters from the patient can still be recognized (24), particularly when using airpolishing equipment (28). However, it is mostly assumed that splashes of water droplets move around 1.2 to 1.8 meter from a patient's oral cavity (6-8, 29, 30). Therefore, and due to the limitation of our operating room, the area of interest for a single operator was defined as a maximum of 1.11 m x 1.35 m. The generated dispersion in this current investigation given in % of the

defined area seems very small. This is not actually the case since the spatter contamination of e.g. 1.01% during the use of CDS alone caused a contaminated area of at least 0.02m2. This adds to 123 x 123 mm (15150 mm2), which equates to the entirely coated palms of the operator, being completely polluted with potentially infectious spatter.

However, through the use of special aerosol reduction devices some investigators have decreased the contamination by nearly 90-97% (31, 32). But those reduction systems are not very common and the spatter development during air polishing is not comparable to those of sonic-/ultrasonic scalers. Jacks (31) discussed that the negligence of the operator to use such special reduction systems could be a result of the difficulty to handle two bulky instruments simultaneously. This corresponded with our observations during the use of the newly developed cannula PS, which tended to be too cumbersome to use. The anticipated improvement in the standard cannula SPS, therefore, failed.

Irrespective of the simple and inexpensive methods for the control of dental aerosols and spatter already available (33), dentists do not apply those methods mostly because of low awareness of health risks, working habits and economic factors (7). Due to the experimental setup of our investigation without air collection, it was difficult to find similar results for aerosol contamination in the studies we cited, which made use of real time aerosol monitoring (grams/cubic meter). It can be assumed that there were inspirable particles emitted due to scaling, which can be termed as aerosols. Nevertheless our study technology only measured spatter, droplets, deposits and non-deposited airborne particles. Therefore, we added the effectiveness of suction during scaling [ml] to discuss potential coolant medium for spatter. A similar effectiveness for STS and PS of nearly 50% and significant result of 6% with a saliva ejector could be found, corresponding to the results of Jacks (31). The results re-emphasized the statement that a lot of coolant medium is potentially present to generate aerosol (fig. 4). In a separate experiment we were able to visualize the spatter production induced through the tip movement during the first two seconds for an area of 0.50 x 0.80 m (fig. 5). According to Timmermann et al. (34), piezoelectric devices, as a result of their linear tip movement (35), should produce less spatter than sonic scalers with almost circular tip movement and a greater amplitude (36). This was in accordance to the spatter production of the two piezoelectric devices in this current investigation, which was significantly lower and more or less target-oriented compared to the sonic scaler (VEC 0.08% and TIG 0.25% versus AIR 2.5%) (fig. 6). According to this, one should carefully consider the choice of instruments, especially when treating potentially infectious patients, and if necessary opt for the use of an ultrasonic scaler.

Due to the higher risk of generating spatter, especially during the use of sonic scalers, only high-speed evacuation systems with a standardized suction volume of around 300 ml/min, as employed in the current investigation (VAS 300 S, Dürr, Bietigheim-Bissingen, Germany), should be used. This recommendation is similar to other studies (37, 38) and shows that the only efficient method for controlling contaminated spatter is the use of a large-diameter high-volume evacuator, instead of e.g. reducing the cooling water supply (28). Heat is produced during operating mode for all power-driven devices (17, 39) and only using cooling medium minimized heat gen-

eration to physiologically tolerable levels (39). Consequently, the necessary cooling during root instrumentation leads to the reality that even small amounts of irrigation can result in potentially contaminating spatter (8). Our results also show that an entire elimination is impossible and, therefore, instrumentation of infectious patients with hand instruments may be recommended (40). Since a salivary ejector can only remove a small volume of air, it's sole use proves ineffective to remove aerosols and spatter (31, 32). However, the use of the special newly developed cannula (PS) for high-volume evacuation in our investigation seems to neither improve spatter reduction (0.18% contaminated area) nor improve the effectiveness of suction (44%) compared with a standard cannula STS (0.17% contaminated area, 47% effectiveness of suction).

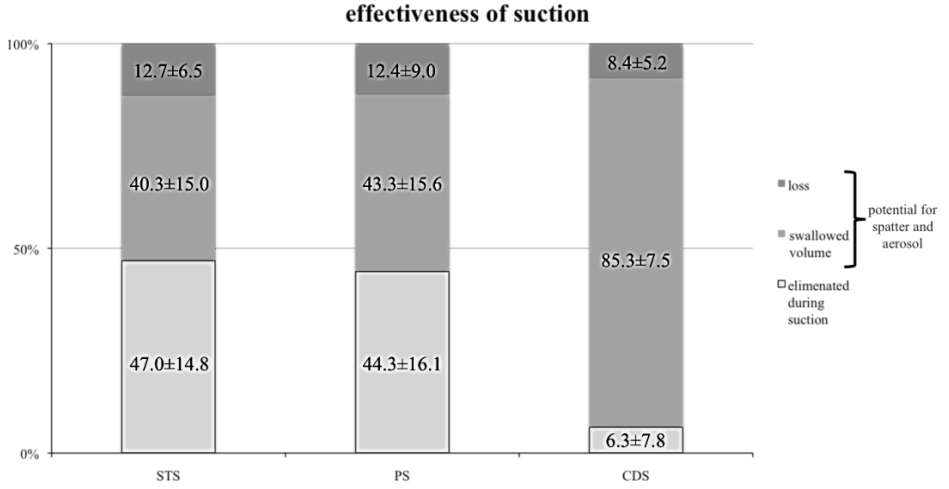

Fig. 4. Illustration of the effectiveness of cooling medium evacuation (%) divided by the three different evacuation methods: standard cannula (STS), the newly developed cannula (PS) and the conventional saliva ejector (CDS).

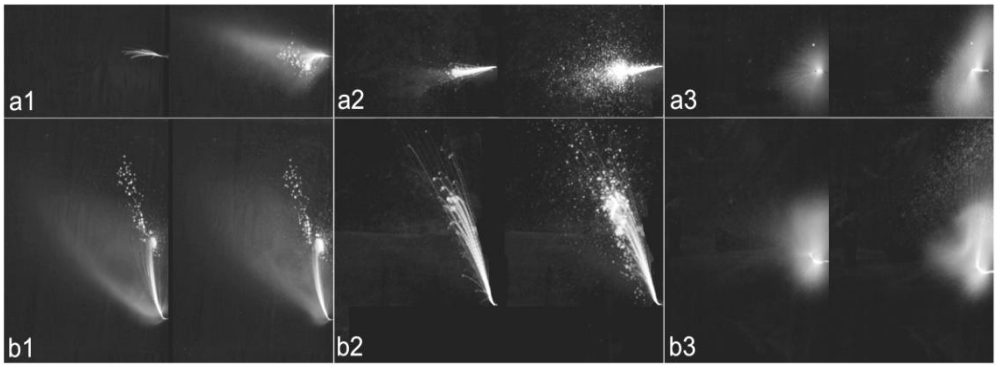

Fig. 5. Spatter and aerosol production right after start and after 2s of tip movement using the two ultrasonic scalers (a/b1: TIG, a/b2: VEC) and one sonic scaler (a/b3: AIR). The upper pictures (a1-a3) show the tip movement in vertical direction and the lower pictures (b1-b3) in horizontal direction.

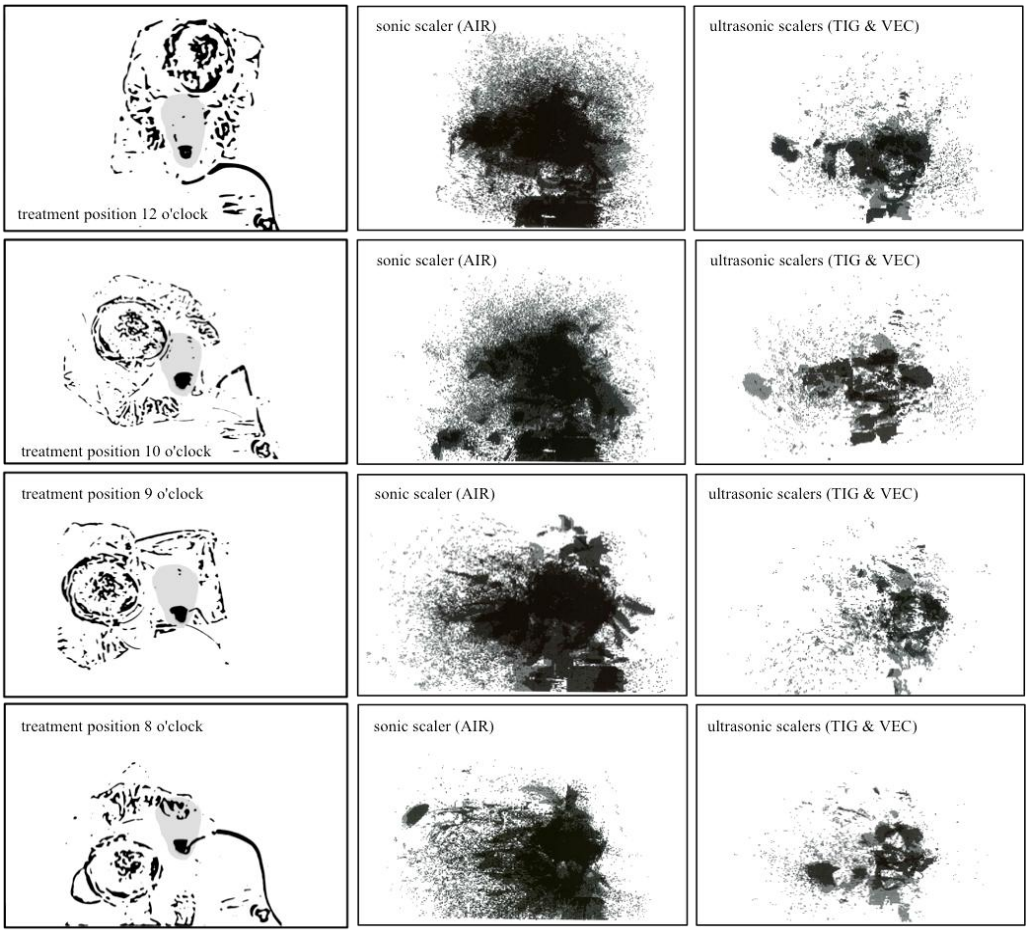

Fig. 6. Area of contamination in the treatment room (measured area: 1,11 m x 1.35 m) according to the treatment positions 8, 9, 10 and 12 o'clock as a summation of 18 trials with a sonic device (AIR) and 18 trials for each of the two ultrasonic devices (TIG and VEC). Due to the identical characteristics, the two ultrasonic scalers were analysed together for this visualization. All three different evacuation systems (STS, PS and CDS) were used. The gradation of the spatter was as follows; black was used when the area was contaminated in all 18 trials, dark grey was used when the area was contaminated in 8 to 17 trials and light grey was used when the area was contaminated in 1 to 7 trials.

Along with the already discussed variables such as instruments and type of suction, the part of the dentition that is being worked on as well as the treatment position greatly influence the occurrence and spreading direction of spatter. In this context, the lips can work as a protection visor, when working from a 12 o´clock position (41). The results of our investigation showed a different picture for the risk of contamination also in this treatment position, especially during the use of the sonic scaler with significantly more spatter production (fig. 6). It could be possible that a

single operator is often unable to work the suction from buccal and therefore spray rebound is unavoidable even from a 12 o'clock position. Another fact to discuss may also be the characteristic of the less flexible lips due to the rubber material and the maximum opening of the manikin head´s mouth. However, the use of the PS as well as the STS seems cumbersome from a subjective point of view.

One limitation of this study as well as other studies was the 2D visualisation of the treatment room (fig. 2) (41). Only the horizontal dimension of the area of interest was photographically documented during treatment. The spatter, which settles on vertical surfaces such as furniture of the room, clothes, the face or face guard, is only conditionally viewable. Even the modified technique, with a fluorescein solution we used to visualize spatter and droplets, could not solve these problems, even though it seemed that the spots produced on horizontal areas could be evaluated more accurately compared with the erythrosine (13). Another aspect that has to be mentioned is that the size of the spatter produced by the sonic and ultrasonic devices seemed different (fig. 5), but the sensitivity of the used detection method failed to measure this. The analysis of the difference between the used and the sucked in volume of cooling medium cannot solve that problem, nevertheless the amount of lost cooling water that can potentially form spatter as well as aerosols was shown (fig. 4).

Despite the limitations of the research design, it can be said that by using the opening of the mouth as a shield, a reduction of the risk of contamination for the posterior teeth can be assumed from all treatment positions. Still a higher risk of infection for the dentist in treatment positions like 12 o´clock for the anterior teeth has to be discussed. It seems that in the treatment position behind the patient's head the face of the operator, eyes and nose are most important for transmission of infections (42), as well as his chest area (30, 43) are the most contaminated area. Therefore it is recommended to use protective means like glasses, masks and especially in treatment positions between 10 and 2 o'clock as a protective shield, which offers the greatest protection in this area. Furthermore, the use of pre-procedural rinsing with an antiseptic mouthwash may be of value in protecting patients and dental professionals due to the reducing effect on the microbiology flora of the oral cavity (43-48).

CONCLUSIONS

The risk of spatter contamination of the area next to the patient's mouth during scaling increases with the use of sonic versus ultrasonic device as well as with the use of saliva ejector versus high-volume evacuation devices. Also the treatment position of the operator and the region of the mouth that is being treated can influence the generation of potentially infectious spatter. It is therefore strongly advised to use high-volume evacuation devices and to wear a visor during treatment in all positions behind the patient's head to protect the face of the operator. Since even under ideal conditions spatter cannot be avoided, it is strongly recommended to follow the common suggestions for protection during dental treatment: use of eye protection, masks, gloves, clothes coverage and rinsing the oral cavity of a patient pre-procedural with antiseptic mouthwash (7, 12, 33, 46, 48).

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NEWS

RELATIONSHIP BETWEEN OBESITY AND ORAL DISEASES

The aim of a study realized by a group of researchers from University of Benin, Nigeria was to determine the relationship between obesity and periodontal status and dental caries experience of a group of nigerian dental patients. Participants were selected from patients attending dental outpatient clinics of the University of Benin Teaching Hospital, Benin City, Nigeria. Their weight and height were measured and body mass index (BMI) estimated in kg/m^2, gingival health assessed using bleeding on probing index, oral hygiene estimated using the simplified Oral Hygiene Index (OHI-S), periodontal health estimated using the Basic Periodontal Examination (BPE) and caries experience was estimated with the Decayed, Missing, Filled teeth (DMFT) index. A few participants (3.8%) were underweight, 52.6% fell within the normal BMI range, 28.2% pre-obese, 12.2% obese class I and 3.2% obese class II. The mean OHI-S score was 2.16 ± 1.13 among the overweight participants and 2.05 ± 1.13 among those who are not (P = 0.543). The mean DMFT score was 3.03 ± 4.25 among the overweight participants and 2.32 ± 3.01 among those who are not (P = 0.223). Sixty-five percent of participants with BPE score of 0, considered to signify periodontal health, had normal BMI while all the participants with the worst BPE score recorded belong to the obese 1 group (P = 0.070). The binary logistic regression revealed that the likely predictor of gingival bleeding in the study is BMI between 35.0 and 39.9 (obese class 2) (P = 0.046, odds ratio = 0.07, 95% confidence interval = 0.01-0.96). It can be concluded that there was no statistically significant relationship between obesity and periodontal status and dental caries experience in the studied group of dental patients. Increased body mass index may however be a predictor of gingival bleeding (Sede MA, Ehizele AO. Relationship between obesity and oral diseases. *Niger J Clin Pract*, 2014 ; 17 (6) : 683-690)

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UTILITY OF AUTOPSY IN MEDICAL EDUCATION - STUDENTS' OPINIONS AND ATTITUDES

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UTILITY OF AUTOPSY IN MEDICAL EDUCATION- STUDENTS' OPINIONS AND ATTITUDES (Abstract): Autopsy has been for centuries part of medical education, providing benefits to medical students in terms of knowledge, skills, and development of respect, empathy and compassion. **Aim:** To evaluate the utility of autopsy in medical education based on the opinions and attitudes of medical students. **Material and methods:** A questionnaire was applied to 219 medical students. The answers were grouped according to two main themes: the utility of autopsy in medical education and students' attitudes toward autopsy. **Results:** Most of the respondents considered that autopsy is useful to society, medical practice and medical education. On average, participants felt "moderately uncomfortable" on exposure to the first autopsy. **Conclusions:** Our study indicates the need for medical education to emphasize the utility of autopsy in the medical practice. Extracurricular activities to deepen the medico-legal knowledge and skills would be beneficial for the students who are particularly interested in this specialty. It is also necessary to take into account the possible negative psychological reactions of the students caused by the first exposure to autopsy and identify adequate methods to solve them. **Keywords**: AUTOPSY, MEDICAL EDUCATION, MEDICAL PRACTICE

Autopsy has been for centuries an important educational tool (1, 2) and the findings during autopsies allowed the achievement of many of the modern medical knowledge (3). Autopsy has many educational roles. It facilitates understanding of the clinical and pathological, physiological and anatomical correlations, improves the observation and communication skills, and provides the students the opportunity to address specific topics, such as: death certificate, violent death, attitudes towards death (4, 5).

However, the use of autopsy in medical education is decreasing due to the progressive change of university curricula, the emergence of teaching alternative technologies, such as video projections, and the overall decrease in the number of autopsies due to the legal, bureaucratic, financial or religious barriers (6, 7). Unfortunately, many times alternative technologies fail to match the immediate impact of exposure to autopsy (6), making less likely that the clinicians who did not attend any autopsy to become supporters of autopsy (4, 5) or identify the need for

this examination (8).

Forensic Medicine is taught at the Iasi Faculty of Medicine in the sixth year of studies. During the practical activity, the students attend five autopsies, and become familiar with the legal procedures, technical aspects of autopsy, death certificate, and ethical issues which stem from the respect for the deceased and his/her family. During the practical activity students are not required to participate effectively in the autopsy but only to attend and learn the procedure.

MATERIAL AND METHODS

The aim of our study was to analyze the opinions of the medical students about the utility of autopsy in medical education and practice. We also sought the respondents' attitudes toward autopsy, i.e. their reaction to the first autopsy and the degree to which they accept and recognize the value of postmortem examination.

We used a quantitative methodology applying a 14-items questionnaire with a 6-point Lichert scale (1- disagree to 6- always agree). The data were analyzed with *SPSS* using the factorial analysis and Mann–Whitney–Wilcoxon test.

The study group comprised 219 sixth year medical students who completed the Forensic Medicine course. They answered the questions anonymously and received no financial compensation for their participation. Before completing the questionnaire, the aim of the study and the questionnaire sections were explained and the subjects were asked to give their written consent to participate in this study. The study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy.

RESULTS AND DISCUSSION

The mean age of the participants was 24.56 ± 0.98 years (range 23 ÷ 29). Gender distribution showed a preponderance of females (139; 63.5%). The majority of the participants were of Romanian nationality (95%), most were Orthodox Christians (92.2%), and 49.3% declared themselves religious persons.

Most of the participants were first exposed to autopsy in the sixth year, during the Forensic Medicine practical activity (196-89.5%). Some of the students were first exposed to autopsy during the first five years of studies during the Anatomy or Pathology classes (tab.I, II).

To investigate the influence of various sociological characteristics we applied the Mann-Whitney-Wilcoxon test (tab. III).

TABLE I

Centralization of the answers from participants

Question	N	Median (quartile interval)	Average ± Standard deviation	Median answer
1. Students should attend more autopsies	219	3 (2÷4)	2.8±1.39	sometimes agree
2. Autopsy will be useful to my future medical activity	219	4 (3÷5)	4.07±1.26	often agree
3. During autopsy I have learned many things that I will use in the medical practice	219	4 (3÷5)	4.11±1.18	often agree
4. Autopsy should be a compulsory part of academic curriculum	219	5 (4÷6)	4.64±1.44	very often agree

Question	N	Median (quartile interval)	Average ± Standard deviation	Median answer
5. Students should actively participate in autopsy	219	3 (2÷4)	2.97±1.53	sometimes agree
6. Autopsy is useful for the progress of medicine	219	6 (4÷6)	5.1±1.14	always agree
7. Autopsy is contrary to religious beliefs	219	1 (1÷3)	1.96±1.25	disagree
8. Exposure to autopsy has influenced my perspective on clinical activity	219	3 (1÷4)	2.81±1.53	sometimes agree
9. I would accept to be autopsied after death	219	5 (3÷6)	4.33±1.85	very often agree
10. I would consent for a deceased close relative to be autopsied	219	5 (3÷6)	4.61±1.64	very often agree
11. Afterlife exists	218	3 (2÷6)	3.72±1.98	sometimes agree
12. Participation to autopsy made me wish to become a forensic pathologist	219	1 (1÷2)	1.71±1.25	disagree
13. Autopsy is useful to society	219	6 (5÷6)	5.17±1.05	always agree
14. Reaction at the first autopsy	218	3 (2÷3)	2.6±1.05	moderately uncomfortable

TABLE II

Results of the factorial analysis

Items	Factorial saturation					Communialities
	F1	F2	F3	F4	F5	
Factor 1- Social utility of autopsy						
Question 9	0.904					0.867
Question 10	0.914					0.877
Question 13	0.673	0.369				0.623
Factor 2- Medical (research, teaching) utility of autopsy						
Question 2		0.785				0.747
Question 3.		0.829				0.720
Question 4		0.536				0.607
Question 6		0.664				0.547
Factor 3- Clinical utility of autopsy						
Question 5			0.666			0.572
Question 8			0.625			0.455
Question 1			0.681			0.473
Question 12			0.523		-0.485	0.546
Factor 4- Sensitivity- discomfort on the first exposure to autopsy						
Gender				0.842		0.714
Question 14				0.508	-0.372	0.507
Factor 5- Religious beliefs						
Question 7					0.598	0.586
Question 11					0.765	0.664
Parameters						
Eigenvalues	2.41	2.34	1.91	1.46	1.39	
% variance	16.03	15.60	12.74	9.72	9.26	Total 63.36%

TABLE III
Results of the Mann–Whitney–Wilcoxon test

Question	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gender	0.789	0.196	0.996	**0.024**	0.065	0.659	0.067	0.569	0.062	0.061	0.055	0.986	0.371	**0.003**
Religious-ness	0.216	0.206	0.495	**0.006**	0.165	**0.033**	0.874	0.386	**0.000**	**0.005**	**0.002**	0.686	**0.030**	**0.002**
Preference for forensics	0.745	**0.002**	**0.001**	**0.023**	**0.002**	0.151	**0.025**	0.175	**0.002**	**0.029**	0.147	-	**0.005**	**0.028**
Nationality	**0.004**	0.760	0.108	0.562	0.147	0.132	**0.007**	0.987	0.223	0.436	0.979	0.624	**0.026**	0.092
Religious affiliation	**0.028**	0.906	0.272	0.499	0.460	**0.040**	0.237	0.793	0.072	0.189	0.732	0.779	0.236	0.589
Discipline	0.766	0.308	0.509	0.274	**0.032**	0.445	0.876	0.847	**0.033**	**0.041**	0.407	0.601	0.287	0.071

We grouped the respondents successively by gender (male/ female), religiousness (Yes / No), the preference for Forensic Medicine specialty (answers "very often disagree" and "always agree" to question 12), nationality (Romanian/ other), religious affiliation (Christian Orthodox/ other religion), and discipline where the participants were first exposed to autopsy (forensic medicine/ another discipline).

I. Utility of autopsy in medical education

Most of the participants considered that autopsy is useful to society and progress of medicine (”always agree”). They also considered that autopsy should be part of the academic curriculum (”very often agree”), a lower level of agreement being expressed by the female participants ($p = 0.024$). The students who acknowledged the utility of autopsy for the progress of medicine agreed with its educational utility and considered that it should be part of the academic curriculum.

The results of other studies also emphasize the educational role of autopsy. Autopsy provides knowledge in many medical fields and facilitates the development of a proper behavior towards the patients and their families (1). Khoo (9) shows that students believe that autopsy is a useful and beneficial experience that should be available to all students. In the study by Ekanem and Akhigbe (10) the majority of students agreed that autopsy is an important educational tool and students should be encouraged to watch and actively participate in this procedure.

The participants in our study partially agreed (”often agree”) that autopsy is useful to their medical practice. However, to a lesser extent they felt that attending an autopsy influenced the way they relate to clinical activity (”sometimes agree”), possibly due to a greater awareness of the risks of medical profession, legal risk included.

Benbow (1) showed that a sizeable proportion of students considered autopsy valuable to the medical practice. In the study by Khoo (9), the participants admitted that attending an autopsy allowed them to reflect on death and made them more prepared to accept patient death.

Most students participating in our study attended and watched autopsies, without being actively involved in the procedure (99.1% witnessed; 0.9% worked effective-

ly). To a lesser extent ("sometimes agree"), they believe that medical students should actively participate in autopsy. We found a statistically significant trend in favour of the active participation in autopsy from students who were first exposed to autopsy during the first five years of studies ($p = 0.032$).

In a study by Conran (11), most students considered that autopsy should be a compulsory part of the medical education, but only a few wanted autopsy on themselves. Khoo (9) found that the students showing interest in actively participating in autopsy, believed that this experience was likely to bring them real benefits.

The participants in our study attended on average six autopsies. Only 56 respondents felt that the students should attend more autopsies ("sometimes agree") assessing that the optimum number of autopsies would be 10 ($8 \div 10.5$). The foreign students advocated to a greater extent for an increased number of autopsies ($p = 0.004$), which may suggest their greater interest in the practical aspects of medical education. Generally, the preference to participate in (and perform) more autopsies is accompanied by a greater recognition of the utility of autopsy in clinical activity and an interest for Forensic Medicine specialty.

Ekanem and Akhigbe (10) found that 69.2% of the participants in their study attended 6- 15 autopsies and 57% of the participants considered that the number of autopsies was enough; however 74% of the participants believed that the students should be encouraged to watch and effectively participate in more autopsies. Sanner and Charlton *et al.* (12, 13) showed that the students are aware that attending a single autopsy is not enough.

II. Students' attitudes toward autopsy

On average the participants in our study felt "moderately uncomfortable" on the first exposure to autopsy. Discomfort was reported to a greater extent by the female participants ($p = 0.003$) and by the religious persons ($p = 0.002$).

The students may experience moderate to serious psychologic reactions on their first exposure to autopsy (14). In the study by Benbow (1), 20% of students acknowledged the utility of autopsy but found it unpleasant and considered they should have been prepared psychologically before the first exposure to autopsy.

It is well recognized that the students should be psychological prepared before attending the autopsy in order to avoid unpleasant reactions and to take advantage of the benefits of attending such a procedure (13, 15, 16). However the potential negative psychological reactions caused by exposure to autopsy is usualy neglected in medical education although autopsy may raise "emotional feelings with regard to human mortality and dignity" (17). He Miao-Xia *et al.* (18) observed that students, after getting over the psychological impact of the first exposure to autopsy became calm, took notes, and focused without getting involved emotionally.

To a large extent ("very often agree") the participants in our study would agree to an autopsy on themselves or a deceased close relative. These findings correlate with the recognition of the social and medical utility of autopsy.

Students who were exposed to autopsy in the first five years of studies years showed less objection to their own autopsy ($p = 0.033$) or the autopsy of a deceased close relative ($p = 0.041$). On the other

hand, the religious persons wanted to a lesser extent an autopsy on themselves (p <0.001) and a close relative (p = 0.005) than the non-religious participants.

Other studies showed that most students would agree with their own autopsy (12, 17), but the thought of it made them feel uncomfortable (12). Khoo (9) found that only 34.1% of the students would agree with an autopsy on themselves or a close relative, but 72.9% would request an autopsy on their patients if necessary. Verma and Benbow (2, 15) found that many students, after attending an autopsy felt stressed knowing that a close person underwent a procedure which ”mutilates the body".

Most participants in our study did not consider autopsy contrary to their religious beliefs. No statistically significant differences were found between religious and non-religious subjects in this respect. This result is consistent with other studies that concluded that generally, religious beliefs are not valid reasons to deny an autopsy because religion does not prohibit autopsy *per se* (9, 19).

We found a partial agreement (”sometimes agree”) on the belief in afterlife. Females (p = 0.055) and religious persons (p = 0.002) agreed to a greater extent that afterlife exists. The respondents who endorsed the existence of afterlife and the conflict between religious beliefs and autopsy reported uncomfortable feelings on the first exposure to autopsy and would not pursue a career in Forensic Medicine.

Williams *et al.* (20) consider that belief in afterlife is a strategy chosen by 40% of medical students and resident physicians to deal with the death of their patients and relatives.

Most of the participants in our study believed that attending an autopsy did not instill the desire of becoming forensic pathologists. We found that the attitude toward autopsy of the students who consider becoming forensic pathologists is generally positive but we did not identify any significant differences between them and their colleagues in terms of gender, religiousness, or the number of autopsies attended. Similarily, Papadodima et al. showed that the students who wish to pursue a career in Forensic Medicine got involved more actively in autopsy but did not identify different characteristics in terms of age, gender, religious belifs, reactions, emotions, attitudes, and lifestyle compared to the rest of their colleagues (14).

CONCLUSIONS

The results of our study show that the students recognize the utility of autopsy for society and medical practice. They also consider that autopsy is an important educational tool. To a large extent, the respondents felt that the number of autopsies provided currently in the curriculum is sufficient and that it is enough for them to watch the procedure without being effectively involved. However, some students considered that attending more autopsies and the hands-on experience are beneficial for their medical education. For these students it would be useful to organize extracurricular activities that would allow them to deepen their knowledge and practical skills in this specialty.

Although students' attitudes toward autopsy are generally positive, on average participants stated that they felt moderately uncomfortable on their first exposure to autopsy. This result supports the fact that the possible negative psychological reactions caused by the first exposure to autopsy should be taken into account, and effective

ways of managing them, such as debriefing or preparatory sessions need to be used.

It would also be useful to mainstream Forensic Medicine among other specialties both conceptually and practically so that the students can perceive autopsy as a part of medical practice and develop a positive attitude toward postmortem examination.

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PREDICTORS OF POSTSURGICAL STRESS AMONG CARDIAC PATIENTS

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PREDICTORS OF POSTSURGICAL STRESS AMONG CARDIAC PATIENTS (Abstract). **Aim:** To explore the role of stress vulnerability, dispositional optimism, fears of surgery and negative affective disposition in prediction of postsurgical stress among cardiac patients who underwent heart surgery. **Material and methods:** The sample included 60 cardiac patients (39 males and 21 females, aged 58.50±10.97 years). All patients completed two sets of standardized questionnaires. Before surgical intervention, stress vulnerability, dispositional optimism and negative affective disposition were measured with N6-Scale (from NEO Personality Inventory–Revised Form), Life Orientation Test-Revised Form and Mental Health Index-5, respectively. Fear of surgery among patients was measured with an 8-item questionnaire designed for the current study. After intervention, patients completed a short postsurgical data form along with the Perceived Stress Scale. **Results:** There was no significant difference in postsurgical stress between the female and male cardiac patients ($t = 0.96$; $p = 0.34$). A similar result was obtained when the presence/absence of postsurgical complications was considered as an independent variable ($t = 1.30$; $p = 0.19$). As expected, a higher level of postsurgical stress was associated with higher stress vulnerability, surgery-related fear, negative affective disposition, and with a lower dispositional optimism score. Moreover, stress vulnerability and negative affective disposition were significant predictors of postsurgical stress. **Conclusions:** Cardiac patients who are prone to stress and negative affective disposition are also at higher risk for postsurgical stress. On its turn, this psychological condition interferes with time and quality of postsurgical recovery. A careful assessment of psychological condition along with counseling before surgical intervention is needed in order to reduce the risk of postsurgical stress and improve medical recovery in cardiac patients. **Keywords:** CARDIAC PATIENTS, STRESS VULNERABILITY, DISPOSITIONAL OPTIMISM, FEAR OF SURGERY, NEGATIVE AFFECTIVE DISPOSITION, POSTSURGICAL STRESS

Surgery is a stressful life event which triggers many physiological, cognitive, and emotional responses among patients due to certain personality traits (i.e. dispositional optimism, emotional instability, confidence in the ability to control postsurgical course of the disease etc.), patient social network (i.e. the level of emotional and social support) or disease-associated clinical characteristics (1). The social, emotional, and cognitive consequences of a surgical intervention have also drawn the attention of professionals and researchers in medical care and social services. The psychological (pre- and post- surgical) state of patients is due to recurrent afflictions, post-surgery complications, mortality rate, and also certain recovery quality indicators (i.e. the

psychological and physical well-being, or the outlook on quality of life) (2).

Usually, the pre-surgical stage of a cardiac intervention is stressful for the patient, as well as for his/her family (3), while recovery is physically and psychologically challenging. The research concerning patients undergoing stress before or during surgery revealed higher levels of anxiety and depression in those cases (1, 3). Nevertheless, the dysfunctional emotional reactivity of cardiac patients is one of the risk factors that explain the high level of postoperative mortality and morbidity (1), the longer postoperative hospital stay and physical recovery time, and the persistence of certain negative emotional states (3).

This research is focused on the variables associated with postoperative stress experienced by cardiac patients who underwent specific surgery. Thus, optimism is important for the postoperative course of cardiac patients. For the scholars this notion embodies the positive expectations (full of hope) a person experiences in a given situation (4). Optimistic stance tends to be a protective factor against functional deterioration and also a consistent indicator of the quality of life in patients with chronic afflictions (5, 6, 7, 8).

Another area of research that has generated numerous empirical clinical and health psychology studies is the relation between personality traits, stress, and health. Neuroticism/emotional instability is one of the most studied personality traits in relation with stress and/or other psychopathological manifestations, and also with adaptation to disease. This type of personality trait describes the general tendency of a person to experience negative emotions (anxiety, sadness, guilt, anger), irrational ideas, and difficulties in impulse control (9). The neurotic state tends to be one of the most important personality factors that determine the vulnerability of a person in stressful situations that influence the functions and adaptation strategies of a person (10).

On the other hand, given the complexity, extent, and risk (predictable and unpredictable complications) of heart surgery, it is natural that most cardiac patients to experience preoperative fear (cognitive preoccupations and unpleasant emotional reactions, accompanied by somatic-neural-vegetative reactions). There is a positive association between the level of preoperative fear and the risk of postoperative stress.

This article summarizes the results of a transversal study designed to explore the role of stress vulnerability (as expression of emotional instability), dispositional optimism, surgery-related fear, and negative affective stance in explaining the individual differences in the level of postoperative experienced by heart surgery patients. The practical applicability of this study consists in the identification of the predisposing individual traits (emotional and cognitive) which can explain the differences in postoperative medical and psychological outcomes between cardiac patients. These findings can be useful in preventive psychological counseling of patients about to undergo surgery and possibly experience a critical stress level.

MATERIAL AND METHODS

Sixty cardiac patients, admitted and operated (October, 2010 - May, 2011) in the "Prof. Dr. I. M. Georgescu" Institute of Cardiovascular Diseases at Iasi were assessed before and after surgery. The 39 male and 21 female patients were aged between 24 and 76 years (M = 58.50 years; SD = 10. 97 years). Over 75% of the patients had at least one cardiovascular disor-

der (high blood pressure, acute myocardial infarction, ischemic cardiomyopathy, coronary insufficiency, stroke), or other type of disorders (diabetes, prostate adenoma, chronic renal failure, plasmacytoma, gastric ulcer), treated over the past 5 years or on therapy. Almost half of the cardiac patients eligible and enrolled in this study underwent coronary bypass. Some of them also underwent other surgical procedure, such as: aortic prosthetic reconstruction, aneurysmectomy, mitral or tricuspid prosthetic reconstruction. The remaining patients underwent: aortic prosthetic reconstruction, mitral or mixed, subvalvular diaphragm resection, atrial myxomectomy, or surgical removal of an atrial septal defect. Surgeries were performed 1 to 28 days after admission (M = 7.40; SD = 5.37). On average, the patients required 15.45 days of postoperative hospital stay (SD = 0.01).

The patients completed two sets of standard questionnaires. The first set included five preoperative questionnaires (on average 5.11 days after admission; SD = 5.77), while the second set consisted in two questionnaires applied to the patients eight days after the surgery (SD = 3.15). Four of the questionnaires (scale N6 – stress vulnerability from NEO Personality Inventory – Revised Form/NEO PI-R, Life Orientation Test-Revised Form/LOT-R, Mental Health Index-5 Items Version/MHI-5, and Perceived Stress Scale/PSS) were taken from the literature, translated, and adapted to the purpose of this study. Three other questionnaires (socio-demographic data and medical history, scale for assessing surgery-related fear, and postoperative medical data) have been developed for this study. Stress vulnerability was measured according to the homonymous scale of NEO PI-R inventory (9). The instrument included eight items. The total score was obtained by summing the score of each item (range 0-32). A high score (range 0-24) was interpreted as high stress vulnerability. For our series of cardiac patients, the value of internal consistency was acceptable ($\alpha = 0.69$). The level of dispositional optimism was assessed with LOT-R (11, 12). The instrument has 10 items, of which 4 are distractors. A high score (range 0-24) signifies a high level of predisposition to conceptualized optimism. For our series patients, $\alpha = 0.65$. Surgery-related fear was assessed using an 8-item scale. The total score was obtained by summing the item scores (range 0-32). A high score was interpreted as a high level of concern regarding heart surgery. For our series of patients the value of internal consistency was acceptable ($\alpha = 0.67$). The negative affective disposition experienced by our patients in the last month was assessed with MHI-5 (13). The instrument is part of the Medical Outcomes Study 36-item Short Form Health Survey and it includes 5 items. A high score (range 0-25) indicates a high level of the negative affective disposition. For our series of patients the value of internal consistency was 0.73.

Postoperatively, the patients completed a short modified version (10 items) of PSS (14). In the original version, the instrument has been developed to evaluate the degree to which individuals believe their life has been unpredictable, uncontrollable, and overloaded during the previous month (15). The assessed items are general in nature rather than focusing on specific events or experiences. In this study a high score (range 0-40) was interpreted as an indicator of a high postoperative stress. The value of the internal consistency was good ($\alpha = 0.82$). More than 25% of the cardiac patients developed clinical complications due to unfavorable postoperative course. Two

patients (1 man and 1 woman) died. Only one of them survived long enough to participate in postoperative assessment. Another patient (who survived) refused it. Thus, PSS scores were obtained from 58 of the 60 patients.

RESULTS

Over 50% of the investigated patients had a moderate postoperative stress score (15-24), 4 patients a moderate to high (score 25-27), and other 5 – a high or very high score (28-36).

There was a positive correlation between the level of postoperative stress and the levels of the stress vulnerability (as personal predisposition), surgery-related fear, and preoperative negative affective disposition, and a negative correlation with the dispositional optimism score (tab.I). In the patients who developed complications due to unfavorable postoperative course, the correlation between postoperative stress level and stress vulnerability ($N = 17$; $r = 0.75$; $p = 0.001$; two-tailed) was stronger than in the patients who did not experience postoperative complications ($N = 41$; $r = 0.53$; $p < 0.001$; two-tailed), but the difference was not significant ($z_{r1-r2} = 1.22$; $p = 0.22$; two-tailed).

TABLE I

Correlations between the measured variables

Variables	M ± SD	Correlations with the level of postoperative stress
1. Postoperative stress	17.50 ± 6.11	-
2. Stress vulnerability	10.90 ± 3.93	0.57 ***
3. Dispositional optimism	13.70 ± 2.96	- 0.38 **
4. Surgery-related fear	13.10 ± 3.29	0.47 ***
5. Negative affective disposition (preoperative)	10.35 ± 4.33	0.45 ***
6. Age	58.50 ± 10.97	- 0.13
7. Preoperative hospital stay (number of days)	7.40 ± 5.37	- 0.06
8. Postoperative hospital stay (number of days)	15.45 ± 10.87	- 0.04

** $p < 0.01$; *** $p < 0.001$

The correlations between postoperative stress level and patients' age, duration of pre- and postoperative hospital stay were not statistically significant.

The difference in PSS scores between female and male patients were not statistically significant (women/18.52 ± 5.53; men/18.52 ± 5.53; $t = 0.96$; $p = 0.34$; two-tailed). Also, the complications due to unfavorable postoperative course had an insignificant influence on postoperative stress (patients with complications/19.11 ± **7**.13; patients without complications /16.82 ± 5.59; $t = 1.30$; $p = 0.19$; two-tailed). However, the patients who developed postoperative complications presented a slightly higher level of stress. The difference between the average score of patients on treatment for diagnosed conditions ($N = 46$; 17.52 ± 6.34), and patients not on treatment ($N = 11$; 17.72 ± 5.53) was not high enough to be significant ($t = - 0.09$; $p = 0.92$; two-tailed).

Stress vulnerability, dispositional optimism, surgery-related fear, and negative affective disposition were all taken into account as independent variables of linear regression model, where the dependent

variable (the criterion) was the level of postoperative stress. The coefficient of multiple correlation was statistically significant ($R = 0.66$; $F = 10.47$; $p < 0.001$; two-tailed). Together with the constant, the independent variables accounted for about 40% of the variation in postoperative stress score distribution, but only stress vulnerability and negative affective disposition were significant predictors (Table II). The score of stress vulnerability accounted for 13.1% of postoperative stress variation, while the negative affective disposition score 5.1%.

TABLE II

Multiple linear regression data

Independent variables	B	SE	β	r^2_{sp}
Stress vulnerability	0.67	0.19	0.42 **	0.131
Dispositional optimism	- 0.12	0.25	- 0.06	0.002
Surgery-related fear	0.21	0.26	0.11	0.007
Negative affective disposition	0.38	0.17	0.26 *	0.051

$p < 0.05$; ** $p < 0.01$ (two-tailed)

DISCUSSION

Scientists unanimously agree that surgery is a stressful factor for those directly involved (the patients), as well as for their family and friends (1, 3, 16, 17). Our correlational data of dispositional optimism and concerns about surgical outcome are all in agreement with those reported by other studies on the mental health and perception of the quality of life among cardiac patients undergoing heart surgery life (5, 7).

In this study the scores of stress vulnerability and negative affective disposition were positive predictors of the level of postoperative stress experienced by cardiac patients. As an aspect of neurosis, stress vulnerability is characteristic to people feeling unable to cope with difficulties, and who are prone to experience helplessness and being emotionally dependent of others (9). Such people are liable to exhibit dysfunctional ways of coping, and difficult recoveries after stressful experiences. On the other hand, the negative affective disposition (i. e. depressive symptoms) is rather common among patients awaiting heart surgery (17, 18, 19).

In a future study we want to continue the analysis of the factors that could explain the patient differences with respect to the psychological and medical indicators of recovery after heart surgery. This would be possible by including other variables, such as: predisposition for anxiety, postsurgical expectancies, perceived self-efficiency, coping abilities (and style) for stressful situations, trust in future and emotional and social support from family.

CONCLUSIONS

Even though the literature emphasizes the importance of preparing a patient for surgery to help them deal with problems resulting from hospital stay, surgery itself, convalescence, rehabilitation period, and increase their resilience to stress (20), many of the medical professionals do not regard this action as an essential part of a comprehensive and integrative medical act (16). In these circumstances it is not surprising that poor psychological status experienced pre-surgically by patients is significantly associated with difficulties of postoperative recovery, this fact emphasizing the importance of psychological factors in the dynamics of medical treatment.

Knowledge by the medical staff of the individual traits of cardiac patients and implementation of specialized pre-and postoperative counseling can help increase resilience to stress and thereby improve the indicators of medical rehabilitation.

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DEVELOPMENT AND OPTIMIZATION OF THE SYNTHESIS OF NEW THIAZOLIDIN-4-ONE DERIVATIVES OF IBUPROFEN

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DEVELOPMENT AND OPTIMIZATION OF THE SYNTHESIS OF NEW THIAZOLIDIN-4-ONE DERIVATIVES OF IBUPROFEN (Abstract): Ibuprofen, an important nonsteroidal anti-inflammatory agent, is one of the most prescribed drugs for the treatment of pain and inflammation from various rheumatic diseases, but some side effects can occur on long-term use. **Aim:** The method for synthesis optimization of new derivatives of Ibuprofen with thiazolidin-4-one moiety, with improved pharmacological and toxicological profile. **Material and methods:** To optimize the derivatization method of free carboxyl group of Ibuprofen (2-(4-isobutylphenyl)propionic acid) the reaction conditions were varied (reagent ratio, catalyst, reaction medium). **Results:** The most favorable method was proved to be the reaction between ibuprofen hydrazone and mercaptoacetic acid, in excess, at 80-85°C, for 6 h with 96% conversion rate. **Conclusions:** The synthesis of 2-phenyl-3-[2-(4-(isobutyl)phenyl)-2-methyl]acetamido-thiazolidin-4-one derivative was optimized in view of applying it as a general procedure for the synthesis of other derivatives with related structure. The chemical structure and molecular weight of the synthesized compound were confirmed by spectral methods (IR, ^{1}H NMR, ^{13}C NMR, HR-MS). **Keywords:** IBUPROFEN, THIAZOLIDIN-4-ONE, SYNTHESIS, OPTIMIZATION.

Development of new nonsteroidal anti-inflammatory drugs (NSAIDs) is the major concern of researchers in the field because of the impact of inflammatory and rheumatic diseases on patient health status. NSAIDs are some of the oldest classes of drugs, the humanity having being related to pain, fever and inflammation from its beginnings. From *Hippocrates* who used a willow bark extract to treat fever and inflammation to the discovery of salicin and then the synthesis of salicylic acid, the researches have evolved and at the end of 19th century, *Hoffman* synthesized the acetylsalicylic acid, which was introduced in therapy under the name Aspirin by Bayer Company (1).

Researches on the discovery of new active drugs, safer than classical therapeutic agents had continued and so Ibuprofen was patented as a new analgesic and anti-inflammatory drug in 1961 by *Dr. Stewart Adams* (Boots Company) and his collaborators. Even if Ibuprofen was proved to be an efficient therapeutic alternative to treat

inflammatory and rheumatic diseases, its long-term use was associated with gastrointestinal and renal side effects. Today, it is known that the toxicological potential of Ibuprofen is related to the lack of the cyclooxygenase (COX) selectivity, an enzyme responsible for prostaglandin synthesis. Ibuprofen is a nonselective COX inhibitor that inhibits COX-2 isoform at the site of inflammation but also COX-1 isoform responsible for the gastric cytoprotective prostaglandin synthesis (2).

To increase the analgesic and anti-inflammatory activity and reduce the side effects, the recent researches were focused on derivatization of carboxyl group of classical NSAIDs with various heterocyclic systems (oxazole, izoxazole, pyrazole, oxadiazole, thiazole, thiadiazole, triazole etc.) (2, 3).

On the other hand, thiazolidin-4-one derivatives are an important class of heterocyclic organic compounds because of their various biological properties: anti-inflammatory and analgesic activity (4), antimicrobial, antifungal (5) and antimycobacterial effects, antioxidant potential (6), antiviral and anti-HIV activity, anticonvulsant effect (7), hypoglycemic or antitumor activity (6).

In order to develop potential new therapeutic agents with anti-inflammatory properties, a general procedure for the synthesis of thiazolidin-4-one derivatives by structural modulation of carboxyl free group of ibuprofen has been optimized.

MATERIAL AND METHODS

Ibuprofen sodium salt, thionyl chloride, hydrazine hydrate, benzaldehyde, mercaptoacetic acid, organic solvents (*p.a.* quality) were purchased from Sigma Aldrich Company.

Fourier transform infrared (FTIR) spectra were recorded on a FT-IR Bomem MB-104 spectrometer (Canada), with a resolution of 4 cm^{-1} after 32 scans between 4000 and 600 cm^{-1}. NMR spectra (^{1}H-NMR, ^{13}C-NMR) were determined using NMR Bruker 400 MHz spectrometer (Germany), and molecular weight was determined by using Maxis Bruker high resolution mass spectrometer.

Chemistry

The synthesis of thiazolidin-4-one derivative of Ibuprofen (9) was realized through two methods (fig. 1).

Method A is based on "one step" reaction between Ibuprofen hydrazide (**5**), benzaldehyde (**6**) and mercaptoacetic acid (**7**) using different ratios between reagents, reaction medium, catalyst, time and reaction temperature.

Method B is a two-step reaction that firstly involves the condensation of hydrazide (**5**) and benzaldehyde (**6**) resulting Ibuprofen hydrazone (**8**), which in the next step is cyclized with mercaptoacetic acid (**7**) using various ratios between reagents, reaction medium, catalyst, time and reaction temperature.

Ibuprofen hydrazide was obtained from ibuprofen (2-(4-(isobutyl)phenyl)propionic acid) (**2**) that was formed by treating the sodium salt (**1**) with hydrochloric acid. After several steps that include the synthesis of acid chloride (**3**), its conversion to corresponding ethyl ester (**4**) and then the reaction with excess hydrazine hydrate, the Ibuprofen hydrazide (**5**) was obtained (8).

General procedure for synthesis of thiazolidin-4-one derivatives of Ibuprofen

Method A. In a corresponding flask were introduced 2-(4-isobutylphenyl) pro-

pionyl hydrazine (MW = 220,31 g/mol), benzaldehyde (MW = 106,12 g/mol, d = 1,0415 g/cm^3) and mercaptoacetic acid (MW = 92,12 g/mol, d = 1,32 g/cm^3). The reaction conditions were modified: reagents ratio (1.5:1:1.3; 1:2:3), reaction medium (anhydrous toluene, dichloromethane = DCM), reaction temperature (120°C, 22°C), time (24h, 45h), in the presence or absence of catalyst (N,N`-dicyclohexyl-carbodiimide, DCC). The reaction mixture was evaporated and the obtained residue was dissolved in ethyl acetate. The organic layer was washed with sodium bicarbonate, sodium chloride (saturated solutions) and water, after which it was dried under anhydrous $MgSO_4$, filtered and concentrated under reduced pressure.

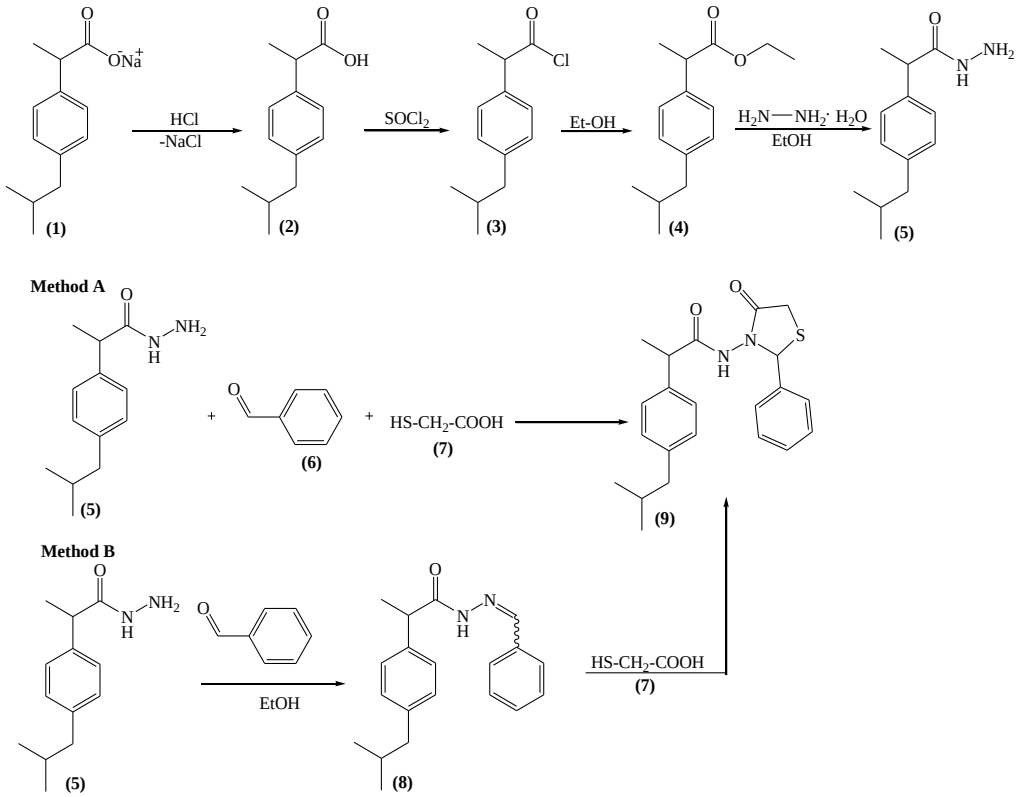

Fig.1. Scheme of the synthesis of Ibuprofen derivative with thiazolidin-4-one structure

Method B. N′-benzylidene-2-(4-isobutylphenyl)propionyl hydrazine (MW = 308.42 g/mol) and mercaptoacetic acid (MW = 92,12 g/mol, d = 1,32 g/cm^3) were introduced in a flask. The reaction conditions were modified: reagents ratio (1:1.5; 1:1.3; 1:1.5; 1:4.5), reaction medium (anhydrous toluene, anhydrous dioxane, acetic acid), reaction temperature (80-85°C, 105°C, 120°C), time (6h, 12h, 24h, 30h, 38h), catalyst (zinc chloride, sodium acetate). The reaction mixture was dissolved in

ethyl acetate and the organic layer was extracted and washed with sodium bicarbonate, sodium chloride (saturated solutions) and water. The organic layer was collected, dried under anhydrous $MgSO_4$ and filtered. After evaporation under reduced pressure, the residue was purified by flash chromatography on silica gel column (dichloromethane:methanol = 9.8:0.2) resulting a colorless residue that was precipitated with diethyl ether to afford a white powder (reaction condition - reagents ratio 1:4.5, reaction temperature 80-85°C, time reaction 6h).

Characterization of 2-phenyl-3-[2-(4-(isobutyl)phenyl)-2-methyl]acetamido-thiazoli-din-4-one derivative (**9**). Mp = 125°C, yield (56,77%), IR (cm^{-1}) 3243 (-NH-), 2959 (CH_{Ar}), 1716 (C=O cyclic), 1666 (-CO-NH-), 1203 (C-N), 694 (C-S).***^{1}H NMR*** (400 MHz, DMSO) – isomers mixture 60/40: δ 10.26 (d, *J* = 3.1 Hz, 1H, -***NH***-), 7.50 – 7.35 (m, 2H, ***Ar-H***), 7.34 – 7.21 (m, 3H, ***Ar-H***), 7.11 (d, *J* = 7.6 Hz, 1H, ***Ar-H***), 7.01 (dd, *J* = 14.8, 7.6 Hz, 3H, ***Ar-H***), 5.82 (5.67*, s, 1H, ***CH*** thiazolidin-4-one), 3.86 (t, *J* = 14.9 Hz, 1H de la ***CH_2*** thiazolidin-4-one), 3.72 (dd, *J* = 14.9, 9.0 Hz, 1H de la ***CH_2*** thiazolidin-4-one), 3.61 – 3.47 (m, 1H, -***CH***-CH_3), 2.39 (d, *J* = 6.7 Hz, 2H, -***CH_2***-CH-$(CH_3)_2$), 1.79 (dt, *J* = 13.4, 6.7 Hz, 1H, -CH_2-***CH***-$(CH_3)_2$), 1.25 (dd, *J* = 24.2, 7.0 Hz, 3H, -CH-***CH_3***), 0.85 (d, *J* = 6.7 Hz, 6H, -CH_2-CH-***$(CH_3)_2$***). ***^{13}C NMR*** (101 MHz, DMSO) – isomers mixture 60/40: δ 172.23 (d, *J* = 4.5 Hz, ***C_q***), 168.77 (d, *J* = 17.4 Hz, ***C_q***), 139.31 (d, *J* = 2.9 Hz, ***C_q***), 138.15 (d, *J* = 6.0 Hz, ***C_q***), 137.95 (d, *J* = 5.1 Hz, ***C_q***), 128.93 (d, *J* = 10.2 Hz, ***CH_{Ar}***), 128.69 (d, *J* = 6.0 Hz, ***CH_{Ar}***), 128.47 (d, *J* = 15.2 Hz, ***CH_{Ar}***), 127.80 (d, *J* = 3.4 Hz, ***CH_{Ar}***), 126.87 (d, *J* = 28.3 Hz, ***CH_{Ar}***), 61.43 (d, *J* = 16.1 Hz, ***CH*** thiazolidin-4-one), 44.22 (d, *J* = 2.8 Hz, -***CH_2***-CH-$(CH_3)_2$), 42.12 (d, *J* = 8.1 Hz, -***CH***-CH_3), 29.62 (d, *J* = 3.3 Hz, -CH_2-***CH***-$(CH_3)_2$), 29.27 (***CH_2*** thiazolidin-4-one), 22.13 (d, *J* = 3.3 Hz, -CH_2-CH-***$(CH_3)_2$***), 18.81 (17.90*, -CH-***CH_3***). ***HRMS (EI-MS)***: m/z calculated for $C_{22}H_{26}N_2O_2S$ was 383,178776 and $[M+H]^+$ found was 383,178716.

RESULTS AND DISCUSSION

The analysis of nuclear magnetic resonance spectra revealed that the application of method A for synthesis of 2-phenyl-3-[2-(4-(isobutyl)phenyl)-2-methyl]acetamido-thiazoli-din-4-one derivative was carried on conversion rate between 40% and 60%. The highest rate (60%) was obtained when anhydrous toluene was used as reaction medium at 120°C and during 24 hours (tab. I).

For method B it was observed that the reagent conversion rates in the final compound was between 34% and 96%, which varied according to the reaction conditions (reagents ratio, reaction medium, temperature, time, catalyst). The highest conversion rate was 96% when the mercaptoacetic acid was used in excess (1:4.5). At temperatures higher than 95°C (120°C) the synthesis of the final compound but also the presence of the mercaptoacetic dimmer that could not be removed by crystallization, precipitation, liquid-liquid extraction, flash chromatography on silica gel column were observed (tab. I).

The chemical structure of the thiazolidin-4-one derivative was confirmed by IR, NMR and HR-MS spectroscopy. In IR spectrum the characteristic absorption bands of the functional groups were identified. The characteristic absorption band of amidic group (CO-NH-) was observed at 1666 cm^{-1} and the cyclic C=O group (thia-

zolidin-4-one) appeared at 1716 cm^{-1}. The characteristic band of C-S bond was identified at 694 cm^{-1} and the NH group at 3243 cm^{-1}. The aromatic ring was observed by the characteristic absorption bands of C-H bond at 2959 cm^{-1}.

TABLE I
Optimization of synthesis of thiazolidin-4-one derivative

Method		Reagents (eq.)		Catalyst (eq.)	Solvent	t°C	Time (h)	C (%)
A	1.[9]	**5** (1.5 eq.)	**6** (1 eq.) **7** (1.3 eq.)	-	anh. toluene	120	24	60
	2.	**5** (1 eq.)	**6** (2 eq.) **7** (3 eq.)	-	anh. toluene	120	45	36
	3.	**5** (1 eq.)	**6** (2 eq.) **7** (3 eq.)	DCC (1.5 eq.)	DCM	22	24	40
B	4.[10]	**8** (1 eq.)	**7** (1.5 eq.)	$ZnCl_2$ (some crystals)	anh. dioxane	105	38	34
	5.[11]	**8** (1 eq.)	**7** (1.5 eq.)	sodium acetate (2.1 eq.)	acetic acid	120	30	48
	6.[12]	**8** (1 eq.)	**7** (3 eq.)	-	anh. toluene	120	24	46
	7.	**8** (1 eq.)	**7** (4.5 eq.)	-	-	105	12	87 (+**7**th dimer)
	8.[13]	**8** (1 eq.)	**7** (4.5 eq.)	-	-	80-85	6	96

C (%) = conversion percentage in the final compound

The aromatic ring protons were identified on ^{1}H-NMR spectrum as multiplet and doublet signals at 7.50-7.01 ppm, and the NH proton appeared at 10.26 ppm. The proton of CH from thiazolidin-4-one appeared as singlet at 5.82 ppm and the characteristic protons of the two aliphatic CH groups were identified as multiplet or doublet signals at 3.61-3.47 ppm or 1.79 ppm. The characteristic protons of CH_2 from thiazolidin-4-one were observed as triplet or doublet signals at 3.86 ppm and 3.72 ppm. The protons of aliphatic CH_2 were identified in ^{1}H-NMR spectrum as doublet signal at 2.39 ppm and the protons of the three CH_3 appeared as doublet of doublet or doublet at 1.25 ppm or 0.85 ppm.

In the ^{13}C-NMR spectrum the aromatic carbons were identified at 128.69-126.87 ppm, the carbon of the thiazolidin-4-one cyclic CH appeared at 61.43 ppm and for the aliphatic CH at 42.12 or 29.62 ppm. The CH_2 carbons were observed at 29.27 ppm for the thiazolidin-4-one cycle and at 44.22 ppm for the aliphatic moiety. The two methyl group (-CH_2-CH-***(CH$_3$)$_2$***) resonances were superimposed in one peak at 22.13 ppm and the third one (-CH-***CH$_3$***)

appeared as two peaks at different values 18.81 ppm and17.90 ppm because the E/Z isomerism.

High Resolution Mass Spectroscopy confirmed the molecular weight of the synthesized compound.

CONCLUSIONS

Two synthesis methods of new thiazolidin-4-one derivative of Ibuprofen were developed in order to be used as general procedure for synthesis of new ibuprofen derivatives. The most efficient conversion rate was obtained when the Ibuprofen hydrazone was reacted with mercaptoacetic acid, at 80-85°C for 6 h. The structure of the synthesized derivative of Ibuprofen was confirmed by Infrared spectroscopy (IR), Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR) and High Resolution Mass Spectroscopy (HR-MS).

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THE CHEMICAL COMPOSITION OF ESSENTIAL OILS ISOLATED FROM SWEET FENNEL FRUITS AVAILABLE AS HERBAL TEA PRODUCTS

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THE CHEMICAL COMPOSITION OF ESSENTIAL OILS ISOLATED FROM SWEET FENNEL FRUITS AVAILABLE AS HERBAL TEA PRODUCTS (Abstract): Sweet fennel fruits (*Foeniculum vulgare* subsp. *vulgare* var. dulce Mill.) are a spice with various uses in medicine, but also in food, pharmaceutic and cosmetic industries. Their biological activities as well as the flavoring properties are due to the essential oil. **Aim**: In this paper, the essential oils obtained from seven Romanian fennel herbal tea products were comparatively characterized in terms of their chemical composition. **Material and methods:** The volatile oils obtained by hydrodistillation, according to the Ph. Eur. 6th edition, were analyzed by GC and GC-MS. **Results**: The volatile oil content of fennel herbal tea products varies widely (28.10-101.00 mL/kg dried fruits), but all the samples meet the requirements of the Ph Eur. 6th edition (min. 20 mL/kg dried fruits). Phenylpropanoid derivatives (52.12%-57.66%) and monoterpenes (39.25%-45.28%) represent the bulk of the fennel volatile oils. Trans-anethole, estragole, limonene, fenchone, α-pinene, γ-terpinene and myrcene were the main compounds in all samples, but their levels vary greatly. **Conclusions**: The Romanian fennel herbal tea products showed important differences in terms of volatile oil content. The qualitative and quantitative differences in the chemical composition of fennel volatile oils were also observed. The most striking variations concern trans-anethole and estragole phenylpropanoid derivatives. Furthermore, these variations could have a significant impact on the flavouring and biological effects of fennel volatile oils. **Keywords:** *FOENICULUM VULGARE*, ESSENTIAL OILS, CHEMICAL COMPOSITION.

Sweet fennel (*Foeniculum vulgare* subsp. *vulgare* var. dulce Mill, Apiaceae), is a valuable medicinal and aromatic plant, native in the Mediterranean area. Fennel fruits and their volatile oil are largely used as a flavouring agent in food, pharmaceutical and cosmetic products. In traditional Romanian medicine, fennel fruits are recommended in the treatment of dyspeptic disorders and symptoms of the upper respiratory tract, but also to prevent flatulence and spasms in nursing infants. The pharmacological effects of fennel are largely atributed to their essential oil. Various experimental studies have reported multiple biological activities for fennel essential oil: hepatoprotective, antioxidant, antithrombotic, antiinflammatory, antidiabetic or antimicrobial (1, 2, 3, 4, 5). The chemical composition of volatile oils is decisive for

the expression of their biological activity, but, in turn, it is influenced by many endogenous and exogenous factors, such as: fruit ripening stage, agronomic practices, pedoclimatic conditions, geographic area or extraction method (6).

Considering those facts, in this paper, the essential oils obtained from seven Romanian fennel herbal tea products were comparatively characterized in terms of their chemical composition.

MATERIAL AND METHODS

Plant samples. Seven herbal tea products of fennel fruits were purchased from local pharmacies in May 2011. All samples were available in 50g packets, came from various inland producers, and they had been manufactured in the same year (2010). The codification of the samples was made as follows: Fares Orastie-FT1; Plafar Bucuresti-FT3; Trident Ilfov-FT4; Dacia Plant batch 11677/1-FT4; Dacia Plant batch 11677/8-FT5; Larix Sovata-FT6; Cyani Ploiesti-FT7.

Essential oils isolation. The essentials oils were obtained from dried fruits by hydrodistillation in the Clevenger apparatus (2 hours), according to the European Pharmacopoeia 6th edition (7). The volatile oils were separated, dried over anhydrous sodium sulfate and kept in a dark glass bottle at 4°C for the analysis.

GC-MS analysis. The GC-MS analysis of the volatile oils was carried out on an Agilent type 7890A gas cromatograph, equipped with an Agilent 5975C mass spectrometer and a DB-5MS capillary column (25 m x 0.25 mm internal diameter, 0.25 µm film thickness). The injection volume was 0.3 µL of pure essential oil. The oil samples were injected in split mode (split ratio: 100/1), using pressured controlled He as carrier gas with a flow rate of 1 mL/min. The injector and detector temperatures were maintained at 250°C. The analyses were performed using the following temperature program: the oven temperature was raised at a rate of 3°C min^{-1} from 40 to 250°C (isothermal for 4 min), then raised to 280°C at 10°C min^{-1} and the final temperature was held for 8 min. The identification of the components was based on the comparison of their mass spectra in the apex of each peak with those of analytical standards from Willey Mass Spectral Library.

RESULTS

The volatile oil content of fennel samples varies widely (28.10-101.00 mL/kg dried fruits) (fig.1). The FT6 (Larix), FT7 (Cyani) and FT1 (Fares) were the richest samples (101 mL/kg; 92.7 mL/kg and 71.1 mL/kg). All volatile oils comply with the requirements of the European Pharmacopoeia 6th edition (min. 20 mL/kg dried fruits).

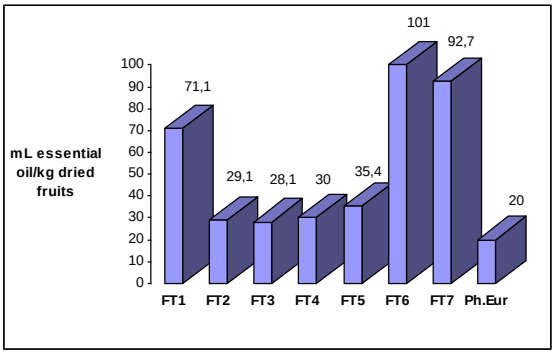

Fig.1. Essential oil content (mL/kg) of dried fennel fruits

The results of the GC-MS analysis are showed in table I.

TABLE I
Compounds identified in fennel essential oils

RT* (min)	Compound	Area (%)						
		FT1	FT2	FT3	FT4	FT5	FT6	FT7
5.47	α-thujene	0.07	0.07	0.06	0.06	0.05	0.08	0.11
5.63	**α-pinene**	**5.90**	**4.76**	**4.36**	**4.72**	**4.21**	**7.38**	**6.25**
5.89	camphene	1.02	0.27	0.26	0.24	0.21	1.08	1.22
6.22	sabinene	0.54	1.73	1.72	1.92	1.49	0.67	0.76
6.32	β-pinene	0.67	0.49	0.46	0.49	0.40	0.89	0.82
6.42	**myrcene**	**2.60**	**1.64**	**1.77**	**1.76**	**1.48**	**2.78**	**3.00**
6.74	α-felandrene	1.30	0.75	0.66	0.77	0.61	1.67	1.81
6.91	α-terpinene	0.07	0.06	0.04	0.05	0.03	0.07	0.08
7.03	o-cimene	1.27	-	-	-	-	-	-
7.11	**limonene**	**6.24**	**17.32**	**17.88**	**18.36**	**16.41**	**7.74**	**7.78**
7.32	t-β-ocimene	0.03	0.11	0.11	0.12	0.09	0.04	0.04
7.55	**γ-terpinene**	**2.22**	**1.68**	**1.52**	**1.74**	**1.65**	**2.90**	**3.52**
7.75	cis-sabinene hydrate	-	0.10	0.11	0.12	0.13	-	-
7.82	trans-sabinene hydrate	0.35	0.09	-	-	-	0.32	0.54
7.99	terpinolene	-	-	-	-	-	-	0.70
8.11	**fenchone**	**18.3**	**7.75**	**7.86**	**7.34**	**9.44**	**17.81**	**15.90**
8.40	1,3,8-p-menthatriene	-	0.02	-	0.02	-	-	-
8.54	fenchol	0.13	-	-	-	-	0.13	-
8.55	p-mentha-2,8-diene-1-ol	-	0.45	0.49	-	-	-	-
8.78	trans-limonene oxide	-	0.79	0.80	0.95	0.71	-	-
9.00	camphor	1.92	0.70	0.64	0.59	0.65	1.48	1.86
9.46	terpineol-4	0.20	0.32	0.23	0.23	0.17	0.14	0.14
9.72	**estragole**	**9.47**	**44.96**	**46.07**	**47.51**	**46.29**	**8.48**	**8.38**
10.00	trans-carveol	0.07	0.35	0.37	0.34	0.40	-	0.06
10.24	fenchil acetate	-	0.62	1.17	0.69	0.77	0.03	-
10.39	D-carvone	0.69	0.43	0.46	0.44	0.35	0.07	0.21
10.67	p-anisaldehyde	1.53	0.32	0.19	0.19	0.47	0.55	0.63
11.02	**(E)-anethole**	**44.08**	**12.12**	**9.43**	**7.15**	**10.11**	**42.68**	**42.24**
11.67	exo-2-hydroxycineole acetate	-	0.09	0.10	0.16	-	-	-
12.09	trans-isoeugenol	-	-	0.05	-	-	-	-
12.10	eugenol	-	0.05	-	0.05	0.06	-	-
12.45	methyl eugenol	0.06	-	-	-	-	0.25	0.10
12.25	α-copaene	-	0.15	0.22	0.20	0.16	-	-
12.38	β-cubebene	-	0.18	0.22	0.22	0.21	-	-
12.86	β-caryophyllene	-	0.09	0.11	0.11	0.10	-	0.02
13.13	trans-β-farnesene	-	0.09	0.13	0.11	0.10	-	-
13.19	1-m-anisyl-1-propanone	0.05	-	-	-	-	0.05	0.18
13.64	germacrene D	0.25	0.42	0.53	0.60	0.45	-	0.76
13.68	anisyl acetone	-	-	-	-	-	0.77	-
13.78	α-muurolene	-	-	0.05	0.11	-	-	-
13.79	α-amorphene	-	0.04	-	-	-	-	-

RT* (min)	Compound	Area (%)						
		FT1	FT2	FT3	FT4	FT5	FT6	FT7
13.88	β-bisabolene	-	0.05	0.07	-	-	-	0.06
14.05	myristicin	0.14	-	-	-	-	0.47	0.33
14.17	Δ-cadinene	-	0.17	0.23	0.23	0.20	-	-
14.28	cis-isoelemicine	-	-	-	-	-	-	0.02
14.29	α-longipinene	-	0.01	-	-	0.03	-	-
14.39	geranyl isobutyrate	-	-	-	-	-	-	0.01
14.73	3-methoxycinnamaldehyde	-	0.03	0.06	0.08	0.05	-	-
14.82	spathulenol	-	-	0.07	0.08	0.07	-	-
14.91	hexadecane	-	-	-	0.12	0.08	-	-
15.21	dillapiole	-	-	0.36	0.31	0.29	0.09	0.16
15.22	trans-isodillapiole	-	0.18	-	-	-	-	-
15.81	1-ethyl-dibenzotiophene	-	-	-	-	-	-	0.10
16.07	heptadecane	-	-	-	0.14	0.12	-	-
16.33	methyl myristate	-	-	-	-	0.04	-	-
17.60	6,10,14-trimethyl-2-pentadecanone	-	0.02	0.06	0.07	0.07	0.02	0.02
17.16	octadecane	-	-	-	0.11	0.07	-	-
18.20	nonadecane	-	-	-	0.01	0.02	-	-
18.45	methyl palmitate	-	-	0.02	0.02	0.12	-	0.04
20.15	6-octadecenoic acid methylester	-	0.02	0.09	0.07	0.55	0.06	0.20
20.38	16-methyl heptadecano-ic acid methylester	-	-	-	-	0.02	-	-
27.41	n-pentatriacontane	-	-	-	-	-	-	0.03
Monoterpene hydrocarbons		20.66	28.90	28.84	30.25	26.63	25.30	26.09
Oxygenated monoterpenes		21.66	11.69	12.23	10.86	12.62	19.98	18.72
Phenylpropanoid derivatives		56.60	57.66	56.16	55.29	57.27	53.34	52.12
Sesquiterpenes		0.25	1.20	1.63	1.66	1.32	-	0.86
Total		**99.17**	**99.49**	**99.03**	**98.60**	**98.93**	**98.70**	**98.08**

*RT, retention time

In the fennel volatile oils were identified a variable number of components, as follows: twenty-six in FT1, forty in FT2, thirty-nine in FT3, forty-two in FT4 and FT5, twenty-eight in FT6 and thirty-five in FT7, representing 99.17%, 99.49%, 99.03%, 98.60%, 98.93%, 98.70% and 98.08%, respectively, of the total oil (tab. I).The major constituents in all samples were: trans-anethole, estragole, fenchone, limonene, α-pinene, γ-terpinene and myrcene. Phenylpropanoid derivatives were the predominant compounds representing between 52.12% and 57.66% of the fennel volatile oils' composition (tab. I, fig. 2). FT2 (Plafar Bucuresti) and FT5 (Dacia Plant lot 2) were the richest samples in such components (57.66% and 57.27%, respectively). The monoterpenes (hydrocarbons and oxygenated derivatives) constitute the second most important structural group of the fennel volatile oils, with values between 39.25% and 45.28%. The highest levels of monoterpenes were found for samples FT6 (Larix) and FT7 (Cyani) (44.81% and 45.28%, respectively). Sesquiterpenes were absent (FT6) or poorly represented (below 2%).

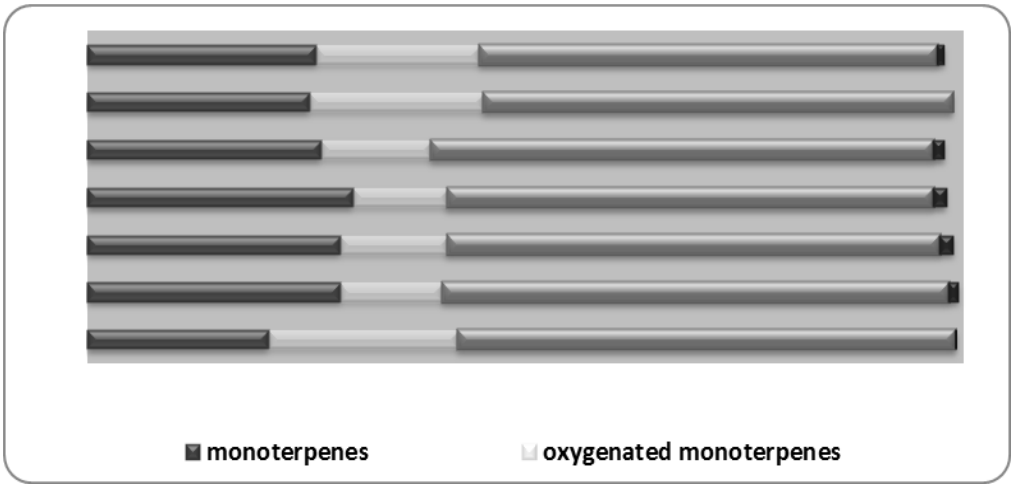

Fig. 2. The percent composition of fennel volatile oils on major component types

DISCUSSION

The content of volatile oil in fennel samples is good, but it varies largely. Thus, in FT6 (Larix), the volatile oil content was about 3.6 times higher than in FT3 (Trident). The variations concern not only the samples from diverse producers, but they were registered at different batches of the same producer (FT4 and FT5 samples), even if they are of lower magnitude (only 1.18 times). Regarding their chemical composition, fennel volatile oils are predominantly composed of phenylpropanoid derivatives (over 50%) and monoterpenes. The number and proportion of the components from each structural group also varies. The main phenylpropanoid compounds encountered in fennel volatile oils, like trans-anethole and estragole, were present in all samples, but there are significant differences regarding their proportion (even about 6 times). Trans-anethole was the major compound only in FT1 (Fares), FT6 (Larix) and FT7 (Cyani) fennel herbal tea products, while in FT2 (Plafar Bucuresti), FT3 (Trident), FT4 and FT5 (Dacia Plant), estragole predominated. It is interesting that the lowest level of oxygenated monoterpenes (10.86%-12.62%) was found in the same samples (FT2-FT5). These differences are even more important as estragole is suspected to be carcinogenic, and its intake should be restricted (8).

CONCLUSIONS

The fennel herbal products were characterized by a highly variable content of volatile oils. The richest samples were Larix-Sovata, Cyani-Ploiesti and Fares-Orastie, with 101 mL/kg, 92.7 mL/kg and 71.1 mL/kg respectively. Trans-anethole, estragole, fenchone, limonene, α-pinene, γ-terpinene and myrcene were the main components in all volatile oils, but they were present in varying proportions. The most striking differences concern the level of trans-anethole and estragole phenylpropanoid derivatives. Trans-anethole was the major compound in Fares (Orastie), Cyani (Ploiesti) and Larix (Sovata) while estragole prevailed in the other samples. Furthermore, these variations could have a significant impact on the flavoring and biological effects of fennel volatile oils.

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NEWS

CLOSTRIDIUM DIFFICILE INFECTION IN LUNG CANCER PATIENTS

Clostridium difficile infection (CDI) is a common nosocomial infection. Lung cancer patients have a high risk of developing CDI because of continuous antibiotic treatment or chemotherapy, prolonged hospitalization, and general weakness. This study aimed to analyze predisposing or associated risk factors for CDI in lung cancer patients receiving chemotherapy. This study was a retrospective review of 188 lung cancer patients who were admitted to the Wonkwang University Hospital between 2008 and 2009. Of the 188 patients, 44 were diagnosed with CDI. The albumin levels were significantly lower and the performance status (PS) score was significantly higher in lung cancer patients with CDI than in those without CDI ($P < 0.05$). In conclusion, clinicians should consider the possibility of CDI occurrence in lung cancer patients receiving chemotherapy, particularly in those with low albumin levels and high PS scores, because most lung cancer patients have a high risk of developing CDI. (Hwang KE, Hwang YR, Seol CH, et al. *Clostridium difficile* infection in lung cancer patients. *Jpn J Infect Dis* 2013; 66(5): 379-82)

Alina Manole

NEW DERIVATIVES OF ARYL-PROPIONIC ACID. SYNTHESIS AND BIOLOGICAL EVALUATION

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NEW DERIVATIVES OF ARYL-PROPIONIC ACID. SYNTHESIS AND BIOLOGICAL EVALUATION(Abstract): **Aim:** To design new derivatives of aryl-propionic acid with potential antibacterial and antioxidant activity. **Material and methods**: New hydrazone of ibuprofen (2-(4-isobutylphenyl)propionic acid) have been synthesized by reaction of ethyl ester of ibuprofen with hydrazine hydrate and then condensation of corresponding hydrazide with various aromatic aldehydes. **Results:** The synthesized compounds were screened for their antibacterial activity against Gram positive (*Staphylococcus aureus*ATCC 25923, *Sarcinalutea*ATCC 9341, *Bacillus cereus*ATCC 14579, *Bacillus subtilis*) and Gram negative bacterial strains (*Escherichia coli*ATCC 25922). Some of them were found to have good antibacterial activity. The antioxidant activity of these compounds was also tested using the total antioxidant capacity test. **Conclusions:** The chemical modulations performed on ibuprofen structure have a good influence on the biological activity of the synthesized compounds. **Keywords:** IBUPROFEN, HYDRAZIDE, HYDRAZONE, ANTIBACTERIAL, ANTIOXIDANT.

The aryl-propionic acid derivatives are a large and important family of nonsteroidal anti-inflammatory agents (NSAIDs) (1). Ibuprofen, chemically called 2-(4-isobutylphenyl) propionic acid, is a well-known drug which has analgesic (pain-relieving), antipyretic (fever-reducing) and anti-inflammatory properties (2, 3). It is particularly known for its use in pain relief from arthritis (4). Long-term use of NSAIDs has been associated with gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity (5). The gastrointestinal (GI) damage is generally attributed to two factors: local irritation by carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandin production, which reduces the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis (6). Studies described that the derivatization of the carboxylate function of representative NSAIDs resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect. Furthermore, certain compounds bearing 1,3,4-oxadizole/thiadiazole and 1,2,4-triazole nucleus have been reported to possess significant anti-inflammatory

activity (7-8). In the last two decades there has been a considerable amount of interest in the role of reactive oxygen species in inflammation. Inflammation is one of the manifestations of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress (9).

In our attempt to discover new and useful agents for treating inflammatory diseases we have replaced the carboxylic acid group with hydrazone group. The synthesized compounds were physicochemically and spectrally characterized and their antioxidant and antimicrobial potential assessed.

MATERIAL AND METHODS

Ibuprofen, hydrazine hydrate, aromatic aldehydes (benzaldehyde, 2-NO_2/3-NO_2/4-NO_2-benzaldehyde, 4-Cl/4-F/4-Br-benzaldehyde, 2-OH/4-OH-benzaldehyde, 2-OCH_3-benzal-dehyde, 4-dimethlaminobenzaldehyde, organic solvents (*p.a.* quality), standard reagents for antioxidant and antimicrobial tests, were purchased from Sigma Aldrich Company. All solvents and reagents were used without further purification.

Chemistry.*Synthesis of ibuprofen derivatives.*Ibuprofen (2-(4-isobutyl-phenyl) propionic acid) (1) was reacted with ethanol in acidic conditions and the resulting ethyl ester (2) was treated with hydrazine hydrate. The corresponding hydrazide (3) was condensed with various aromatic aldehydes, resultingnew hydrazone derivatives (4-14) of ibuprofen (fig. 1).

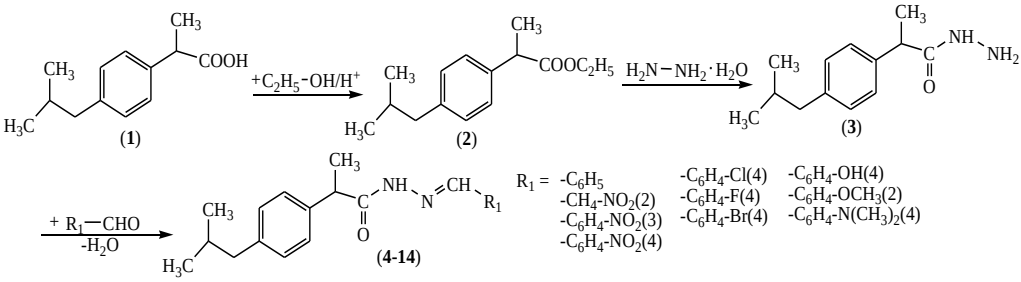

Fig. 1. Synthesis of new hydrazones of ibuprofen

Synthesis of ethyl ester of ibuprofen (2). Ibuprofen (0.08 mol, 16.5 g) was treated with ethanol (70 ml) in the presence of sulphuric acid (2 ml) as catalyst. The mixture was heated under reflux for 8 h, and then the solution was neutralized with sodium carbonate 100 g/L until pH 8. The product was extracted 3 times with ethyl ether, dried with sodium sulphate, filtered and evaporated under reduced pressure. The product was a yellow liquid, b.p. 260-262°C. IR (cm^{-1}): 1750 (CO), 3350 (C-OH).

Synthesis of ibuprofen hydrazide(3).The ethyl ester of ibuprofen (0.07 mol) a solution of hydrazine hydrate 98% (0.3 mol) and ethanol (60 ml) were added. The mixture was heated under reflux for 12 h, and then concentrated to ¼ of initial volume. The product was treated with cold water when the hydrazide of ibuprofen was separated under stirring as white solid, m.p. 70-73°C. IR (cm^{-1}): 1645 (CO-NH), 3420, 3280 (NH_2).

General procedure for synthesis of 1-(R-benzyliden-hydrazinyl)-2-(isobutylphenyl)-

propionamide(4-14).To a solution of ibuprofen hydrazide (0.012 mol, 2.83 g) (3) in ethanol (50 ml), aromatic aldehydes (0.012 mol) and glacial acetic acid (2-3 drops, pH 4-5) were added. The reaction mixture was heated under reflux for 5 h. After cooling the obtained solid was filtered off and recrystallized from ethanol.

Characterization by FTIR Spectroscopy. Fourier transform infrared (FTIR) spectra were recorded on a FT-IR Bomem MB-104 spectrometer (Canada). FTIR spectra were acquired after 32 scans between 4000 and 600 cm^{-1}, with spectral resolution of 4 cm^{-1}. Spectra processing was carried out with a Grams/32 program (Galactic Industry Corporation).

Antioxidant assays. *Determination of total antioxidant capacity*.The antioxidant activity of tested compounds was evaluated by the phosphomolybdenum method according to the procedure of (10) with minor modifications. Briefly, 300 µL sample solution (500 µg/ml) in DMSO was mixed with the reagent solution (2 ml, 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The samples were incubated at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank (DMSO mixed with reagent solution). Ascorbic acid (AA) was used as standard antioxidant in the same concentration of the samples.

Antimicrobial assessment. The antimicrobial activity was evaluated using Gram positive (*Staphylococcus aureus*ATCC 25923, *Sarcinalutea*ATCC 9341, *Bacillus cereus*ATCC 14579, *Bacillus subtilis*,), and Gram negative (*Escherichia coli*ATCC 25922) bacterial strains. An extended-spectrum beta-lactamase-producing strain, *Escherichia coli*CTX-M-14, was also used. Antimicrobial activity was evaluated by agar disc diffusion method according to described protocols (CLSI, 2012). Sterile stainless steel cylinders (50 mm internal diameter, 100 mm height) were applied on the agar surface in Petri plates. 200 µL of the tested compounds (4-14) (10 mg/ml) were added to each cylinder. Commercially available discs containing ampicillin (25 mg/disc), chloramphenicol (30 mg/disc) were used. The plates were incubated at 37°C for 24 h. After incubation the diameters (mm) of inhibition zones were measured.

RESULTS AND DISCUSSION

Chemistry. The physical measurements of the compounds are presented in tab. I and the characteristic IR spectroscopic bands are given in tab. II.

In the IR spectra of the hydrazone derivatives of ibuprofen (compounds 4-14) the characteristic absorption bands of all functional groups were observed. The absorption band for the C=O group was in the range of 1656-1673 cm^{-1} and for the C=N group in the range of 1528-1548 cm^{-1}. The characteristic band for the NH group appears in the range of 3144-3275 cm^{-1}, and due to the phenyl ring (C-H arom) the absorption band was observed at 2947-2965 cm^{-1}. The bands of the benzilydene ring substituents appeared in the range of 1520-1537 cm^{-1} (NO_2 group), 778 cm^{-1} (C-Cl), 1142 cm^{-1} (C-F), 706 cm^{-1} (C-Br), 3043-3053 cm^{-1} (Ar-OH), 3070 cm^{-1} (OCH_3) and 2838 cm^{-1} (CH_3). The presence of these bands confirmed the chemical structure of the synthesized compounds.

Antioxidant assays. *Determination of total antioxidant capacity*. The assay for total antioxidant capacity is based on the reduction of Mo^{+6} to Mo^{+5} by the tested com-

pounds followed by formation of a green phosphate/ Mo^{+5} complex at acidic pH. The results of the tested compounds are listed in tab. III. The test was carried out in triplicate. Data were statistically analyzed by the analysis of variance (ANOVA) ($p < 0.05$) and were expressed as means ± SD. The most active compound was derivative (12) obtained through condensation of hydrazide of ibuprofen with 4-hydroxibenzaldehyde. For this compound the absorbance was 0.3862± 0.0551, being four times more active than unsubstitutedbenzylidenhydrazone derivative (4) (0.1070±0.019). Compared to ascorbic acid (AA) all tested compounds were less active in similar conditions.

TABLE I

Physical characteristics of ibuprofen derivatives (4-14)

Sample	R	Yield (%)	Mol. weight	m.p.°C
4	-C6H5	76.17	308.43	125-130
5	-C6H4-NO2(2)	91.49	353.42	133
6	-C6H4-NO2(3)	85.02	353.42	155-158
7	-C6H4-NO2(4)	69.08	353.42	190
8	-C6H4-Cl(4)	91.06	342.87	160
9	-C6H4-F(4)	83.57	326.41	125-130
10	-C6H4-Br(4)	93.11	387.32	160
11	-C6H4-OH(2)	74.82	324.42	155
12	-C6H4-OH(4)	98.73	324.42	160
13	-C6H4-OCH3(2)	93.71	338.46	110
14	-C6H4-N(CH3)2(4)	61.82	351.49	130-135

TABLE II

Characteristic spectral bands of ibuprofen derivatives (4-14)

Sample	R	*IR characteristic band (cm^{-1})*				
		-NH-	C –H arom	C=O	C=N	C-R/ R
4	-C6H5	3275	2951	1673	1545	-
5	-C6H4-NO2(2)	3180	2954	1656	1538	1521 (NO_2)
6	-C6H4-NO2(3)	3198	2954	1662	1541	1537 (NO_2)
7	-C6H4-NO2(4)	3184	2952	1670	1532	1520 (NO_2)
8	-C6H4-Cl (4)	3203	2952	1659	1534	778 (C-Cl)
9	-C6H4-F (4)	3144	2953	1672	1548	1142 (C-F)
10	-C6H4-Br (4)	3201	2950	1668	1531	706 (C-Br)
11	-C6H4-OH (2)	3197	2948	1663	1542	3053 (Ar-OH)
12	-C6H4-OH (4)	3197	2965	1658	1534	3043 (Ar-OH)
13	-C6H4-OCH3 (2)	3194	2953	1656	1548	3070 (OCH_3)
14	-C6H4-N(CH3)2 (4)	3165	2947	1664	1528	2838 (CH_3)

TABLE III

Absorbance of tested compounds at concentration of 500 μg/ml

Sample	R	Absorbance	Sample	R	Absorbance
4	-C6H5	0.1070±0.019	**10**	-C6H4-Br (4)	0.1569±0.0389
5	-C6H4-NO2(2)	0.0377±0.0398	**11**	-C6H4-OH (2)	0.1408±0.03902
6	-C6H4-NO2(3)	0.0892±0.01864	**12**	-C6H4-OH (4)	0.3862±0.0551
7	-C6H4-NO2(4)	0.1278±0.0067	**13**	-C6H4-OCH3 (2)	0.1135±0.0129
AA	-	1.00 ± 0.4052			

Antimicrobial assessment. The inhibition zone diameter (mm) of the tested compounds is listed in tab. IV. According to the obtained results some compounds were active on Gram positive bacterial strains, especially *Staphylococcus aureus* ATCC 25923. The most active compounds were 6, 7 and 12, their inhibition zone diameter being 20 mm (6, 7) and 21 mm (12). The compound with broad antibacterial spectrum was compound 9 which was active on *Staphylococcus aureus* ATCC 25923 (15 mm), *Sarcinalutea* ATCC 9341 (12 mm) and *Bacillus cereus* ATCC 14579 (14 mm).

TABLE IV

Inhibition zone diameter (mm) of the tested compounds (4-14)

Sample	*S. aureus*	*S. lutea*	*B. cereus*	*B. subtilis*	*E. coli*[a]	*E. coli*[b]
4	0	12	10	8	0	0
5	0	0	9	0	0	0
6	20	0	0	0	0	0
7	20	0	0	0	0	0
8	17	0	9	0	0	0
9	15	12	14	8	0	0
10	12	0	0	0	0	0
11	14	0	0	0	0	0
12	21	0	0	0	0	0
13	14	0	0	0	0	0
14	14	0	0	0	0	0
DMSO	10	0	0	0	0	0
Ampicillin	19	33	0	20	20	0
Chloramphenicol	25	35	25	27	26	19

[a]*Escherichia coli* ATCC 25922, [b]*Escherichia coli* CTX-M-14

CONCLUSIONS

Some new hydrazones of ibuprofen have been synthesized and optimal conditions of reaction were established for obtaining compounds with high yields and advanced purity. The synthesized compounds have been physically characterized (melting point, yield, molecular formula,

molecular weight, solubility in different organic solvents), and their chemical structure was confirmed by FT-IR spectroscopy. Their antibacterial and antioxidant activities have been also evaluated. Some compounds have antimicrobial and antioxidant activity.

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THE POLYPHENOLIC CONTENT OF COMMON *LAMIACEAE* SPECIES AVAILABLE AS HERBAL TEA PRODUCTS IN ROMANIAN PHARMACIES

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THE POLYPHENOLIC CONTENT OF COMMON *LAMIACEAE* SPECIES AVAILABLE AS HERBAL TEA PRODUCTS IN ROMANIAN PHARMACIES (Abstract): The plants belonging to *Lamiaceae* family are widely used in medicine, cosmetic and food industry. Besides volatile oils, the main constituents of these plants are polyphenols. These compounds are secondary metabolites with multiple biological activities useful in the prevention and treatment of many disorders. **Aim**: To determine the flavonoids, hydroxycinnamic acids and total polyphenols content in several aromatic *Lamiaceae* plants available in Romanian pharmacies as herbal tea products*: Melissa officinalis, Lavandula officinalis, Rosmarinus officinalis, Mentha piperita, Thymus vulgaris* and *Salvia pratensis*. **Material and methods**: The quantitative determination of different types of polyphenols was performed in ethanolic extracts 70% and 50%, respectively, of each sample, using spectrophotometric methods. **Results and discussion**: The total polyphenols levels in the investigated products ranged between 3.10% and 6.51% (expressed as g GAE/100 g dry weight), the largest amount being identified in *Melissa officinalis* (6.51%). The samples of *Salvia pratensis* and *Mentha piperita* were the richest in flavonoid derivatives expressed as g rutin/100 g dry weight (1.83% and 1.53%, respectively), the optimal extraction being with 70% ethanol. The highest content in hydroxycinnamic acids expressed as g rosmarinic acid/100 g dry weight was found in *Melissa officinalis* sample (4.15%). **Conclusions**: There is a wide variability of polyphenolic content not only by species, but also depending on the polyphenols type or solvent used for extraction. The best extraction rate of total polyphenols in the investigated *Lamiaceae* species was obtained with 50% ethanol. **Keywords**: *LAMIACEAE*, POLYPHENOLS, QUANTITATIVE STUDY

Polyphenols are secondary plant metabolites characterized by high structural diversity and considerable potential biological action. Depending on the chemical structure there are ten main classes and among these, flavonoids and polyphenolic acids are the most abundant compounds. Antioxidant capacities represent one of the major biological properties of polyphenols, an intrinsic action as well as a possible mechanism to explain many other effects of these constituents (antiatherosclerotic, anti-inflammatory, and antitumor). As antioxidants, polyphenols protect cell constituents from oxidative damage, limiting the risk of oxidative stress-related diseases. Experi-

mental and epidemiological studies have highlighted the role of polyphenols in preventing cardiovascular disease, cancer, osteoporosis, diabetes and neurodegenerative diseases as well as aging (1).

The *Lamiaceae* family is one of the most important plant families comprising about 200 genera, of which 40% are aromatic plants (2). Although *Lamiaceae* species are known primarily for their volatile oil content, their therapeutic activities as well as other properties reflect the presence of other groups of chemical components, of which the most important are the polyphenols. Such compounds frequently encountered in *Lamiaceae* family are flavonoids and polyphenolic acids (mainly hydroxylcinnamic acids). Of the aromatic plants belonging to *Lamiaceae* family, the most popular and commonly used are: *Melissa officinalis* L. (lemon balm), *Lavandula officinalis* Chaix ex Vill. (lavender), *Thymus vulgaris* L. (thyme), *Mentha piperita* L. (peppermint), *Rosmarinus officinalis* L. (rosemary) and *Salvia pratensis* L. (meadow sage). These plants are mainly used for digestion stimulation, as spasmolytic, carminative and antimicrobial or antioxidant agents. The amount of polyphenols present in plants varies inter and intraspecifically, in the latter case depending on many factors, such as: area and harvesting time, origin (cultures or spontaneous flora), or processing methods including extraction type. Because polyphenols and volatile oils are bioactive markers for *Lamiaceae*, the content differences may lead to variations in therapeutic action (3).

The aim of this study was to determine the total amount of specific phenolic compounds (flavonoids, hydroxycinnamic acid derivatives, and total polyphenols) in alcoholic extracts obtained from several aromatic *Lamiaceae* plants available in Romanian pharmacies as herbal tea products. To estimate the true level of these compounds in plants, 50% and 70% ethanol was used as extraction solvent, given the fact that a better overall extractability for a wide spectrum of polyphenols can be obtained using hydro alcoholic solutions. This way, the quantitative variation of polyphenols was observed both in terms of species, but also depending on the solvent used for extraction.

MATERIAL AND METHODS

Chemicals and apparatus

All chemicals and reagents were analytical grade and were purchased from Sigma Aldrich Chemical Co. (Germany) and Merck (Germany). All spectrophotometric measurements were performed on ABLE-JASCO V 550 UV-VIS spectrophotometer.

Plant samples

Herbal tea products made from the leaves of *Melissa officinalis*, *Mentha piperita*, *Salvia pratensis*, *Rosmarinus officinalis*, flowers of *Lavandula officinalis* and aerial parts of *Thymus vulgaris* were purchased from local pharmacies. All samples were available in 50 g packets and come from the same manufacturer and same production year (2010).

Plant extraction

The plants (1 g) were ground into a fine powder in a mill and were extracted under reflux with 100 ml solvent, for 30 minutes. Then, extracts were filtered; after cooling, the filters were rinsed with the same solvent and the filtrates and rinsing were added in a volumetric flask and diluted ad 100 *ml*. For each sample, the extractions were performed using both ethanol 70% (v/v)

and ethanol 50% (v/v) as solvents. For the extraction of hydroxycinnamic acid derivatives the method described in European Pharmacopoeia 6th edition (*Melissae folium* monograph) was used (4).

Quantitative investigations

Before performing the quantitative determinations the polyphenolic spectrum was established. Thus, rutin and luteolin-7-glucoside are the major flavonoids and rosmarinic and caffeic acids are the predominant hydroxycinnamic acid derivatives in all samples.

- Determination of flavonoids

It was performed by a spectrophometric method based on the formation of yellow colored complexes with aluminium chloride, according to Romanian Pharmacopoeia 10th edition. Optical density was measured at a wavelength of 430 nm. Rutin was chosen as standard. The calibration curve was performed using rutin in the concentrations range of 4-16 μg/ml. The total flavonoid content was expressed as g rutin/100 g dry plant material (5).

- Determination of hydroxycinnamic acids

Total hydroxycinnamic acid derivatives were quantified in alcoholic extracts (ethanol 50%, v/v) using a European Pharmacopoeia 6th edition assay (*Melissae folium* monograph). The method is based on the formation of carmine red colored complexes in the presence of sodium nitrite and sodium molybdate, in alkaline medium. Optical density was measured at a wavelength of 505 nm. Rosmarinic acid was used as standard. The concentration range of rosmarinic acid for calibration curve was of 3-6 μg/ml. The amount of hydroxy-cinnamic acid derivatives was expressed as g rosmarinic acid/ 100 g dry plant material (4).

- Determination of total polyphenols

Total phenolic content of plant extracts was determined by Folin-Ciocalteau reagent (Singleton & Rossi method) using gallic acid as standard. Absorbance was measured at a wavelength of 765 nm. A standard curve was calculated using gallic acid concentrations in the range of 0.39-12.5 μg/ml. The results were expressed as gallic acid equivalents (GAE) (g gallic acid per 100 g dry plant material) (6).

All determinations were performed in triplicate and results are presented as means ± standard deviations.

RESULTS

Dependening on the extract type, flavonoids content varied between 0.35% and 1.83% for the 70% ethanolic extracts, and between 0.83% and 1.78% for the 50% ethanolic extracts (table I). The flavonoids were present in greater amounts in both extracts obtained from *Salvia pratensis* (1.83%; 1.78%) and *Mentha piperita* tea products (1.53%; 1.41%). Also, the level of hydroxycinnamic acid varied widely (0.43%-4.15%) between investigated species, the highest amount was found in *Melissa officinalis* (4.15%), followed by *Rosmarinus officinalis* (1.96%) (tab. I).

The total polyphenols amount ranged from 3.6 to 5.16 g GAE/100 g dry weight in 70% ethanolic extracts, while in 50% ethanolic extracts it varied between 3.10 to 6.51 g GAE/100 g dry weight (table II); 50% ethanolic extracts of *Melissa officinalis*, *Rosmarinus officinalis* and *Salvia pratensis* are polyphenol richest products (6.51%, 5.09% and 4.59%, respectively). Both ethanolic extracts of *Lavandula officinalis* had the lowest content of polyphenols (3.07%; 3.10%).

TABLE I

Flavonoids and hydroxycinnamic acid derivatives content (%) in *Lamiaceae* extracts

Sample	Total flavonoids (g rutin/ 100g dry weight)		Total hydroxycinnamic acids (g rosmarinic acid /100g dry weight)
	70% ethanol (v/v)	50% ethanol (v/v)	
Melissa officinalis	0.76±0.01	0.83±0.00	4.15±0.05
Lavandula officinalis	0.35±0.01	0.42±0.00	0.51±0.06
Mentha piperita	1.53±0.00	1.41±0.00	0.80±0.07
Thymus vulgaris	0.94±0.03	1.26±0.03	0.43±0.08
Salvia pratensis	1.83±0.00	1.78±0.01	0.63±0.09
Rosmarinus officinalis	0.97±0.00	1.02±0.00	1.96±0.17

TABLE II

The percentage amount of polyphenols in *Lamiaceae* extracts

Sample	Total polyphenols (g gallic acid/100 dry weight)	
	70% ethanol (v/v)	50% ethanol (v/v)
Melissa officinalis	5.16±0.01	6.51±0.01
Lavandula officinalis	3.07±0.01	3.10±0.01
Mentha piperita	4.11±0.06	4.32±0.07
Thymus vulgaris	3.67±0.03	4.56±0.04
Salvia pratensis	3.72±0.11	4.59±0.03
Rosmarinus officinalis	4.01±0.06	5.09±0.10

DISCUSSION

Polyphenols are metabolites of particular interest in determining antioxidant properties and other biological activities of *Lamiaceae* species. The polyphenols level found in the investigated species is relatively good, but modest compared to values reported in literature for *Mentha piperita* (6-12%) and *Lavandula officinalis* (5-10%) (1). Apart from *Mentha piperita* and *Salvia pratensis*, in the investigated species flavonoids are minor components of the polyphenolic spectrum, accounting for less than 30%. Also, hydroxycinnamic acid derivatives represent less than 20% of the polyphenolic distribution, except for *Melissa officinalis* (over 60%) and *Rosmarinus officinalis* (over 30%). For each plant, the use of 50% ethanol lead to better extractibility of total polyphenols, in most species the increase exceeding 20%. In the case of *Mentha piperita* and *Salvia pratensis* species, a larger amount of flavones was extracted using 70% ethanol, probably due to the presence of more pronounced lipophilic structures (methoxylated flavonoids). In the leaves of *Melissa officinalis*, European Pharmacopoeia requires a minimum content of 4.0% of hydroxycinnamic derivatives, expressed as rosmarinic acid, so that the investigated sample corresponds to pharmacopoeia

requirements (4).

CONCLUSION

The quantitative composition of polyphenols varies greatly between investigated *Lamiaceae* species. *Salvia pratensis* and *Mentha piperita* samples are the richest in flavonoid derivatives, the optimal extraction being with 70% ethanol. The highest content in hydroxycinnamic acids is found in 50% ethanolic extracts of *Melissa officinalis* and *Rosmarinus officinalis* plants. The *Lavandula officinalis* sample shows the lowest levels of flavonoids and total polyphenols, in both 70% and 50% ethanolic extracts. The solvent that provides a better extraction of total polyphenols in the investigated *Lamiaceae* species is 50% ethanol.

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NEWS

OLMESARTAN HEXETIL: A NEW ESTER PRODRUG OF OLMESARTAN

A new prodrug of olmesartan named olmesartan hexetil was synthesized. In vitro and in vivo studies were performed on olmesartan hexetil in comparison to olmesartan medoxomil, a known prodrug of olmesartan with long-lasting antihypertensive activity. The olmesartan hexetil exhibited higher effects than olmesartan medoxomil, probably due the hexetil moiety that increased lipophilicity. The new prodrug was rapidly hydrolyzed to olmesartan in rat liver microsomes and plasma. In vivo studies on male rats showed a good absorption from gastrointestinal tract. The pharmacokinetic parameters values were higher than those of olmesartan medoxomil. The results are intended to be a promise for the discovery of a new angiotensin receptor blockers effective in the treatment of hypertension (El-Gamal MI, Anbar HS, Chung HJ et al. Discovery of olmesartan hexetil: a new potential prodrug of olmesartan. Bioorg Med Chem Lett 2013; 23: 1347-1350).

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EFFICACY OF ANTIBIOTIC PROPHYLAXIS FOR PREVENTING INTRATHORACIC INFECTIONS AFTER THORACOSTOMY FOR TRAUMATIC HAEMO/PNEUMOTHORAX – EXPERIENCE OF ORADEA COUNTY EMERGENCY HOSPITAL

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EFFICACY OF ANTIBIOTIC PROPHYLAXIS FOR PREVENTING INTRATHORACIC INFECTIONS AFTER THORACOSTOMY FOR TRAUMATIC HAEMO/PNEUMOTHORAX –EXPERIENCE OF ORADEA COUNTY EMERGENCY HOSPITAL (Abstract): The **aim** of the paper is to observe the effectiveness of prophylactic administration of antibacterials against empyema and pneumonia after tube thoracostomy for traumatic collections. **Material and methods:** Observational retrospective study over a ten years period (2002-2011), at the Oradea County Emergency Hospital on 939 patients with chest tube drainage for traumatic haemo/pneumothoraces. The morbidity by intrathoracic infections was 5,5% in the curative antibiotic group. **Results:** The median number of risk factors for surgical infections and case severity were not statistically different (*p=0.9653 and p=0,6601*) between cases with antibioprophylaxis and curative treatment, but the incidence of intrathoracic infection in the prophylaxis group (n=86) was half (2,3%). Antibioprophylaxis was effective in over 95% of the cases and it associated in-hospital length of stay, length of stay in the ICU and costs of care significantly (*p<0.0001, p<0.0001, p=0.0046*) lesser than of those patients treated with curative regimen. The overall mortality was 8.6% within the curative regimen group with an attributable mortality to infections of 17.39%; but it was only 2.3% and respectively 0 within the prophylaxis group. **Conclusions:** Antibiotic prophylaxis for intrathoracic infections after tube thoracostomy for traumatic collections was justified by case severity and risk factors and was effective and cost-efficient, but it should be administered selectively. **Keywords**: ANTIBIOPROPHYLAXIS, TUBE THORACOSTOMY, TRAUMA, INTRATHORACIC INFECTIONS.

The literature oscillates between the opinion that antibioprophylaxis after tube thoracostomy for traumatic collections lowers the incidence of empyema and pneumonia(1) as opposed to non-administration of antibacterials, and the finding that administrated before, during or after tube thoracostomy the antibacterials do not change the outcome(2). Some authorities consider that the level of evidence is good enough to recommend prophylaxis for pneumonia, but not for empyema (3). More recent studies are encouraging the use of prophylaxis by some institutions; meanwhile others remain very selective with this indication (4, 5). The benefit from the antibacterial must be

significant, because of the risk of bacterial resistance generation and dissemination with excessive use of antibacterial drugs (6).

MATERIAL AND METHODS

The study is a retrospective observational review over ten years (2002-2011) conducted in the Emergency County Hospital Oradea, on all admitted trauma patients who underwent tube thoracostomy for haemo/pneumothorax, exclusively on those who needed urgent thoracostomy in the first 48 hours. We identified the risk factors for infection after a selected (7, 8, 9, 10, 11) list (COPD, TB, diabetes mellitus, heart diseases, liver diseases, renal diseases, neoplasms, alcohol consumption, obesity, age over 65 years, leucocytosis, polytrauma, multiple rib fractures or flail chest, more than one chest drain) and their association with antibacterial prescriptions, surgical procedures, intrathoracic infections (pneumonia/empyema) and with mortality. We retrospectively noticed the prescribing and non-prescribing of antibacterial drugs and pneumonia and/or empyema developing after 48 hour from tube thoracostomy. We considered as prophylactic regimen the prescribing of one antibacterial drug in one or two doses or at most for 24 hours, and as curative regimen, the prescribing of one antibacterial for more than 24 hours or the prescribing of more than one antibiotic drug. We compared the in-hospital length of stay, the length of stay in the ICU, the morbidity by intrathoracic infections and the mortality, respectively after the prophylactic administration, the curative administration and the non-administration of antibacterial drugs.

As the dedicated trauma severity scores (AIS, ISS) were used neither in prehospital nor in the hospital settings and as also the physical/physiological status scores (ASA, APACHE) were not consistently evaluated in all patients, we assessed the severity of cases as the presence of serious traumatic lesions in more than one anatomical region of the body (polytrauma).

The statistical analysis was performed with the MedCalc program, version 9.4.3.0 (MedCalc Software, Mariakerke, Belgium). The results were represented as the 'null' hypothesis presence probability (*p*), with the *p value* <0.05 proving the statistical significance of the difference between variables.

RESULTS

In our hospital, between 01.01.2002 and 31.12.2012 were admitted 939 patients with thoracic trauma who underwent closed chest tube thoracostomy with the intent of definitive treatment for haemo/pneumotoraces. Ninety-eight percent of the patients suffered a blunt chest trauma (n=922). Most of the 654 patients (70%) were prescribed antibacterial drugs in curative regimen; 86 of them (9%) were prescribed antibiotics in prophylactic regimen, and 199 (21%) received no antibacterial agents. But only 78 of 654 (12%, *p<0.0001 Chi-square test*) of those who received antibacterial agents in curative regimen had a diagnosis of infection or solid evidence of infection documented in their paper forms, such as the presence of pus in respiratory aspirates or pleural fluid, the identification of pathogen strains in the biologic specimens, or sensitivity test results.

The logistic regression analysis showed that two risk factors were statistically significant associated with the antibiotic prescription (n=740): leucocytosis [OR 4.3584 (CI 95%: 2.05-9.26) *p=0.0001*] and polytrauma [OR 1.9032 (CI 95%:1.22-2.96) *p=0.0045*].

Morbidity. We found 42 septic complications (21 pneumonias and 21 empyemas) in 40 patients (4.24%), with 2 patients associating both of the infections. Of all 40 infected cases, 34 (85%) were cured by treatment and 6 died (15%). In the non-antibiotic group of patients (n=199), 2 manifested empyema, and 2 of those who received antibiotic prophylaxis also suffered of intrathoracic infections after the pleural drainage. The rest of 36 of the infected patients (90%) belonged to the curative antibiotic group. The infectious morbidity with pneumonia/empyema in the curative group was 5.5%, in the prophylaxis group was 2.3% and in the non-antibiotic group was 1%. The patients with septic complications had longer mean hospital stay: 18 days (CI 95%:14.5-21.5) comparative to 9 days (CI 95%:8-9) (*p<0.0001 Mann-Whitney test*), and median costs of care higher than uninfected patients: 5184RON (CI 95%: 2303-8616) comparative to 2588 RON (CI 95%: 2445-2769). (*p<0.0001 Mann-Whitney test*)]. The retrospective analysis with logistic regression did not find the prophylactic or curative antibiotic prescribing to lower the risk for infection outcomes (pneumonia and empyema) (OR=0.4087; CI 95%: 0.09-1.72).

Mortality. There were 28 deaths (3%) in ten years. In the curative treatment group died the largest number (23 deaths) of patients (82%), 4 of those died with intrathoracic infections. The overall mortality in this group was 3.5%, and the specific mortality by intrathoracic infections was 17.39%. In the non-antibiotic group the overall mortality was 1.5% (3 deaths), with no deaths due to infections. In the prophylaxis group the overall mortality was 2.3% (2 deaths) and no specific infectious mortality. Among the patients with intrathoracic infections the mortality was 15%. Mortality was statistically significant more frequently associated with two risk factors: the polytrauma condition [OR=19.898; CI 95% (6.81-54) *p=0.0001*] and age over 65 years [OR=2.5080; CI 95% (1.06-5.88) *p=0.0045*].

Prophylaxis. Two patients in the prophylaxis group suffered intrathoracic infections and were converted to curative antibacterial regimen with successful recovery. Eighty-two of 86 of the patients with antibiotic prophylaxis (>95%) were discharged in good recovery. The median hospital length of stay of the prophylaxis patients was 7.5 days (CI 95% 7-8), the median length of stay in ICU was 1 day (CI 95% 1-1), and the cost of care was 2196 RON (CI 95%: 1879-2842) and all the three variables were significantly lesser than those for the curative group (*p<0.0001, p<0.0001 and p=0.0046* respectively *Mann-Whitney test*). Although the prophylaxis patients also had lesser lengths of stay in the ICU than the non-antibiotic patients, the median cost of care did not significantly differ between the prophylaxis and non-antibiotic groups (*p=0.1584 Mann-Whitney test*).

DISCUSSION

Tube thoracostomy is the definitive treatment for 85-90% of the chest trauma patients (3, 4, 15). Trauma lowers immunity, jeopardizes tissues making them vulnerable to infection and contaminates spaces that are normally sterile. Thoracostomy itself may care intrathoracic infection either by procedural contamination or by missing complete evacuation of the pleural collections that secondary become infected (1, 3). The incidence of pneumonia and bacterial empyema after closed tube thoracostomy is reported as

2-35% and carries mortality as high as 30% (12, 13). Forty percent of the traumatic haemo-thoraces, complicated by respiratory insufficiency, empyema or fibrothorax will need thoracostomy with the associated morbidity (14, 15).

The curative prescribing of the antebacterials is justified by infection or by penetrating contaminated or grossly dirty wounds (16, 17, 18). In our hospital the prescribing of antibacterials is strongly associated with leucocytosis (neutrocytosis) and with polytrauma. The risk of prescribing antibiotics was 4,3 times higher in the presence of leucocytosis and 1.9 times higher in the presence of polytrauma. But it is well known the fact that trauma is an activator of the systemic and local inflammatory response and that the activation and mobilization in great numbers of inflammatory white blood cells both in the circulatory torrent and organs (affected or not by lesions), in trauma setting does not imply infection or antibacterial fight (16,17,19), without pertinent evidence of the presence and multiplication of bacteria. In these cases, white blood cell count based antibacterial prescribing is not justified without other arguments (19). Should the antibiotic be mandatory, only the prophylactic regimen was justified (20). The polytrauma setting by severity may involve not only ventilatory but also haemodynamic assisting or control, circumstances that may elicit preventing infection, but also only in a prophylactic regimen (16, 19, 20).

The incidence of one intrathoracic infection at every 25 tube thoracostomies for trauma over ten years would intuitively demand 25 antibacterial prophylactic administrations in order to avoid one infection. But actually it would be justified to select patients for prophylaxis instead of routine prophylaxis. In fact, the morbidity by empyema and pneumonia was 1% in the non-antibiotic group but more than double (2.3%) in the prophylaxis group which means more than superfluous prophylaxis. That is why the actual study suggests selective prophylaxis.

CONCLUSIONS

Antibiotic prophylaxis for intrathoracic infections after tube toracostomy for traumatic haemo/pneumothoraces was justified in the presence of the risk factors and case severity and demonstrated efficacy and cost effectiveness.

Antibiotic prophylaxis in our hospital should be selective with this indication.

Unjustified antibacterial therapy in curative regimen must be abandoned because of the useless and long exposure of hospital pathogens to antibacterials, with emerging and dissemination of bacterial resistance. In trauma setting, the antibacterials are adjuvants for the treatment, while in pneumonia they are the basic treatment, and the loss of the effectiveness of the basic treatment of nosocomial pneumonia would become (again) an immense danger concerning the actual mortality of 30% or more.

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MAIN NEUROENDOCRINE FEATURES AND THERAPY IN PRIMARY SLEEP TROUBLES

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MAIN NEUROENDOCRINE FEATURES AND THERAPY IN PRIMARY SLEEP TROUBLES (Abstract): Insomnia is a sleep trouble in which the patient has difficulties in falling or in staying asleep. There are patients who fall asleep easily, but wake up too early; others have troubles in falling asleep and a third category has troubles with both falling and staying asleep. Independent of the type of insomnia, the final result is a poor-quality sleep, responsible for depressive or irritable mood, loss in concentration, learning and memory capacities. Sleep is essential to emotional and physical health. Inadequate sleep over a period of time is increasing the risks for obesity, diabetes, heart disease and depression. People suffering of chronic insomnia show an increased predisposition for psychiatric problems. People who had sleep troubles reported impaired ability to fulfill tasks involving memory, learning, logical reasoning and mathematical operations. New studies show that insomnia might be a result of the decrease of gamma-aminobutyric acid (GABA), a neurochemical responsible for the decrease of activity in many brain areas. Lower brain GABA levels were also found in people with major depressive disorder and anxiety disorders. Hypnotics, such as benzodiazepines are acting increasing the activity of the GABA neurons. Exposure to stress is associated with a greater risk for insomnia, with individual differences. Stress activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Increased activity of HPA axis is stimulating the secretion of corticotropin-releasing hormone, further inducing sleep disruption. Insomnia is also associated with depression and anxiety disorders, in which the HPA axis is characteristically overactive. People who show predisposition to sleep troubles have a hyperactive sympathetic nervous system, they are usually suffering from hyperarousal and they have a more intense response to stressful events. Primary sleep troubles (insomnia) has no apparent causes, is lasting more than one month, and is affecting approximately a quarter of the adult population. Secondary insomnia is associated with chronic heart and/or lung diseases, medication which interfere with onset or duration of sleep, constant change of the sleep habits, restless leg syndrome, etc. Besides lifestyle changes and cognitive-behavioral therapy, in the treatment of insomnia are used hypnotic medicines, advised to be prescribed on short-term cures of one or two weeks. Benzodiazepines are inducing and maintaining sleep. Longer use is responsible for severe side effects – dependency and withdrawal syndrome, daytime drowsiness and dizziness, low blood pressure, memory troubles and change in the melatonin secretion during night-time period. For these reasons were created non-benzodiazepines hypnotics – zolpidem, zaleplon, which are as effective as benzodiazepines, but have fewer side effects. Nevertheless the use of these hypnotics is also restricted to 7 – 10 days. Zopiclone (Imovane) another short-acting non-benzodiazepine hypnotic

has a different chemical structure, but a pharmacologic profile similar to that of the benzodiazepines; the treatment should be of maximum four weeks. Besides generally known concerns related to the use of hypnotics (residual sedative effects, memory impairment, rebound insomnia, abuse, dose escalation, dependency and withdrawal problems) it was signaled a risk of death associated with the use of current hypnotic medications. **Key words:** SLEEP TROUBLES, INSOMNIA, HYPNOTIC MEDICATION

DEFINITION, OCCURENCE, TYPES, SYMPTOMS AND RISKS OF PRIMARY SLEEP TROUBLES

Insomnia is a sleep trouble characterized by the difficulty in falling or in staying asleep. There are patients who fall asleep easily, but wake up too soon, others have troubles in falling asleep and finally, the third group has troubles with both falling and staying asleep. Indifferent of the type of insomnia, the result is a poor-quality sleep. This situation is responsible for an inactive mind along the day and the lack of refreshness after sleep. In this view, insomnia is the most common cause of depressive or irritable mood, loss in concentration, learning and remembering. Sleep is essential to physical and emotional health. Inadequate sleep over a period of time is associated with obesity, diabetes, heart disease and depression (Stores 2007, Schroeder 2005). Emotional and mental benefits of sleep are significant. Some people with chronic insomnia show an increased predisposition for psychiatric problems. People who had sleep troubles reported impaired ability to fulfill tasks involving memory, learning, logical reasoning and mathematical operations (Estivill 2003).

In contrast with the secondary insomnia, which is related with other diseases or troubles, being the most common type, the primary insomnia appears without any coexisting cause, it lasts at least one month and is found at approximately a quarter of the adult population. Secondary insomnia is found in eight people of ten with insomnia. Some of the major causes of secondary insomnia are – heart and/or lung chronic diseases, medicines which interfere with onset or duration of sleep, substances like caffeine, tobacco and alcohol, constant change in sleep routine or a poor sleep environment, restless legs syndrome. Main symptoms associated with insomnia are poor concentration, impaired motor coordination and memory troubles, depressive or irritable mood (Silber 2005, Sateia 2009, Panossian 2009).

NEUROENDOCRINE FEATURES OF THE PRIMARY SLEEP TROUBLES

Many adults suffering from insomnia complain of an inability to “shut down” their mind at night. New studies show that this is a result of the decrease of gamma-aminobutyric acid (GABA). People with primary insomnia with more than six months have 30 % less GABA, a neurochemical responsible for the decrease of activity in numerous brain areas. Lower brain GABA levels have also been found in people with major depressive disorder and anxiety disorders. Primary insomnia has a lot of features in common with these conditions, being as well a critical risk factor (Winkelman 2009, Riemann 2003, Roth 2007). In this view, the GABA deficiencies found in people with mood and anxiety

disorders, might be linked to sleep disturbances. Many hypnotic medications, most effective in treating insomnia, are benzodiazepine receptor antagonists (BzRAs) which increase the activity of the GABA neurons. Besides sleep, GABA is involved in cognitive, memory and psychomotor functions.

Exposure to stressful events is usually associated with a greater risk for insomnia, with individual differences. Stress factors activate the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (HPA). In insomniacs there is an increased activity of HPA axis, which by secretion of corticotropin-releasing hormone is further producing sleep disruption. Sleep loss is also activating HPA axis. Insomniacs have reduced melatonin levels at night, while corticotropin-releasing hormone was demonstrated to reduce nocturnal melatonin levels, in normal subjects (Passarella 2008).

These data suggest that HPA hyperactivity may be involved in neuroendocrine changes that lead to insomnia. Furthermore, insomnia is associated with depression and anxiety disorders, in these illnesses the HPA axis is characteristically hyper activated. People which show predisposition to sleep troubles have an overactive sympathetic system; they are more likely to suffer from hyper arousal and have a more intense response to stressful events (Benca 2005, Roth 2007).

THERAPY OF THE PRIMARY SLEEP TROUBLES

Treatment options for insomnia include lifestyle changes, cognitive-behavioral therapy and sleep or hypnotic medications. Lifestyle changes are focusing on good sleep habits: dark and quiet bedroom, with adequate temperature; avoidance of overintake of caffeine, alcohol, tobacco or other stimulants; avoidance of medicines that disrupt sleep; stable bedtime habits – going to bed at the same hour. Cognitive-behavioral therapy includes relaxation training and biofeedback at bedtime, to reduce anxiety; positive thinking and replacing worries related to falling asleep; talking to therapists; reduction of time spend in bed while awake (Smith 2002, Morin CM 2009). Usually, sleeping pills are prescribed only for one or two weeks, helping to establish a regular sleep schedule. Sleep medication can become habit-forming, thus it is considered that sleep medication works best as a short-term treatment, combined with lifestyle and behavior changes.

Benzodiazepines are used for short-term relief from insomnia. They induce sleep and they maintain sleep. Examples such as diazepam (Valium), lorazepam (Activan) or quazepam (Doral) have troubling side effects – dependency and withdrawal syndrome, daytime drowsiness and dizziness, low blood pressure, especially in older people and memory troubles (Cotroneo 2007). They affect the melatonin secretion, during the night-time period (Garfinkel 1999). Using such hypnotics may also worsen sleep apnea and other breathing disorders (Budur 2007, Schutte-Rodin 2008).

Zopiclone (Imovane) is a short-acting hypnotic. Zopiclone is a cyclopyrrolone derivative, with a structure unrelated to other hypnotics. However, the pharmacologic profile of zopiclone is similar to that of the benzodiazepines. In sleep laboratory, studies of one to 21 days duration in man, the hypnotic reduced sleep latency, increased the sleep-duration and reduced the

number of nocturnal awakenings. Zopiclone delayed the onset of REM sleep, but did not reduce consistently the total duration of REM periods. The course of treatment should be of maximum 4 weeks as duration. The most common adverse reaction of zopiclone is taste alteration (bitter taste). Drowsiness, dizziness, somnolence, asthenia, may be signs of intolerance or excessive doses (Dundar 2004). Non-benzodiazepines hypnotics, zolpidem and zaleplon are short-acting, being as effective as benzodiazepines. They have fewer side effects and lesser withdrawal symptoms. They are used as alternative therapy and to treat the dependency and withdrawal problems induced by benzodiazepines. However the use of these hypnotics is also restricted to 7 – 10 days. Some studies have signaled dependency and withdrawal symptoms with these medicines too. Expert opinion is strongly against long-term drug treatment because of possible residual sedative effects, memory impairment, falls, respiratory depression, rebound insomnia, abuse, dose escalation, dependency and withdrawal difficulties and an increased risk of death possibly associated with the use of current hypnotic medications (Wagner 2000, Barbera 2005, Morin A K 2007).

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NEWS

IMPULSIVITY COULD BE DECREASED BY HIGHER DOPAMINE LEVELS

Dopamine is a neurotransmitter that plays a key role in motivations, mood, learning, cognition, behavior and attention. Higher levels of dopamine in the frontal cortex of the brain decrease impulsivity, according to a study conducted by Kayser et al. Impulsivity characterizes subjects with frontal lobe damage and behavioral disorders and is a common risk factor associated with substance abuse. To test the hypothesis that lower dopamine in the frontal cortex contributes to impulsivity by impairing corticostriatal function, researchers performed a randomized, double-blind, placebo-controlled study in which they administered the brain penetrant catechol-O-methyltransferase inhibitor tolcapone or placebo to healthy subjects performing a delay discounting task. The results of the study showed that tolcapone significantly increased choice of delayed monetary rewards and also changed corticostriatal connectivity by inducing a decrease in the coherence between ventral putamen and pregenual cingulate cortex. In conclusion, the study states that raising cortical dopamine levels attenuates impulsive choice by changing corticostriatal function (Kayser AS, Allen DC, Navarro-Cebrian A et al. Dopamine, corticostriatal connectivity, and intertemporal choice. *J Neurosci* 2012; 32(27): 9402-9).

Teodora Vremera

DATE MICROBIOLOGICE ACTUALE PRIVIND INFECȚIA TRACTUSULUI RESPIRATOR INFERIOR ÎN FIBROZA CHISTICĂ PARTEA I: MICROORGANISME IZOLATE ŞI SEMNIFICAȚIA LOR CLINICĂ

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CURRENT MICROBIOLOGICAL DATA ON LOWER RESPIRATORY TRACT INFECTION IN CYSTIC FIBROSIS. PART I: MICROORGANISMS ISOLATED AND THEIR CLINICAL SIGNIFICANCE (Abstract): Cystic fibrosis (CF) is a disease that degrades the local defense mechanisms of the lower respiratory tract. As a result, patients develop recurrent infections that progressively deteriorates antiinfection defenses and represent the major cause of mortality. A hallmark of those infections is the diversity of microorganisms isolated from broncho-alveolar lavage or sputum. The clinical significance of isolates is different, the recognition of the role in altering of respiratory function being important in guiding antibiotic therapy. Current data support pathogenicity in CF of *S. aureus*, *P. aeruginosa* and *B. cepacia* complex, the latter two being considered "preterminal bacteria". *S. aureus* was the most common cause of mortality and morbidity in preantibiotic era. Today, life expectancy was improved with the introduction of antistaphylococcal antibiotics. Hypermutation of *P. aeruginosa* - the most common species isolated from patients with CF, is the essential factor in the development of multi-resistance to antibiotics. *As P. aeruginosa, B.cenocepacia* is a virulent species that cause infections with poor prognosis. Microorganisms with secondary role *(H. influenzae, S. maltophilia*, atypical Mycobacteria, *Aspergillus* spp) whose clinical significance has not been clarified yet, require further studies. **Key words**: CYSTIC FIBROSIS, RESPIRATORY TRACT INFECTION, MICROBIAL PATHOGENS, CLINICAL SIGNIFICANCE

Fibroza chistică (FC) este o afecțiune congenitală care degradează, încă din perioada de sugar, mecanismele de apărare locală ale tractusului respirator inferior (TRI): secreția de mucus capătă o consistență vâscoasă şi *clearance*-ul muco-ciliar devine nefuncțional. Ca o consecință, pacienții dezvoltă repetate infecții acute, care într-o etapă mai tardivă devin cronice cu exacerbări, ce deteriorează progresiv apărarea antiinfecțioasă şi constituie cauza majoră de mortalitate prin insuficiență cardiopulmonară. Progresele recente în domeniu au condus la prelungirea vârstei la care apare declinul ireversibil al funcției respiratorii.

O particularitate a acestor infecții este reprezentată de diversitatea microorganis-

melor izolate din lavajul bronho-pulmonar sau spută, în diverse asociații, care variază în raport cu vârsta pacienților. Semnificația clinică a izolatelor este diferită, recunoaşterea rolului jucat de anumite specii în alterarea funcției respiratorii fiind importantă în orientarea antibioterapiei. În plus, în infecția cronică apar modificări fenotipice la unele tulpini, ceea ce determină dificultăți în identificarea acestora la nivel de specie prin metode convenționale.

Pe baza unor sinteze recente din literatură (1, 2, 3) şi a numeroase studii publicate în ultimii ani, ne-am propus să detaliem mai jos aceste aspecte cu impact în ameliorarea diagnosticului microbiologic, al strategiei terapeutice şi măsurilor de prevenție.

Staphylococcus aureus este, din punct de vedere istoric, prima specie asociată cu infecția TRI la pacienții cu FC, care poate determina infecții acute şi cronice, responsabile de amplificarea răspunsului inflamator local. În perioada preantibiotică a reprezentat cea mai frecventă cauză de morbiditate şi mortalitate la copilul de vârstă mică, speranța de viață fiind ulterior ameliorată odată cu introducerea antibioticelor antistafilococice.

Colonizarea nazală cu *S.aureus* este un factor de risc pentru infecții ulterioare pulmonare cu acelaşi genotip (4). Sunt autori care susțin că profilaxia continuă cu antibiotice determină o reducere a numărului de spitalizări la copii sub vârsta de cinci ani, alții consideră că ar favoriza suprainfecția cu *Pseudomonas aeruginosa*, microorganism cu agresivitate mai importantă (5).

În ultimii ani a crescut progresiv frecvența infecțiilor determinate de tulpini meticilino-rezistente la toate vârstele, mai ales la adolescenți. Un studiu realizat în SUA a raportat o creştere a prevalenței de la 2,1% în 1996 la 17,2% în 2005 (6). Este posibilă transmiterea acestor tulpini între pacienți cu FC sau de la persoane fără această boală de fond; condițiile de spitalizare constituie un factor de risc. Date contradictorii apar în literatură privind consecințele pe termen lung ale infecțiilor cu tulpini meticilino-rezistente; infecțiile cu tulpini comunitare, recunoscut mai virulente, pot explica declinul mai rapid al funcției respiratorii (7, 8, 9).

De la pacienții tratați cu trimethoprim-sulfametoxazol sau aminoglicozide poate fi izolat un fenotip particular, *S.aureus* cu colonii mici, ce prezintă auxotrofism pentru hemină, menadionă sau timidină (10). Aceste tulpini se dezvoltă lent (după 48-72 ore) şi prezintă unele caractere întâlnite la stafilococii coagulazo-negativi: cultivă sub formă de colonii nehemolitice, nepigmentate, manito-negative, cu testul coagulazei pe lamă negativ (11); în aceste condiții există riscul de a nu fi depistate de microbiolog. Ele au fost izolate şi din alte infecții persistente şi recidivante ca osteomielite, artrite septice sau infecții tegumentare.

Pseudomonas aeruginosa este cea mai frecventă specie izolată din sputa pacienților cu FC, cu variații în raport de vârstă: aproximativ 30% la copii în primii 5 ani de viață şi peste 80% la adulți (4, 11, 12). La vârste mici este prezent intermitent, consecutiv tratamentului cu antibiotice sistemice şi locale. Infecțiile devin ulterior cronice, cu acutizări intermitente care la un moment dat apar refractare la tratament şi conduc la deces (13,14). Terapia precoce cu antibiotice, când infecția este pentru prima data detectată, are un efect benefic întrucât realizează eradicarea temporară a *P. aeruginosa* şi întârzie trecerea spre infecția cronică (15).

În stadiul cronic, sub efectul stresului oxidativ, sunt izolate tulpini modificate,

consecutiv unor mutații, care sintetizează în exces o matrice extracelulară din alginat ce le conferă avantaje adaptative la condițiile locale: aderență sporită la suprafața epiteliului respirator, rezistență la mecanismele de apărare (opsonizare, fagocitoză), acces redus al antibioticelor către celulele bacteriene ce poate explica în parte neconcordanța între sensibilitatea *in vitro* și răspunsul terapeutic (16, 17, 18, 19). Coloniile au un aspect mucos, lipsite de pigmentul caracteristic, prezența lor fiind recunoscută ca o cauză majoră a deteriorării pulmonare ("fenotip hipermutator"). Microbiologul va consemna prezența acestor colonii mucoide a căror izolare de la un pacient cu diagnostic neprecizat impune investigații pentru FC, indiferent de vârstă (13).

În plus, în infecțiile cronice, mai ales după tratamente cu tobramicină sau colistin în aerosoli, au fost izolate tulpini cu multiplicare lentă care dezvoltă colonii mici, dar relevanța clinică a acestora este neconcludentă (20). În cazul tulpinilor atipice, studii comparative au demonstrat că identificarea biochimică cu minigalerii API 20NE riscă să conducă la neconcordanțe semnificative în raport cu tehnicile moleculare (21, 22).

Hipermutația reprezintă factorul esențial în dezvoltarea multirezistenței la antibiotice (18, 23). În timp ce tratamentul prelungit cu agenți terapeutici anti-*Pseudomonas* este folosit pentru evitarea declinului rapid al funcției respiratorii, în paralel are loc selecția de mutanți rezistenți care sunt responsabili in final de eşec terapeutic. A fost dovedit că rata mutației genelor care controlează sinteza pompelor de eflux și producerea de beta-lactamaze la tulpinile "hipermutante" este mult mai mare decât la tulpinile sălbatice. În aceste condiții este recomandat ca în orice stadiu al bolii să se recurgă la asociații sinergice de antibiotice și nu la monoterapie.

În infecții cronice au fost descrise tulpini "epidemice" responsabile de *cross*-infecții, care necesită măsuri de control, cele mai eficiente fiind izolarea pacienților necolonizați de cei infectați (4, 24, 25).

***Burkholderia cepacia* complex** (BCC) este un grup taxonomic care cuprinde în prezent 17 specii identificate prin tehnici moleculare, diferențierea pe baza caracterelor fenotipice prezentând un risc crescut de eroare (22, 26). Sunt bacili gram-negativi cu habitat în mediul extern (sol și plante), unii fitopatogeni, rar prezenți în infecții la om. *B.cenocepacia* și *B.multivorans* sunt cel mai frecvent izolate de la pacienții cu FC, prevalența cea mai mare, de aproximativ 5%, fiind înregistrată la adulți cu vârsta de 18-25 ani (6, 17, 27). Utilizarea mediilor selective a crescut şansa de izolare a acestora (28).

În general, prognosticul acestor infecții este sever: infecții cronice pulmonare evoluează cu episoade de exacerbare mai ales în faza tardivă a bolii pe care o agravează (aproximativ un sfert dintre bolnavi mor în primul an de la debutul acestei infecții). Dintre speciile din BCC, *B.cenocepacia* este considerată cea mai virulentă, dar pacienții pot fi colonizați cu tulpini cu patogenitate diferită, ceea ce face dificilă o predicție a evoluție. Perioadele de acutizare nu s-au dovedit a fi determinate de achiziția unei alte tulpini decât cea inițială (17, 29).

BCC prezintă niveluri înalte de rezistență la majoritatea antibioticelor, după cure repetate putând fi selectate clone panrezistente (11). Studii de tipare moleculară au evidențiat transmiterea unor tulpini între pacienți spitalizați, dar în infecții cu *B. multivorans* sunt recunoscute surse în special din mediul natural. Măsurile de control

al acestor infecții depind de capabilitatea laboratorului de a izola şi identifica aceste bacterii.

La aproximativ 20% dintre cei infectați poate să apară o formă clinică severă numită "sindromul cepacia" care evoluează cu pneumonie gravă, bacteriemie şi deces (4, 11, 30). În Marea Britanie şi Canada a fost descrisă o clonă, ET12 (*electrophoresis type* 12), înalt patogenă şi transmisibilă, responsabilă de infecții rapid fatale.

Ca şi *P.aeruginosa*, specii BCC au potential de a produce în exces un polizaharid extracelular (diferit chimic de alginat) dar aceste tulpini mucoide sunt considerate mai puțin virulente. Conversia fenotipului mucoid în non-mucoid a fost indusă *in vitro* sub efectul ciprofloxacinei şi ceftazidimei dar nu cu meropenem (31).

De menționat o altă specie, *B.gladioli*, foarte asemănătoare fenotipic cu BCC, capabilă să determine mai ales colonizări tranzitorii (32). Medii selective folosite în izolarea BCC care conțin gentamicină inhibă dezvoltarea acestei specii.

***Stenotrophomonas maltophilia* şi alți bacili gram negativi nefermentativi** (*Achromobacter* spp, *Pandoraea* spp, *Ralstonia* spp, *Inquilinus limosus*). Sunt bacterii ubicvitare, dificil de identificat la nivel de specie prin teste biochimice (21). Au fost izolate în FC din probe de spută, precum şi în alte tipuri de infecții de predilecție la imunocompromişi (6, 4, 33, 34). Prezența în căile respiratorii poate fi inițial tranzitorie, dar *S.maltophilia* şi *A. xylosoxidans* au fost puse în evidență în faza avansată a bolii, când pacienții devin cronic colonizați (2). Mai mulți autori pun în discuție semnificația clinică a acestora (11, 17, 35, 36, 37); un studiu recent consideră că infecțiile cronice (nu şi colonizări intermitente) cu *S. maltophilia* reprezintă un factor de risc pentru exacerbările pulmonare (38).

Haemophilus influenzae a fost izolat cu o frecvență de aproximativ 20% la copii cu FC sub vârsta de 1 an, 32% între 2-5 ani iar la adulți sub 10% (6). Aceste date urmează dinamica colonizării la purtători, ceea ce impune criterii cantitative de diagnostic care să diferențieze infecția de contaminarea cu flora oro-faringiană. În ambele condiții sunt prezente tulpini necapsulate, neinfluențate de vaccinul anti-*H.influenzae*. Deşi este luat în discuție rolul real al acestei specii în FC, majoritatea clinicienilor tratează exacerbările acute cu antibiotice (11).

Streptococcus pneumoniae, un recunoscut patogen implicat în infecții ale TRI, este rar izolat de la pacienți cu FC (1, 35).

Micobacterii atipice. FC nu reprezintă un risc pentru infecția cu *Mycobacterium tuberculosis* dar în ultimii 20 ani a sporit numărul cazurilor colonizate sau cu infecții determinate de micobacterii atipice. Un studiu multicentric din SUA a evidențiat o prezență a acestora prin examene microscopice şi culturi repetate în medie la 13% (7-24%) din cazuri, asociate cu modificări radiologice (39). Au fost izolate de la toate grupele de vârstă dar prevalența este semnificativ mai joasă înainte de 15 ani. Pacienții sunt în special adulți cu funcție respiratorie bună, care prezintă concomitent infecții pulmonare cu *S.aureus* şi mai rar cu *P.aeruginosa*.

M.avium complex *şi M.abscessus* sunt cel mai frecvent izolate; *M.abscessus* este întâlnit la toate grupele de vârstă în timp ce *M.avium* complex mai ales la adulți (40, 41). Este posibil ca infecții cu *M.abscessus* să fi fost anterior descrise ca determinate de *M.fortuitum* complex sau *M.chelonae* din cauza dificultăților de identificare sau

schimbărilor taxonomice.

Micobacteriile atipice, prezente intermitent şi în număr mic în spută, sunt considerate de contaminare sau colonizante, fără indicaţii de tratament antibacterian.

Alte bacterii izolate de la pacienţi cu FC. Bacterii normal prezente în cavitatea bucală şi căile aeriene superioare pot fi puse în evidenţă în număr semnificativ în probe de spută şi lavaj bronho-alveolar. Dintre acestea, grupul *Streptococcus milleri* (*S. constellatus, S. intermedius, S. anginosus*), izolat pe mediu selectiv McKay, a fost asociat cu pusee de acutizări respiratorii. Administrarea de antibiotice, lipsite de activitate pentru patogenii recunoscuţi, a determinat ameliorarea manifestărilor clinice (42, 43).

În FC, tensiunea scăzută de oxigen în secreţiile căilor respiratorii poate oferi condiţii locale favorabile pentru dezvoltarea bacteriilor anaerobe: *Prevotella, Bacteroides, Veillonella, Propionibacterium, Peptostreptococcus, Actinomyces*. Prin determinări cantitative, realizate pe probe purulente de spută, au fost izolate > 10^5 UFC/ml bacterii anaerobe. Lichefierea acestor produse prin sonicare şi izolarea în număr important face improbabilă prezenţa din flora oro-faringiană; în plus, a fost demonstrată persistenţa acestora prin izolarea din probe repetate de la acelaşi pacient (44). În acest context, este luată în discuţie influenţa bacteriilor anaerobe în evoluţia clinică a bolii; ca şi în alte infecţii cronice mixte (sinuzite, abcese pulmonare), este posibil ca unele dintre acestea să interacţioneze cu speciile recunoscut patogene, contribuind la modularea virulenţei prin sinergism microbian.

Alte specii (*Serratia marcescens, Enterobacter cloacae, Klebsiella pneumoniae* şi alte enterobacteriacee, *Acinetobacter* spp, *Comamonas* spp, *Moraxella* spp, *Corynebacterium* spp etc) pot coloniza tranzitoriu TRI sau sunt considerate contaminanţi (35).

***Aspergillus* spp şi alţi fungi.** Diferite specii *Aspergillus* ubicvitare în natură, în special *A. fumigatus*, sunt izolate din sputa pacienţilor cu FC cu o frecvenţă care variază în limite largi. La majoritatea cazurilor prezenţa este tranzitorie, fără relevanţă clinică (11). Tratamentul intensiv cu antibiotice pentru infecţia cu *P. aeruginosa* favorizează această colonizare, care atunci când devine persistentă poate afecta funcţia respiratorie; administrarea de antifungice poate ameliora manifestările clinice (45). Rar sunt posibile infecţii sistemice. La copii peste vârsta de 4 ani şi la adulţi colonizaţi cronic este descrisă o entitate clinico-radiologică, aspergiloza bronho-pulmonară alergică, care beneficiază de tratament cu steroizi (46).

Scedosporium apiospermum, un alt fung filamentos saprofit cu largă distribuţie în natură a fost izolat din sputa pacienţilor cu FC; un studiu european a raportat o incidenţă de 8,6%. Importanţa sa în evoluţia bolii rămâne controversată dar a fost dovedită implicarea în infecţii invazive la cei cu transplant pulmonar (47, 48).

Specii *Candida*, în special *C. albicans*, izolate din produse provenite din TRI sunt în cele mai multe cazuri expresia unui dismicrobism consecutiv curelor repetate cu antibiotice (35, 49). Nu există dovezi certe care să susţină un rol semnificativ al speciilor *Candida* în FC.

Virusuri. La pacienţii cu FC pot evolua infecţii cu virusul respirator sincitial, virusurile gripale, paragripale, adenovirusuri şi mai recent metapneumovirus, fără ca aceştia să fie mai receptivi comparativ cu populaţia generală. Manifestările clinice sunt însă mai severe prin afectarea funcţiei de

clearance bacterian a aparatului mucociliar deja deteriorată, fiind admis un sinergism între flora bacteriană din TRI şi virusurile respiratorii (2, 4, 50). Virusul respirator sincițial a fost cel mai frecvent identificat, urmat de virusurile gripale A şi B. Această categorie de pacienți, indiferent de vârstă, au indicație de terapie şi profilaxie specifică antigripală.

CONCLUZII

Datele actuale din literatură susțin patogenitatea certă în FC a *S.aureus*, *P. aeruginosa* şi *B. cepacia*, ultimele două fiind considerate "bacterii preterminale". Microorganisme cu rol secundar, *H. influenzae*, *S.maltophilia*, *A. xylosoxidans*, micobacterii atipice, *A .fumigatus* şi virusurile respiratorii, a căror semnificație clinică nu a fost încă clarificată, necesită studii suplimentare.

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PPARs: FUNCȚII FIZIOLOGICE ŞI ROLURI FARMACOLOGICE ALE AGONIŞTILOR ÎN BOLILE UMANE (Nota II)

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PPARs: PHYSIOLOGICAL FUNCTIONS AND PHARMACOLOGICAL ROLES OF AGONISTS IN HUMAN DISEASES. Note II (Abstract) Increasing attention paid to the main family of peroxisome proliferator activated receptors - PPARs is generated, on one hand by the multiple functions of its members in numerous metabolically active tissues, and on the other hand by the therapeutic benefits expresed by some specific ligands that are used in certain metabolic diseases treatment plan. PPARα stimulates the β-oxidative degradation of fatty acids and controls plasma lipid transport through the mediated action upon the triglycerides and fatty acids metabolism and by modulation of biosynthesis and catabolism of bile acids in the liver. PPARγ promotes adipocytes differentiation and fat storage. PPARβ/δ is involved in control and management of adipogenesis. While PPARα mediates the hypolipemiant actions of fibrates, PPARγ is the receptor for thiazolidinediones (glitazones) reccomended in type 2 diabetes treatment; by binding to PPARγ, glitazones modulates transcription of genes involved in lipid and carbohydrate metabolism. **Key words:** PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS, AGONISTS, FIBRATES, THIAZOLIDINEDIONE, DIABETES MELLITUS, LIPIDS METABOLISM.

PPARs (peroxisome proliferators-activated receptors) reprezentați de un trio de receptori nucleari (PPAR-α, -γ, -β/δ) acționează ca factori de transcripție, activați de ligand. Fiecare din aceste izotipuri formează obligatoriu heterodimeri cu partenerul specific, receptorul acid 9-cis-retinoic (RXR). Heterodimerii PPARs: RXR (factori de transcripție funcționali) reglează exprimarea genelor țintă prin legarea la o secvență din ADN cunoscută ca PPRE (peroxisome preliferator response elements) amplasată în domeniile reglatoare ale genelor. După legarea agoniştilor, conformația PPARs se modifică astfel încât eliberează corepresorii permițând recrutarea cu afinitate mare a coactivatorilor care remodelează cromatina şi comunică astfel cu maşinăria transcripțională celulară ce va induce inițierea transcripției (1).

PPARs modulează transcripția genei prin două mecanisme: **a**. transactivare ADN-dependentă care implică legarea la PPRE a genelor țintă şi heterodimerizarea cu RXR şi **b**. mecanismul transrepresiei, o cale ADN-independentă, realizată prin interferența cu alți factori de transcripție ce pot explica, spre exemplu, acțiunea antiin-

flamatoare a PPAR-γ. În ultima decadă s-a acordat o deosebită atenţie acestor factori datorită implicării lor în reglarea diferitor gene cu funcţii fiziologice variate şi efecte farmacologice distincte ale agoniştilor (2).

PPAR-α, clonat în 1990, identificat şi la om, se exprimă preponderent în ţesuturi active metabolic, care cer energie, precum ficat, muşchi scheletic, intestin, cortex renal (3) şi celule cu leziuni aterosclerotice (celule endoteliale, celulele muşchiului neted, monocite/ macrofage)(1).

Mecanism de acţiune. PPAR-α activat după legarea ligandului sau după fosforilare, induce modificări conformaţionale ale receptorului care permit recrutarea de activatori şi facilitarea transcripţiei genei ţintă. Descoperirea liganzilor specifici PPARs şi identificarea multiplelor nivele de control a acestor factori a permis o mai bună înţelegere a mecanismului molecular de activare ligand-dependentă şi a rolului lor de ţinte moleculare pentru droguri utile în tratamentul bolilor metabolice umane.

Liganzii PPARα, sunt compuşi naturali sau sintetici. Liganzii naturali sunt reprezentaţi de: **a**. acizi graşi (AG) – liganzi primari care activează genele implicate în consumul, transportul intracelular, catabolismul oxidativ al AG şi **b**. eicosanoizi derivaţi din acid arahidonic pe calea lipoxigenazei sau cicloxigenazei (4). Liganzii sintetici sunt activatori farmacologici folosiţi în tratamentul bolilor metabolice precum dislipidemia (5). Legarea agonistului induce o conformaţie activată a receptorului care determină disocierea proteinelor corepresor şi permite recrutarea diferenţiată a coactivatorilor (6) care vor declanşa un răspuns biologic specific.

Agoniştii PPARα precum derivaţii acidului fibric (fibraţii): clofibrat, gemfibrozil, fenofibrat, bezafibrat, ciprofibrat sunt folosiţi în virtutea beneficiului lor terapeutic, pentru tratamentul hiperlipidemiei.

PPAR-α şi metabolismul AG, triglicеridelor (TG) şi a fracţiei LDL (low density lipoprotein). PPARα este recunoscut ca ţintă primară a clasei de droguri hipolipidemiante – a fibraţilor.

Cea mai bine caracterizată acţiune a agoniştilor PPARα priveşte metabolismul lipidelor şi lipoproteinelor la nivel hepatic şi extrahepatic. Aproape fiecare faţetă a metabolismului acizilor graşi este sub controlul transcripţional al PPARα (transport, sinteză, depozitare, mobilizare, activare, β-oxidare) (6) acţionând ca un senzor molecular al AG endogeni şi al derivaţilor lor (3), precum şi a proteinelor esenţiale implicate în metabolismul lor (7). Efectele benefice ale fibraţilor se datorează în parte creşterii exprimării genelor implicate în β-oxidarea AG, catabolismului crescut al particulelor bogate în TG şi producerii reduse de VLDL (very low density lipoprotein) (8) care, indirect scad sdLDL (small dense LDL), cresc formarea particulelor HDL şi dirijează excesul de colesterol spre excreţie hepatică în bilă.

PPARα activat de fibraţi creşte activitatea LPL (lipoprotein lipazei) responsabilă de activitatea lipolitică în ficat, muşchi (9) şi macrofage (10). Acest efect promovează lipoliza şi catabolismul lipoproteinelor bogate în TG (VLDL şi LDL), diminuându-le nivelul plasmatic.

În ficat, PPARα reglează exprimarea enzimelor căii de producere a corpilor cetonici şi ai gluconeogenezei (11).

Lipsa alimentaţiei este compensată prin lipoliză din ţesutul adipos care eliberează acizi graşi disponibili pentru oxidare în ţesuturile periferice şi conversia la corpi cetonici în ficat (11) a căror sinteză este stimulată la diabetici de PPARα.

PPARα reglează metabolismul glicerolului hepatic prin promovarea gluconeogenezei din glicerolul produs în ţesutul adipos în cursul lipolizei şi importat în ficat (12).

Fibraţii, activatori ai PPARα, reduc exprimarea apolipoproteinei (apo) C-III care inhibă atât activitatea LPL de a hidroliza TG cât şi consumul de lipoproteine remanente bogate în TG.

În hepatocitele umane, exprimarea apo A-V activator al lipolizei este reglată pozitiv de agoniştii PPARα. Administrarea agoniştilor PPARα cresc circulaţia apolipoproteinei A-V din ser şi rapotul apo A-V/apo C-III (13).

PPAR-α şi fracţia HDL.

- Fibraţii cresc transcripţia apolipoproteinelor majore ale HDL, apo AI şi apo AII (14) şi în consecinţă creşterea concentraţiei HDL. La persoanele tratate cu fibraţi scade incidenţa evenimentelor cardiovasculare, iar la pacienţii cu diabet tip 2 caracterizaţi prin hiper-trigliceridemie moderată şi concentraţie mică de colesterol HDL, fibraţii scad incidenţa infarctului de miocard.
- Activatorii PPARα cresc transportul invers de colesterol (RTC-reverse cholesterol transport) prin accelerarea efluxului de colesterol din celulele periferice şi creşterea consumului lui de către ficat de unde colesterolul este eliminat prin bilă, fie direct, fie după metabolizare la acizi biliari. PPARα influenţează calea RTC prin reglarea efluxului de colesterol din macrofage (3).

Efectele pleiotrope ale fibraţilor. În plus de principalele efecte benefice în homeostazia lipidică induse de fibraţi, diferite studii efectuate *in vitro* şi *in vivo* au demonstrat numeroase efecte pleiotrope suplimentare. PPARα, activaţi de fibraţi, se exprimă în sistemul cardiovascular, în celulele endoteliale, celulele muşchiului neted vascular, monocite, macrofage (1).

- Principala acţiune a PPARα la nivelul cordului este aprovizionarea cu energie prin activarea genelor care reglează consumul şi oxidarea mitocondrială a AG (15).
- Fenofibratul, (denumit iniţial procetofen), asigură protecţia miocardului, prevenind instalarea şi evoluţia hipertensiunii, hipertrofia cardiacă, insuficienţa cardiacă, miocardita, disfuncţia vasculară endotelială (16, 17).
- Fenofibratul, ca agonist al PPARα îmbunătăţeşte funcţia endotelială prin exprimarea crescută şi eliberarea de NO (18).
- PPARα reglează exprimarea proteinelor cheie implicate în toate stadiile aterogenezei incluzând inflamaţia vasculară, instabilitatea plăcii şi tromboza (19). Activarea PPARα poate avea efecte antiaterogene directe asupra diferitor tipuri de celule din peretele vascular, de ex. scăderea exprimării moleculelor de adeziune, a factorului tisular, Il-6 (interleukinei-6), endotelinei-1.
- Interacţiunea cu diferiţi factori de transcripţie (VCAM-vascular cell adhesion molecule, ICAM-intercellular adhesion molecule, PECAM-platelet endothelial cell adhesion molecule, molecule de adeziune plachetare şi endoteliale, CRP-C-reactive protein ş.a.) PPARα inhibă gene proinflamatoare (16). De asemeni, activarea PPARα scade inflamaţia celulară prin inhibarea căilor de semnalizare precum AP-1 (activator protein-1), factorul nuclear kappa B, care împreună scad stresul oxidativ vascular şi sistemic.

PPARγ factor de transcripţie ce acţionează ca receptor pentru tiazolidindione (TZD)(glitazone) creşte transcripţia genelor sensibile la insulină (20), şi este cel mai bine caracterizat, în parte datorită potenţia-

lului terapeutic pentru tratamentul diabetului (21) şi al consecinţelor legate de sindromul metabolic (22). După descoperire, glitazonele au devenit foarte populare şi frecvent prescrise datorită sensibilităţii crescute la insulină fără să determine hipoglicemie şi implicării şi în alte condiţii patologice umane (23).

Mecanism de acţiune. Glitazonele ca agonişti selectivi ai PPARγ, acţionează prin legarea la PPARγ. Când este activat de ligand, PPARγ formează heterodimerul PPARγ:RXR care se leagă la ADN şi reglează transcripţia genetică şi translaţia unei mari varietăţi de proteine implicate în diferenţierea celulară, metabolismul glucidic şi lipidic.

Liganzii PPARγ. PPARγ leagă şi este activat de compuşi (liganzi) care apar în mod natural precum 15-deoxi-Δ12,14 – prostaglandin J2 (15-PGJ2), diferite eicosanoide şi compuşi sintetici precum TZD (24). Faptul că liganzii se deosebesc prin capacittea lor de a interacţiona cu coactivatori explică diferitele răspunsuri biologice descrise.

Primii agonişti sintetici identificaţi pentru PPARγ au fost TZD care ameliorează rezistenţa la insulină (diabet zaharat non-insulino-dependent, NIDDM). TZD utilizate în tratamentul diabetului zaharat de tip 2 includ: ciglitazone, pioglitazone (Actos), troglitazone (Rezulin), rosiglitazone (Avandia), rivoglitazone, balaglitazone compuşi cu profil de securitate diferit, astfel că cele mai utilizate clinic sunt pioglitazone şi rosiglitazone.

Efectele PPARγ-dependente ale TZD. Funcţiile celulare ale PPARγ se exprimă abundent în ţesutul adipos şi celulele epiteliale ale colonului şi multe alte ţesuturi precum muşchi scheletic, ficat, inimă, intestin, monocite/macrofage, pneumocite, miocite (1).

Defectul de început în dezvoltarea diabetului de tip 2 este rezistenţa la insulină-factor de risc major, caracterizat prin scăderea transportului glucozei şi a metabolismului ei în muşchiul scheletic (25).

Prin activarea PPARγ, TZD cresc exprimarea proteinelor implicate atât în metabolismul glucidic, cât şi lipidic.

▪ TZD contribuie la scăderea rezistenţei la insulină, atenuarea hiperinsulinismului, îmbunătăţirea sensibilităţii la insulină dovedită în ţesutul adipos, ficat, muşchi scheletic. Pot reface sensibilitatea la insulină prin scăderea citokinei TNF-α (26) şi creşterea exprimării adiponectinei (27).

▪ Activarea PPARγ creşte exprimarea şi translocarea transportorilor 1 şi 4 de glucoză de la suprafaţa celulei, crescând consumul de glucoză în ficat şi muşchiul scheletic.

▪ La pacienţi cu control glicemic neadecvat, TZD reduc nivelul hemoglobinei glicate (glicozilate) (Hb A1c) şi al glucozei plasmatice în condiţie de post.

În plus, de eficacitatea în controlul glicemiei prin sensibilitatea la insulină, TZD au **efecte pleiotrope** asupra metabolismului lipidelor, presiunii sanguine, funcţiei endoteliale, celulelor muşchiului neted vascular, macrofage, inflamaţiei, aterosclerozei, plăcii aterosclerotice, coagulării, albuminuriei ş.a. în funcţia imună a monocitelor/macrofagelor (28, 29, 30, 31, 32).

▪ Se consideră că TZD şi alţi agonişti PPARγ îşi exercită efectele pleiotrope direct în ţesutul adipos, care în final duc la sensibilizarea la insulină în muşchiul scheletic şi ficat (33).

▪ În ţesutul adipos, activarea PPARγ duce la inducţia multor gene implicate în eliberarea şi consumul de acizi graşi având

drept consecinţă scăderea nivelului de acizi graşi liberi şi trigliceride şi în final la reducerea depozitelor de grăsimi în muşchi, ficat şi viscere .

▪ TZD cresc secreţia de adiponectină şi scad producerea de rezistină şi a factorului de necroză tumoral (TNF-α) (34), în contextul inflamaţiei acute. La persoanele obeze s-a observat producere crescută de TNF-α în ţesutul adipos implicat ca factor cauzativ în obezitatea asociată cu rezistenţa la insulină şi patogeneza diabetului de tip 2. De asemenea, TZD cresc cHDL, cresc mărimea/scad densitatea LDL. Alte efecte PPARγ-dependente includ: inducţia diferenţierii adipocitare, inhibarea angiogenezei indusă de factorul de creştere endotelial vascular (VEGF), modificarea nivelului leptinei.

▪ În contextul rezistenţei la insulină şi a diabetului de tip 2, dislipidemia se caracterizează prin modificarea lipidelor plasmatice, în principal prin oxidare şi glicare. Aceste lipide modificate sunt implicate în iniţierea aterogenezei. În virtutea efectelor favorabile asupra profilului lipidic (35), pioglitazona poate avea efecte pozitive asupra sistemului cardiovascular (36) şi a complicaţiilor cardiovasculare. TZD modifică favorabil distribuţia grăsimilor şi îmbunătăţesc factorii de risc cardiovasculari precum presiunea sanguină, markeri inflamatori, acidul uric, întârzie evoluţia aterosclerozei, atenuează nivelul circulator al mediatorilor proinflamatori (37). De asemenea, TZD reduc nivelul plasmatic al chemokinelor, proteinei-1 monocitare chemotactice (MCP-1), proteinei antifibrinolitice, inhibitor-1 al activatorului plasminogenului, moleculei de adeziune a celulei endoteliale, C-selectinei, ICAM-1 (38) şi au efect antioxidant (39).

▪ Deşi TZD utilizate terapeutic sunt considerate ca droguri fără probleme, s-au observat unele efecte comune colaterale care includ: câştig în greutate (40), retenţie de apă care reprezintă un risc semnificativ pentru pacienţii cu funcţie ventriculară scăzută. Retenţia de sodiu şi expansiunea volumului plasmatic au fost legate de stimularea PPARγ în epiteliul ductului colector renal.

▪ De asemenea, glitazonele scad formarea osului şi a masei osoase şi cresc frecvenţa fracturilor cel puţin la femei (41). Un risc crescut pentru boala coronariană s-a observat cu rosiglitazonă (41), în timp ce s-a dovedit că rosiglitazona scade evoluţia aterosclerozei (42).

Această listă este departe de a fi completă, dar poate oferi o idee asupra multiplelor gene reglate după stimularea PPARγ.

Pentru că mulţi pacienţi cu rezistenţă la insulină manifestă dislipidemie, a fost practic judicios să se producă agonişti duali PPARα/γ în tratarea acestor bolnavi. Aceşti agonişti, denumiţi glitazari, combină efectul de sensibilizare la insulină ale activării PPARγ, cu efectul de scădere a lipidelor, ale agoniştilor α care scad trigliceridele, cresc nivelul HDL şi sensibilitatea la insulină şi reducerea riscului cardio-vascular. De asemenea, în tratamentul diabetului zaharat tip 2 se pot administra pioglitazonă în combinaţie cu metformin, sulfoniluree, exenatide, insulină (43) ş.a. Terapia cu aleglitazar (agonist dual PPARα/γ) reduce hiperglicemia şi modifică în mod favorabil nivelul c-HDL şi al trigliceridelor. În continuare, acest glitazar se studiază clinic dacă reduce riscul major de infarct miocardic, stroke, moarte, la pacienţi cu diabet şi boli arteriale.

Terapia combinată poate fi o importantă paradigmă pentru tratamentul şi încetinirea aterosclerozei, a bolilor coronariene, a

dezordinilor de co-morbiditate metabolică caracterizate prin disfuncţie endotelială şi rezistenţă la insulină (44).

PPAR β/δ a fost clonat în 1990.

Distribuţia. Exprimarea PPARβ/δ este esenţială în toate tipurile de celule şi ţesuturi. Nivele mari a acestei izoforme se găsesc în intestin subţire, colon, inimă, ţesut adipos, creier (1).

Mecanismul de acţiune impune, ca şi în cazul celorlalte izotipuri, formarea de heterodimeri cu RXR (PPAR:RXR), dar se deosebeşte de acestea prin selectivitate şi sensibilitate la liganzi care la rândul lor diferă prin capacitatea de a interacţiona cu coactivatori, ceea ce explică diferite răspunsuri biologice. Liganzii PPARβ/δ sunt agenţi sintetici eficace şi selectivi precum acizii fenoxiacetici 4-tiosubstituiţi (45).

Efectele PPARβ/δ. Deşi sunt disponibile informaţii limitate asupra funcţiilor biologice ale acestui receptor datorită lipsei unui antagonist utilizat curent în clinică pot contribui la mai puţina cunoaştere a acţiunilor lui. Exprimarea PPARβ/δ în aproape toate tipurile de celule şi ţesuturi sugerează rolul lor potenţial fundamental în biologia celulară şi posibelele efecte ale agoniştilor.

◊ Studii recente au arătat implicarea lui în reglarea metabolismului glucidic, în transportul şi oxidarea acizilor graşi (46, 47), implicarea în diferenţierea epidermică, maturarea şi vindecarea rănilor pielii (48).

◊ Activarea PPAR β/δ are efecte benefice directe asupra homeostaziei vasculare prin protejarea celulelor endoteliale de inflamaţie şi apoptoză, scăderea, scăderea inflamaţiei şi promovarea angiogenezei.

Aceste efecte conferă un potenţial promiţător agoniştilor PPAR β/δ pentru tratamentul diferitor afecţiuni.

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REZISTENȚA PRIN METALO-BETA-LACTAMAZE LA IZOLATE CLINICE DE *PSEUDOMONAS AERUGINOSA* REZISTENTE LA CARBAPENEME

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METALLO-BETA-LACTAMASE-MEDIATED RESISTANCE AMONG CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES (Abstract): **The aim** of our study was to evaluate the antimicrobial susceptibility profile and the presence of metallo-beta-lactamases (MBLs) among carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates. **Material and methods.** A total of 84 *P. aeruginosa* clinical isolates collected between January 2007- February 2011 from four university hospitals in Iasi (North-East region of Romania) were randomly selected. Antimicrobial susceptibility testing was performed according to CLSI 2010 (*Clinical and Laboratory Standards Institute*) guidelines. The isolates were tested for MBLs using EPI (EDTA-phenanthroline-imipenem) phenotypic test and polymerase chain reaction (PCR) for bla_{VIM} and bla_{IMP}. **Results.** Fifty-eight carbapenem resistant strains were identified, from which 24 (41,3%) were positive for VIM - type MBLs. No IMP - type MBL was detected. All MBL-producing isolates displayed a MDR (multidrug resistant) phenotype, two of them were XDR (extensively drug-resistant). Colistin remained the most effective antibiotic. **Conclusions**. The high proportion of MBL producing *P. aeruginosa* clinical isolates urges the need for a better use of antibiotics and for efficient infection control measures to prevent dissemination of MBL producers. This is the first report of VIM-like enzymes in *P. aeruginosa* isolates from the Iasi area. **Key words**: *PSEUDOMONAS AERUGINOSA*, CARBAPENEM-RESISTANCE, METALLO-BETA-LACTAMASES

Pseudomonas aeruginosa este un frecvent patogen nosocomial, implicat într-o gamă largă de infecții şi care, prin rezistența intrinsecă la numeroase antibiotice şi prin acumularea de noi mecanisme de rezistență, pune mari probleme de tratament şi de control al infecției.

Rezistența la imipenem rezultă adesea prin pierderea porinei *OprD* sau prin creşterea expresiei pompelor de eflux. Carbapenemazele (incluzând serin carbapenemazele de clasă A şi D şi metalo-β-lactamazele - MBL) sunt enzime emergente de mare interes, deoarece conferă rezistență la carbapeneme, antibiotice valoroase împotriva bacililor Gram-negativi multirezistenți. Metalo-beta-lactamazele (MBL) transferabile aparțin clasei moleculare B, cu zinc în centrul activ, nu sunt influențate de inhibitorii terapeutici de β–lactamaze şi conferă rezistență la toate β-lactaminele, exceptând aztreonamul. Genele ce codifică

MBL se găsesc pe elemente genetice mobile, împreună cu alți determinanți de rezistență, astfel încât prezența lor constuie un marker pentru multidrog rezistență (1). Prima MBL transferabilă, IMP-1 a fost descoperită în Japonia spre sfârşitul anilor 80' la tulpini de *Pseudomonas aeruginosa* multirezistente la antibiotice. În ultimii 10 ani 9 tipuri de MBL au fost descrise, pe baza secvenței de aminoacizi, ulterior acestea diseminând rapid printre tulpini de *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacteriaceae*. Cele mai frecvente şi mai răspândite la nivel mondial sunt enzimele IMP (*imipenemase*) şi VIM (*Veronese imipenemase*); SPM (*Sao Paulo metallo-β-lactamase*), endemică în America de Sud, a fost importată recent în Elveția, GIM (*German imipenemase)*, SIM (*Seul imipenemase)* şi AIM (*Australian imipenemase*) au fost semnalate în Germania, Coreea de Sud, respectiv Australia (2). Enzimele KHM (*Kyorin Hospital metallo-β-lactamase*) şi DIM (*Dutch imipenemase*) au fost identificate doar la tulpini de *Citrobacter freundii* în Japonia, respectiv *Pseudomonas stutzeri* în Olanda. Deşi depistată inițial la tulpini de *Klebsiella pneumoniae* şi *Escherichia coli* din India, MBL NDM-1 s-a transmis la *P. aeruginosa*, primul izolat fiind raportat în Serbia (3).

Prevalența MBL la tulpini de *P. aeruginosa* variază semnificativ între țări, între oraşe şi chiar între centrele medicale: 1,3% în Italia la nivel național, respectiv 9,2% la Napoli; 0,1% în Spania, 7,5 - 44% în Brazilia, 1,9% în Japonia (1).

Studiile noastre au identificat prezența VIM-2 şi IMP-13 la două izolate de *P. aeruginosa* din Galați (Pa16GL), respectiv Iaşi (Pa247), tulpina producătoare de IMP-13 din Iaşi fiind înrudită clonal cu cea responsabilă de o izbucnire epidemică în sudul Italiei (4).

În acest studiu ne-am propus investigarea profilului de sensibilitate la antibiotice şi determinarea frecvenței MBL la tulpini de *P. aeruginosa* rezistente la imipenem, izolate în 4 spitale universitare din Iaşi.

MATERIAL ŞI METODE

Tulpinile bacteriene. Lotul a inclus 84 tulpini de *Pseudomonas aeruginosa* izolate în 4 spitale universitare din Iaşi: Spitalul Clinic Universitar de Boli Infecțioase (58 tulpini), Spitalul Clinic Universitar Județean de Urgențe "Sf. Spiridon" (12 tulpini), Centrul de Cardiologie Iaşi (8 tulpini), Spitalul Clinic Universitar "Dr. C. I. Parhon" (6 tulpini), în perioada ianuarie 2007 - februarie 2011. Majoritatea izolatelor au provenit din puroi şi urină (41,8%, respectiv 40,3%), restul din spută, hemocultură, aspirat traheal, lichid cefalorahidian, exsudat faringian.

Testarea sensibilității la antibiotice am efectuat-o conform standardelor CLSI (*Clinical and Laboratory Standards Institute*) 2010 (5). Am testat prin metoda difuzimetrică sensibilitatea la piperacilină-tazobactam (TZP), ceftazidim (CAZ), cefepim (FEP), aztreonam (ATM), imipenem (IPM), ciprofloxacin (CIP), tobramicină (TOB), gentamicină (GM), amikacină (AK) şi colistin (COL) (*Oxoid Ltd., Basingstoke*, Hampshire, Marea Britanie). Concentrațiile minime inhibitorii (CMI) la IPM şi CAZ le-am determinat prin metoda microdiluțiilor în bulion. Procentele de rezistență la CAZ şi IPM au fost calculate pe baza punctelor de ruptură recomandate de CLSI 2010 pentru categoria total sensibil (izolatele intermediare le-am considerat rezistente).

Detectarea fenotipică a producerii de metalo-β-lactamaze am efectuat-o prin testul fenotipic EPI (*EDTA-phenanthroline-imipenem*), care are la bază susceptibilitatea MBL la inhibiția prin chelatori de metale, amestecul de EDTA şi o-fenan-

trolină (6). Acest test constă în determinarea CMI la imipenem în absența şi în prezența amestecului chelator. O scădere de ≥4 ori a valorii CMI pentru imipenem în prezența chelatorilor este considerată ca sugestivă pentru producerea de MBL.

Analiza prin moleculară prin *polymerase chain reaction* (PCR).

Pentru detectarea genelor de tip bla_{VIM} şi bla_{IMP} am efectuat un multiplex PCR cu următorii primeri (*Eurogentec*, Liège, Belgia):

IMP-DIA-FWD (5'-GGAATAGAGTGGCTTAATTCT-3'),
IMP-DIA-REV (5'-GTGATGCGTCYCCAAYTTCACT-3'),
VIM-DIA-FWD (5'-CAGATTGCCGATGGTGTTTGG-3'),
VIM-DIA-REV (5'-AGGTGGGCCATTCAGCCAGA-3') (7),

care generează ampliconi de 361 bp pentru bla_{IMP} şi de 523 bp pentru bla_{VIM}. Reacția a cuprins: ADN matriță 2 µl (vezi mai jos), 50 pmol din fiecare primer, dezoxinucleozidtrifosfați 200 µM, 1 U de polimerază Taq (*Fermentas International Inc.*, Canada), $MgCl_2$ 1,5 mM şi soluție tampon asigurat de producător până la un volum final de 50 µl. Matrița ADN am obținut-o astfel: din cultura proaspătă pe agar Mueller-Hinton am preparat o suspensie densă în 500 µl apă distilată, pe care am fiert-o 15 min. Resturile celulare distruse le-am îndepărtat prin centrifugare (5 min, 13000 rpm), iar supernatantul l-am folosit ca sursă de ADN pentru amplificare, în termocilor *Corbett CG1-96* (*QIAGEN*). Parametrii reacției au fost: 3 min la 94°C, 30 cicluri de 1 min la 94°C, 1 min la 52°C, 1 min la 72°C, şi o extensie finală de 10 min la 72°C. Produşii de amplificare au fost separați prin electroforeză în gel de agaroză 1,5%, cu scală de 100 bp (*Promega*, SUA) la putere de 100 V, 1,5 ore, folosind un aparat *Scie-Plas HU13W*, Marea Britanie.

Controlul de calitate l-am asigurat utilizând tulpina de *Pseudomonas aeruginosa* ATCC 27853, iar tulpinile de *P. aeruginosa* Pa16GL şi Pa247 au reprezentat martorii pozitivi.

REZULTATE ŞI DISCUȚII

Prin antibiograma difuzimetrică, din 84 de tulpini *P. aeruginosa* incluse în studiu 54 au fost rezistente la IPM, 55 la CAZ, 47 la FEP, 57 la CIP, 50 la TZP, 11 la ATM, 55 la GM, 57 la TOB, 53 la AK, niciuna la COL.

Analiza comparativă a rezultatelor antibiogramei difuzimetrice şi ale antibiogramei prin metoda microdiluțiilor a reîncadrat în valori de sensibilitate tulpinile testate astfel: 2 tulpini rezistente şi 5 sensibile la IPM au fost încadrate ca intermediare (CMI=8 mg/L); față de CAZ, 3 tulpini rezistente au fost clasificate ca intermediare (CMI=16 mg/L), una rezistentă ca sensibilă şi 2 sensibile ca rezistente.

Valorile CMI la CAZ şi IPM pentru tulpinile investigate s-au încadrat între 1 - ≥ 128 mg/L (fig.1), cu predominanța izolatelor cu rezistență înaltă la cele două antibiotice (CMI ≥ 128 mg/L).

Pentru urmărirea modificărilor în timp ale rezistenței bacteriene, standardele internaționale recomandă prezentarea datelor de supraveghere a rezistenței ca valori CMI_{50} sau CMI_{90} (8). Tulpinile de *P. aeruginosa* studiate s-au remarcat prin valori ridicate ale CMI_{50} şi CMI_{90} atât pentru IPM (32 mg/L, respectiv >128 mg/L), cât şi pentru CAZ (32 mg/L, respectiv 128 mg/L) şi procente crescute de rezistență la IPM şi CAZ (69,1%, respectiv 60,8%). Conform definițiilor propuse în 2011 (9), 55 de tulpini (65,47%) au prezentat fenotip MDR (*multidrug resistant*), fiind rezistente la cel

puțin un antibiotic din cel puțin trei clase diferite: peniciline anti-*Pseudomonas* şi un inhibitor de β-lactamază, cefalosporine anti-*Pseudomonas*, carbapeneme, aminoglicozide, fluorochinolone, polimixine; 9 tulpini (10,71%) au fost XDR (*extensively drug-resistant*), deoarece au prezentat rezistență la cel puțin un antibiotic din cel puțin şase din categoriile de antibiotice menționate. Nu am depistat nicio tulpină PDR (*pandrug-resistant*), definită ca rezistentă la toate categoriile de antibiotice.

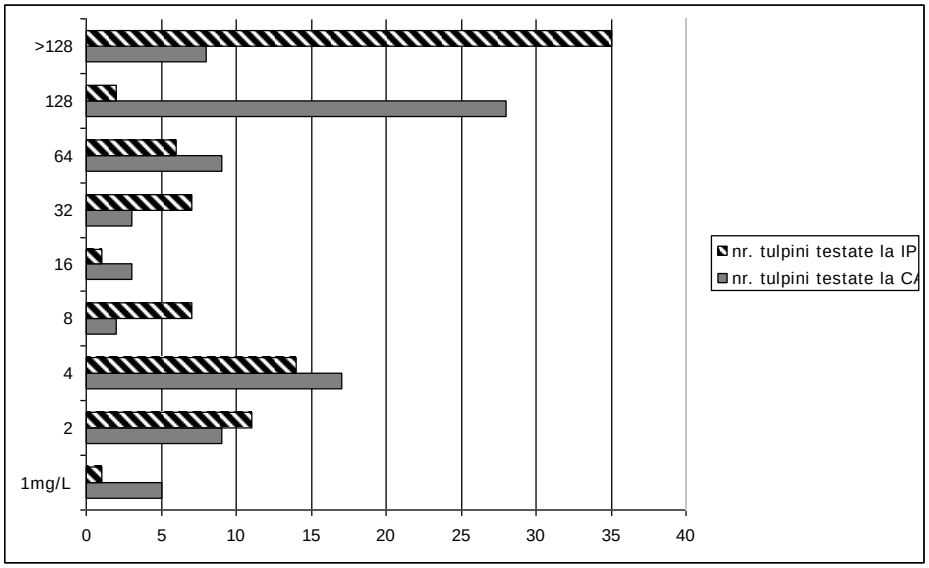

Fig. 1. Distribuția CMI (mg/L) la ceftazidim (CAZ) şi imipenem (IPM) pentru cele 84 tulpini de *P. aeruginosa* studiate.

Din totalul de 84 tulpini de *P. aeruginosa*, pentru 24 (28,5%) am obținut rezultate pozitive la testul fenotipic EPI, reprezentate de o scădere de ≥4 ori a CMI pentru imipenem în prezența chelatorilor.

Prin multiplex PCR am detectat gene codante pentru MBL tip VIM la 24 izolate din lotul investigat (fig. 2). Nu am identificat în acest studiu nici o genă bla_{IMP} şi nici gene pentru MBL, la tulpinile sensibile la imipenem.

Majoritatea tulpinilor producătoare de MBL au provenit din urină (15 tulpini), restul fiind izolate din puroi (5), aspirat traheal (2), spută (1) şi hemocultură (1).

Tulpinile de *P. aeruginosa* producătoare de MBL au prezentat fenotipul MDR, două dintre ele fiind XDR. Ele au fost rezistente la ceftazidim (CMI între 32-128 mg/L), cefepim, imipenem (cu rezistență de nivel înalt CMI>128 mg/L), ciprofloxacin, gentamicină, tobramicină, amikacină, toate fiind sensibile la colistin. Aztreonamul s-a dovedit al doilea antibiotic, ca eficiență, doar 2 tulpini fiind rezistente, sugerând asocierea şi a altor mecanisme de rezistență la beta-lactamine.

Izolatele pozitive pentru gena bla_{VIM} au fost depistate în toate cele patru spitale: o tulpină la Centrul de Cardiologie Iaşi, 2 tulpini la Spitalul Clinic Universitar "Dr. C. I. Parhon", 5 la Spitalul Clinic Universitar Județean de Urgențe "Sf. Spiridon" şi 16 la Spitalul Clinic Universitar de Boli In-

fecțioase.

În ansamblu, proporția tulpinilor producătoare de MBL a fost de 28,5% din întregul lot şi de 41,3% din izolatele intermediare şi rezistente la imipenem. Am detectat numai MBL tip VIM, acestea fiind cel mai frecvent raporatate şi la nivel mondial (1, 2).

Prevalența rezistenței la carbapeneme mediate prin MBL la tulpini de *P. aeruginosa* variază semnificativ între țări. În Polonia, din 311 izolate, 22% au fost rezistente la carbapeneme, dintre acestea 60% produceau MBL, iar majoritatea au fost de tip VIM (10). În Brazilia MBL au fost identificate la 30,4% din 69 tulpini de *P. aeruginosa* rezistente la imipenem izolate din hemoculturi (11). Un studiu din Croația a identificat 6 tulpini producătoare de VIM-2 dintre 100 tulpini rezistente la imipenem sau meropenem (12). Din 104 tulpini rezistente la imipenem izolate în Nord-Vestul Iranului 23% produceau MBL, predominant VIM (13). În Norvegia şi Suedia între 1999 şi 2007 au fost raportate numai 4, respectiv 9 tulpini de *P. aeruginosa* producătoare de MBL (2). Un studiu recent realizat în Germania semnalează că 1,6% dintre 489 izolate de *P. aeruginosa* şi 11,7% dintre tulpinile cu sensibilitate redusă la imipenem sunt producătoare de MBL, procente similare cu cele raportate în Italia şi Coreea unde însă diversitatea MBL este mai mare (14).

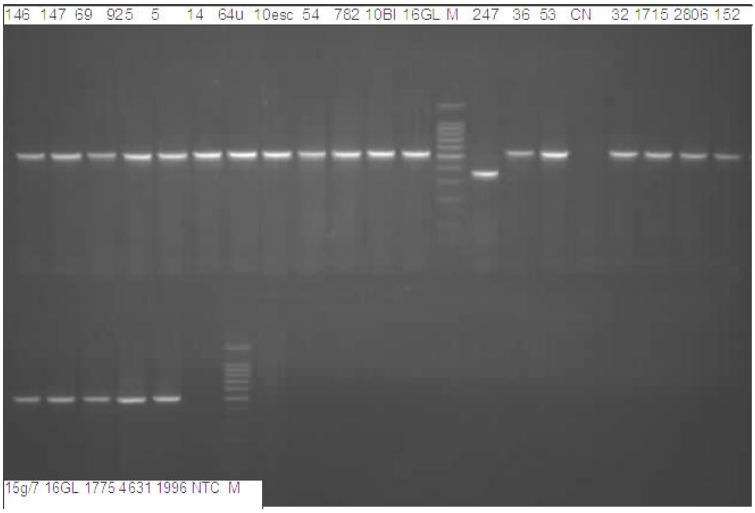

Fig. 2. Multiplex PCR pentru detectarea genelor de tip bla_{VIM} şi bla_{IMP}. M = 100 bp DNA ladder; CN= control negativ; NTC= *non template control*. 16GL= martor pozitiv pentru gena bla_{VIM}, cu fragment amplificat de 523 bp; 247= martor pozitiv pentru gena bla_{IMP}, cu fragment amplificat de 361 bp. Tulpinile testate posedă un amplicon de 523 bp, ceea ce sugerează genă de tip bla_{VIM}.

CONCLUZII

În acest studiu a fost depistată pentru prima dată gena codantă pentru MBL de tip VIM la tulpini de *P. aeruginosa* rezistente la carbapeneme izolate în spitale universitare din Iaşi.

Procentele de rezistență înregistrate la imipenem (69,1%) şi la ceftazidim (60,8%) trag un semnal de alarmă asupra utilizării corecte, restrictive a acestor antibiotice în

terapia infecțiilor produse de bacili Gram-negativi (*Enterobacteriaceae, P. aeruginosa, Acinetobacter* spp). Supravegherea în condiții standardizate a sensibilității la antibiotice a tulpinilor de *P. aeruginosa* este utilă pentru cunoaşterea spectrului de sensibilitate actual al bacteriilor la antibiotice, cu implicații directe în limitarea răspândirii tulpinilor multidrog rezistente.

Proporția mare a tulpinilor producătoare de MBL (28,5%) limitează opțiunile terapeutice în infecțiile cu *P. aeruginosa*, doar la colistin şi impune măsuri pentru prevenirea infecției nosocomiale şi a transferului orizontal al acestor bacterii.

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INFLUENŢA FACTORILOR DE STRES ASUPRA FUNCŢIEI HEPATICE ŞI METABOLISMULUI LIPIDIC PE UN MODEL DE HIPERTENSIUNE ARTERIALĂ LA ANIMALE DE LABORATOR

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THE INFLUENCE OF STRESS FACTORS ON LIVER FUNCTION AND LIPID METABOLISM IN AN ANIMAL MODEL OF ARTERIAL HYPERTENSION (Abstract): **Aim**: to investigate the influence of some stress factors on hepatic function and lipid metabolism in an experimental-induced hypertension. **Material and methods**: The experiment was carried out on Wistar rats, treated intraperitoneally, for 4 weeks, as follows: Group 1: saline solution (0.5ml/100g weight); Group 2: saline solution + stress; Group 3: Endotelin-1; Group 4: Endotelin-1 + stress; Group 5: Endotelin-1 + cholesterol diet (0.2g/kbw/day); Group 6: Endotelin-1 + cholesterol diet + stress. Endotelin-1 0.25nmol/kbw was initially administered, followed by 0.5nmol/kbw 15 min later, 2 times/week. Stress-inducing factors were immobilization and water immersion. In the 28^{-th} day of the experiment blood pressure was measured and blood samples were taken from retro-orbitary plexus to assess glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) activity, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides levels. **Results**: Using immobilization and water immersion as chronic stress, our study proved an increase of GOT, GTP and total and LDL cholesterol in rats with endotelin-1-induced hypertension. **Conclusions**: In this animal model of endothelin-1-induced hypertension the blood pressure increased significantly under chronic exposure to stress, reaching the highest values when associating stress and experimentally induced dyslipidemia. **Key words**: STRESS, CHOLESTEROL, BLOOD PRESSURE.

Hipertensiunea arterială (HTA) este considerată una dintre cele mai importante probleme de sănătate publică din ţările dezvoltate, ea evoluând, de multe ori, fără a fi diagnosticată, monitorizată şi tratată adecvat, deşi este extrem de uşor de depistat. Persoanele cu HTA nu prezintă mult timp nici un simptom, motiv pentru care această boală a fost supranumită "ucigaşul tăcut".

Stresul obligă organismul să fie într-un continuu proces de adaptare. Starea de stres experimentată în mod regulat, pentru perioade lungi de timp, este cea care poate produce HTA, prin accelerarea bătăilor inimii şi prin vasoconstricţia indusă de eliberarea neurohormonilor. Acest fond predispune la HTA (1). Tensiunea arterială poate creşte în condiţii de stres şi stare de tensiu-

ne, cel puţin pe moment, când persoana se confruntă cu agentul stresor, această reacţie făcând parte din răspunsul general al organismului la stres. (2,3) Deşi există o astfel de relaţie demonstrată între cele două elemente (HTA şi stres), încă nu s-a stabilit dacă diminuarea stimulilor psihologici şi a situaţiilor stresante din jur poate reduce pe termen lung şi HTA (4).

Stresul mental cronic creşte presiunea arterială, precum şi nivelul colesterolului, conducând, astfel, la HTA cronică şi dislipidemie.

Studiile efectuate au pus în evidenţă existenţa unor corelaţii între diferitele tipuri de stres, reactivitatea organismului şi modificările presionale (5,6).

Scopul studiului a constat în cercetarea influenţei unor factori de stres asupra funcţiei hepatice şi a metabolismului lipidic, pe un model de HTA indusă experimental la animale de laborator.

MATERIAL ŞI METODĂ

Pentru experiment au fost utilizate 6 loturi de câte 6 şobolani Wistar (180-200g), cu repartiţie uniformă pe sexe, proveniţi de la Institutul de Sănătate Publică din Iaşi, trataţi conform schemei:

L1 = SF = Ser fiziologic (0,5 ml/100g corp/zi);

L2 = SF+s = Ser fiziologic (0,5 ml/100g corp/zi) + stres indus de imobilizare şi imersie în apă;

L3 = ET-1 = Endotelină-1 (0,25 nmol/kg corp, i.p., iar după 15 min 0,5 nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni);

L4 = ET-1+s = Endotelină-1 (0,25 nmol/kg corp, i.p., iar după 15 min 0,5 nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni) + stres indus de imobilizare şi imersie în apă;

L5 = ET-1+col = Endotelină-1 (0,25 nmol/kg corp, i.p., iar după 15 min 0,5 nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni) + dietă cu colesterol (0,2 g/kg corp/zi, 4 săptămâni);

L6 = ET-1+col+s = Endotelină-1 (0,25 nmol/kg corp, i.p., iar după 15 min 0,5 nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni) + dietă cu colesterol (0,2 g/kg corp/zi, 4 săptămâni) + stres indus de imobilizare şi imersie în apă.

Substanţele au fost administrate conform schemei de mai sus, începând cu ora 8 dimineaţa, timp de 28 de zile. După 28 de zile de tratament au fost evaluate valorile tensiunii arteriale şi s-a recoltat sânge din plexul retroorbitar, pentru determinarea următorilor parametri: valorile transaminazei glutamic oxaloacetice (TGO), transaminazei glutamic piruvice (TGP), lactat dehidrogenazei (LDH), bilirubinei serice totale, colesterolului total, LDL-colesterolului şi HDL-colesterolului, trigliceridelor şi glicemiei. Aceşti parametri au fost determinaţi cu un analizor pentru teste biochimice VITROS 750 XRC, folosind reactivi Johnson&Johnson. Rezultatele au fost apoi prelucrate statistic folosind testul *t-Student* al programului Microsoft Office Excel.

Cercetările care au fost efectuate în cadrul studiului s-au realizat în strictă concordanţă cu normele Comisiei de Etică a Universităţii şi reglementările etice internaţionale, privind lucrul pe animale de laborator.

REZULTATE ŞI DISCUŢII

Modelul experimental realizat poate fi folosit pentru studiul rolului endotelinei în etiopatogeneza HTA indusă experimental la animale de laborator, constituind, în acelaşi timp, o alternativă mult mai puţin costisitoare, la utilizarea şobolanilor modificaţi

genetic care dezvoltă HTA. (7,8,9)

Cercetările noastre anterioare (10) au demonstrat că administrarea repetată de Endotelină-1 a determinat creşterea valorilor tensiunii arteriale la şobolanii Wistar. Aceste valori sunt semnificativ statistic crescute în condiţiile expunerii la stres comparativ cu animalele care nu au fost menţinute în aceste condiţii experimentale. Cele mai ridicate valori ale tensiunii arteriale au fost înregistrate la şobolanii Wistar cărora li s-a administrat Endotelină-1, dietă cu colesterol şi au fost expuşi unui stres cronic (fig. 1).

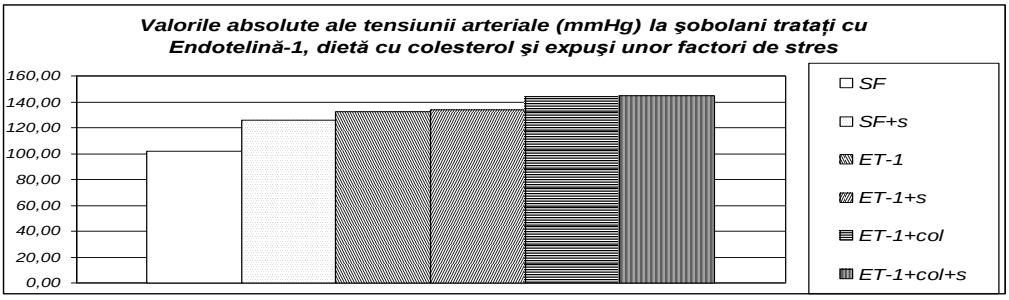

Fig. 1. Valorile absolute ale tensiunii arteriale după 28 de zile

În cursul experimentelor, s-a constatat, însă, creşterea TGO (fig. 2) şi TGP (fig. 3) la toate loturile analizate, comparativ cu lotul martor. Creşterea transaminazelor este efectul alterării permeabilităţii membranei celulare din cauza dezechilibrului masiv indus de stres. Nu s-au înregistrat modificări semnificative statistic ale valorilor LDH sau ale bilirubinei serice totale, comparativ cu lotul martor.

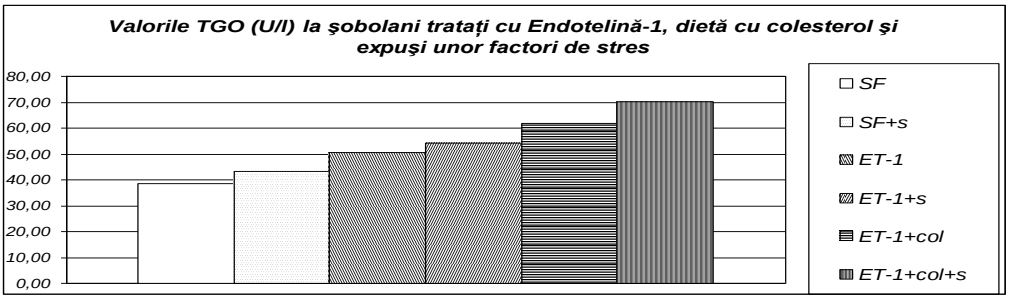

Fig. 2. Valorile TGO după 28 de zile

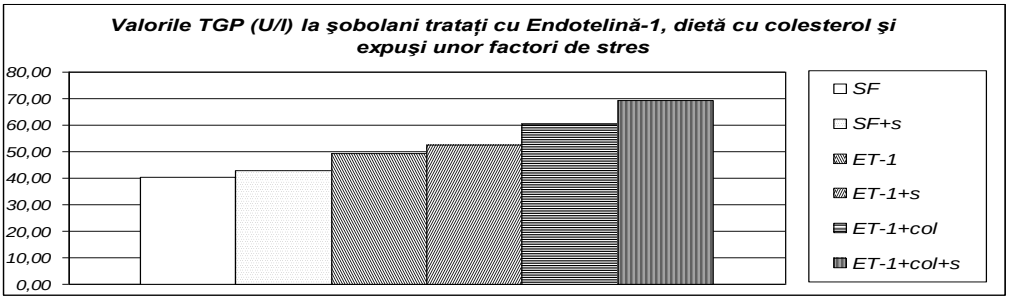

Fig. 3. Valorile TGP după 28 de zile

Pe de altă parte, s-a constatat scăderea trigliceridelor (fig. 7) şi creşterea colesterolului total (fig. 4), pe seama creşterii LDL-colesterolului (fig. 5) şi scăderea HDL-colesterolului (fig. 6). Efectele cele mai puternice au fost înregistrate la animalele cu HTA indusă experimental cu endotelină-1, cu dietă cu colesterol plus menţinerea în condiţii de stres. Modificările biologice sunt datorate stimulării induse de stres a axei hipotalamo-hipofizo-suprarenaliene şi a sistemului nervos simpatic, cu eliberarea secundară de catecolamine şi glucocorticoizi.

Răspunsul la stres include multipli hormoni: ACTH, catecolamine, ADH, angiotensină, glucagon şi GH. Creşterea colesterolului este atribuită creşterii corespunzătoare a sintezei acestuia prin mobilizarea lipidelor din depozite datorită activităţii simpatice şi a axei hipotalamo-hipofizo-suprarenaliene. Trigliceridele scad având în vedere relaţia inversă dintre acizii graşi liberi şi trigliceride, acestea din urmă reprezentând o sursă rapidă de energie.

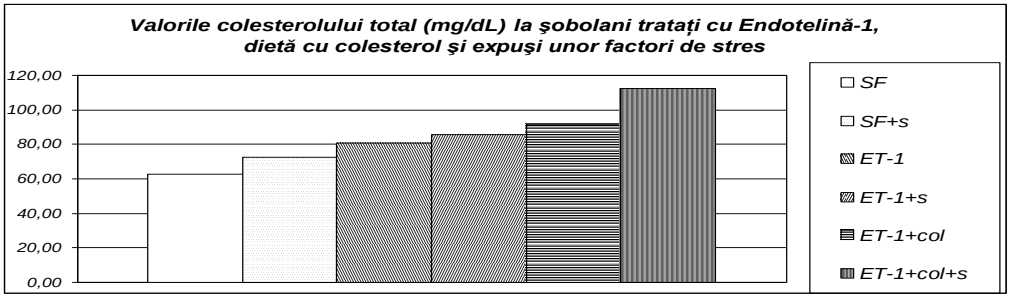

Fig. 4. Valorile colesterolului total după 28 de zile

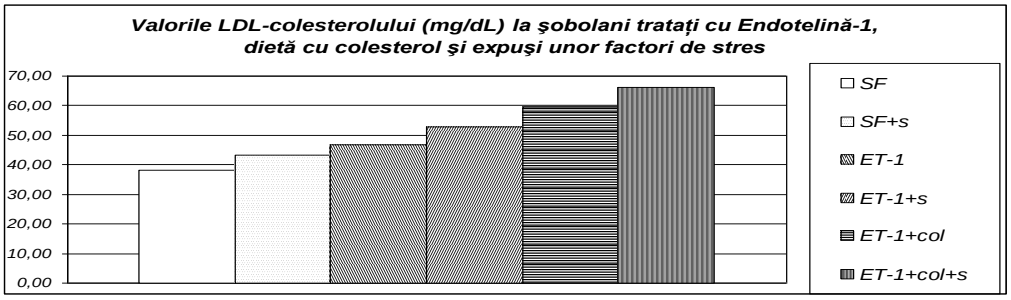

Fig. 5. Valorile LDL-colesterolului după 28 de zile

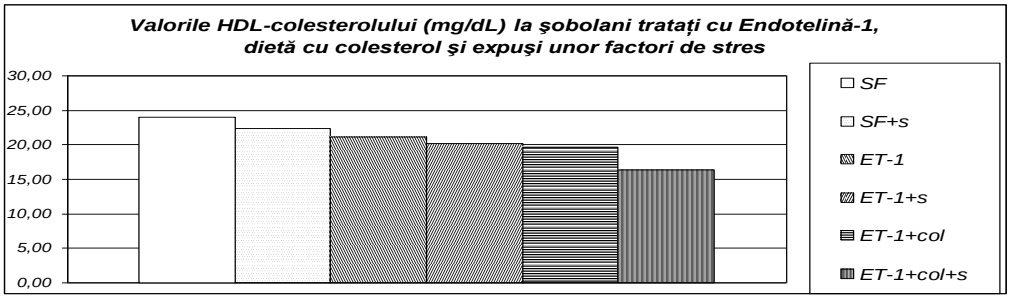

Fig. 6. Valorile HDL-colesterolului după 28 de zile

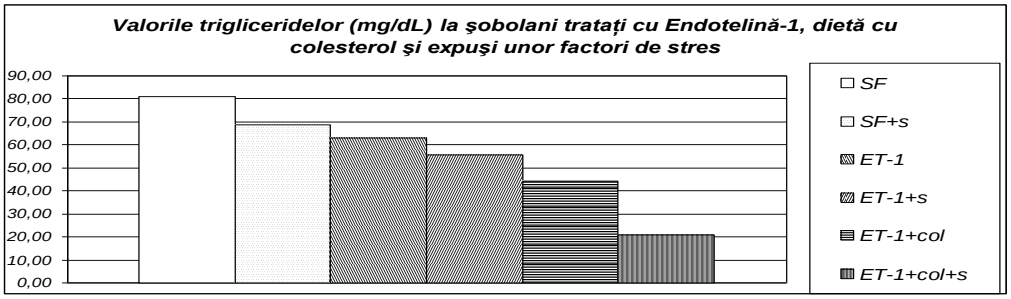

Fig. 7. Valorile trigliceridelor după 28 de zile

CONCLUZII

În condiţiile experimentale realizate, s-a constatat că, după expunerea la un stres cronic, şobolanii Wistar cu HTA indusă de endotelina-1 au prezentat alterarea funcţiei hepatice (creşterea TGO şi TGP), la toate loturile analizate, comparativ cu lotul martor. Pe de altă parte, s-a constatat scăderea trigliceridelor şi creşterea colesterolului total, pe seama creşterii LDL-colesterolului şi scăderea HDL-colesterolului. Efectele cele mai puternice au fost înregistrate la animalele cu HTA indusă experimental, cu dietă cu colesterol plus menţinerea în condiţii de stres.

Condiţiile de stres cronic în care au fost menţinute animalele cu HTA indusă experimental cu endotelină-1 au determinat o creştere a valorilor tensionale semnificativ statistic mai puternică comparativ cu nivelurile acestui parametru la animalele neexpuse la stres. Cele mai importante creşteri ale tensiunii arteriale au fost înregistrate la animalele de laborator expuse stresului şi prezentând, în acelaşi timp, dislipidemie indusă experimental.

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EVALUĂRI CLINICO-EPIDEMIOLOGICE ASUPRA ACCIDENTELOR RUTIERE, COMPARATIV, ÎN UNITĂȚI DE PRIMIRE URGENȚE. OBSERVAȚII ÎN TREI JUDEȚE ALE MOLDOVEI, ROMÂNIA, ÎN PERIOADA 2005-2010

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COMPARATIVE CLINICAL-EPIDEMIOLOGICAL ASSESSMENTS OF ROAD TRAFFIC ACCIDENTS. OBSERVATIONS IN EMERGENCY ROOMS FROM THREE COUNTIES OF MOLDAVIA, ROMANIA, DURING 2005-2010 (Abstract). **Aim:** to assess road traffic injuries according to some descriptive parameters. **Material and methods:** The study was carried out on a series of 5374 patients admitted to emergency rooms at Iasi (n=3050), Botosani (n=1187), and Vaslui (n=1137), between 2005 and 2010. **Results**: Most frequently, the patient was a young, male adult, with cranial cerebral trauma, leg fracture, forearm contusion, or polytrauma, presenting to the emergency room most commonly in the month of August, weekends, and interval noon – dawn interval. **Conclusions**: The impact of road trauma epidemic would facilitate a better adaptation of the medical staff to case dynamics and increase the efficacy of primary prevention programs. **Key words**: ROAD TRAUMA EPIDEMIC, INJURY, EPIDEMIOLOGY

În Uniunea Europeană, accidentele de circulație sunt principala cauză de deces în rândul turiştilor, reprezentând 50% din cauzele de deces, 20% din internările în spital şi 30% din cazurile unui serviciu de urgențe (1, 2, 3, 4).

În România, circulația rutieră a cunoscut o dezvoltare semnificativă în ultimii 17 ani. În condițiile în care infrastructura rutieră a rămas în aproximativ aceiaşi parametri ca în anii 1990, parcul de autovehicule şi numărul posesorilor permiselor de conducere s-au dublat (peste 5 milioane). Peste 2500 de persoane decedează anual în evenimente rutiere, echivalentul dispariției de pe hartă a unei localități rurale de mărime medie, iar 6000 de persoane sunt rănite (5, 6).

Rapoartele statistice ale Poliției Rutiere arată că cele mai multe accidente rutiere din România sunt cauzate de viteza neregulamentară şi neadaptarea acesteia la con-

diţiile de drum (29%), urmate de incidentele provocate de pietonii care traversează strada fără să se asigure (25%), depăşirea neregulamentară (12%), neacordarea de prioritate (9%).

MATERIAL ŞI METODE

Studiul a fost efectuat, în perioada 2005-2010, pe un lot total de 5374 pacienţi din care internaţi în secţiile U.P.U. ale Spitalului Clinic de Urgenţe „Sf. Ioan" Iaşi (n=3050), Spitalului Judeţean Botoşani (n=1187) şi Spitalului Judeţean Vaslui (n=1137). Cazuistica din cei 6 ani calendaristici corespunde unui număr total de 2191 zile de activitate medicală în UPU, timp în care s-au inregistrat, în medie, 1,39 cazuri pe zi la U.P.U. Iaşi, 0,54 la U.P.U. Botoşani şi 0,51 cazuri pe zi prezentate în U.P.U. Vaslui. Culegerea datelor a fost realizată retroprospectiv, utilizându-se registrele de internare şi externare ale spitalului şi foile de observaţie ale pacienţilor. Prelucrarea datelor s-a efectuat cu ajutorul programului de statistică SPSS 16.0.

REZULTATE ŞI DISCUŢII

1. Incidenţa anuală a cazurilor de accidente de trafic rutier, în judeţele Iaşi, Botoşani şi Vaslui, în perioada 2005-2010, a arătat o tendinţă crescătoare a numărului de cazuri, în toate judeţele, cu un maxim în anul 2008, de 673 cazuri la Iaşi; 240 la Botoşani şi 285 cazuri la Vaslui (fig. 1). În ultimii doi ani luaţi în studiu au fost înregistrate mai puţine cazuri de accidente rutiere, aspect observat şi de alţi autori şi pus pe seama implementării mai eficiente a unor programe de prevenţie a accidentelor de circulaţie în general şi a celor rutiere, în special (3, 4, 5).

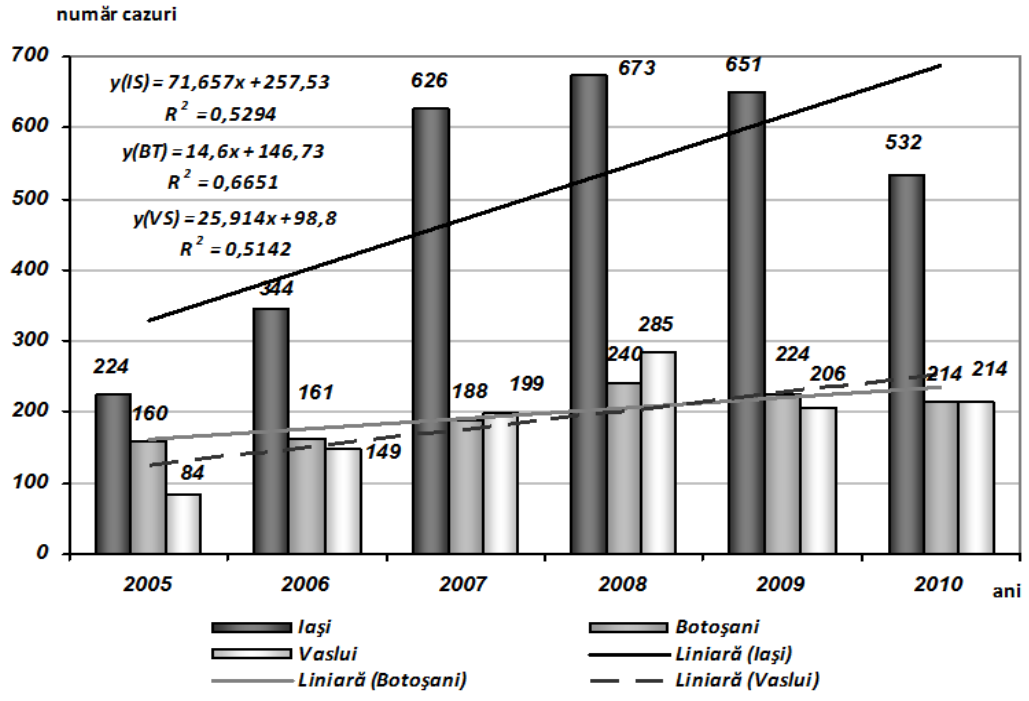

Fig. 1. Repartiţia anuală a numărului de cazuri de accidente de trafic rutier, comparativ în judeţele

Iaşi, Botoşani şi Vaslui, în perioada 2005 - 2010

2. Repartiția pe sex a cazurilor de accidente rutiere, în județele Iaşi, Botoşani şi Vaslui, în intervalul de timp studiat, arată că sexul masculin a fost mai frecvent implicat (cu un maxim de 67,2% în Vaslui), față de feminin (39,2% maxim în Iaşi), cu un raport bărbați / femei (calculat cumulativ pentru întreg lotul studiat şi toții anii) de aproximativ 1,8 (fig. 2).

Şi alți autori, în cercetările lor, publicate în țară şi străinătate, evidențiază faptul că bărbații sunt mai frecvent implicați în accidente rutiere decât femeile, luând în considerație atât riscul ocupațional (angajații conducători auto bărbați sunt mai numeroşi), cât şi faptul că temperamentul feminin este mai prudent, atât la volan, cât şi ca pieton (3, 5, 6, 8).

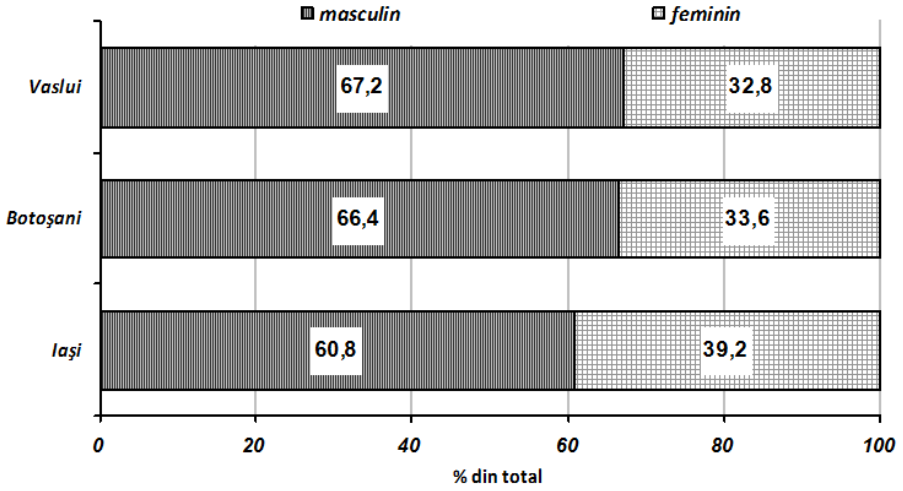

Fig. 2. Repartiția pe sex a numărului de cazuri de accidente de trafic rutier, comparativ în județele Iaşi, Botoşani şi Vaslui, în perioada 2005 - 2010

3. Distribuția pe grupuri de vârstă. Analiza de varianță ANOVA şi testul Bonferroni au arătat că, la Botoşani, au existat diferențe semnificative în ceea ce priveşte prezența în UPU a cazurilor din grupurile de vârstă 19-30 ani, comparativ cu cele peste 61 ani (F=4,947; p=0,000588). La Iaşi, aceleasi teste au arătat diferențe semnificative între toate grupurile de vârstă (F=19,197; p=0,000), grupul de vârstă <18 ani fiind exclus din această comparație. La Vaslui, diferențe semnificative au fost între grupurile de vârstă < 18 ani şi 19-30 ani (F=3,355; p=0,009681) (tab. I, II).

TABELUL I

Categorii statistice ale vârstei, comparativ în cele trei județe studiate

Indicatori statistici	Iaşi	Botoşani	Vaslui
Nr. total cazuri valide	3050	1187	1137
Media	40,44	39,12	34,49
Eroarea standard a mediei	0,321	0,508	0,575
Mediana	37	36	31
Modulul	20	20	19
Deviația standard	17,739	17	19,39
Minim	6	6	1
Maxim	96	98	89

TABELUL II

Distribuţia pe grupuri de vârstă, comparativ în cele trei judeţe studiate

Grupuri de vârstă (ani)	Jud. Iaşi		Jud. Botoşani		Jud. Vaslui	
	Nr.	%	Nr.	%	Nr.	%
≤ 18	70	2,3	71	6,0	249	21,9
19-30	1097	36,0	404	34,0	314	27,6
31-45	790	25,9	309	26,0	237	20,8
46-60	624	20,5	241	20,3	215	18,9
≥ 61	469	15,4	162	13,6	122	10,7
Total	**3050**	**100,0**	**1187**	**100,0**	**1137**	**100,0**

Şi alte cercetări, efectuate în diferite ţări, prezintă rezultate similare cu cele obţinute de noi, referindu-se la faptul că grupul de vârstă al adultului tânăr este cel mai frecvent implicat în producerea accidentelor rutiere (3, 8, 9, 10).

4. Repartiţia cazurilor în relaţie cu data, ziua şi ora prezentării. Analizând data prezentării pacienţilor în UPU, dacă s-a comparat cu o distribuţie egală / uniformă, s-au observat diferenţe semnificative între lunile anului, atât la Botoşani ($\chi2$=99,224, p=0,000), cât şi la Iaşi ($\chi2$=64,027, p=0,000) şi Vaslui ($\chi2$= 74,050, p=0,000), cu o frecvenţă mai mare în august (Botoşani şi Vaslui) şi octombrie (Iaşi).

Diferenţe semnificative, comparativ pe zilele săptămânii, apar doar la Iaşi ($\chi2$=14,655, p=0,023) şi la Vaslui ($\chi2$=19,133, p=0,004). S-a constatat o frecvenţă mai mare a accidentelor în zilele de weekend, cu o medie de aproximativ 2 cazuri / zi, în toate cele trei judeţe.

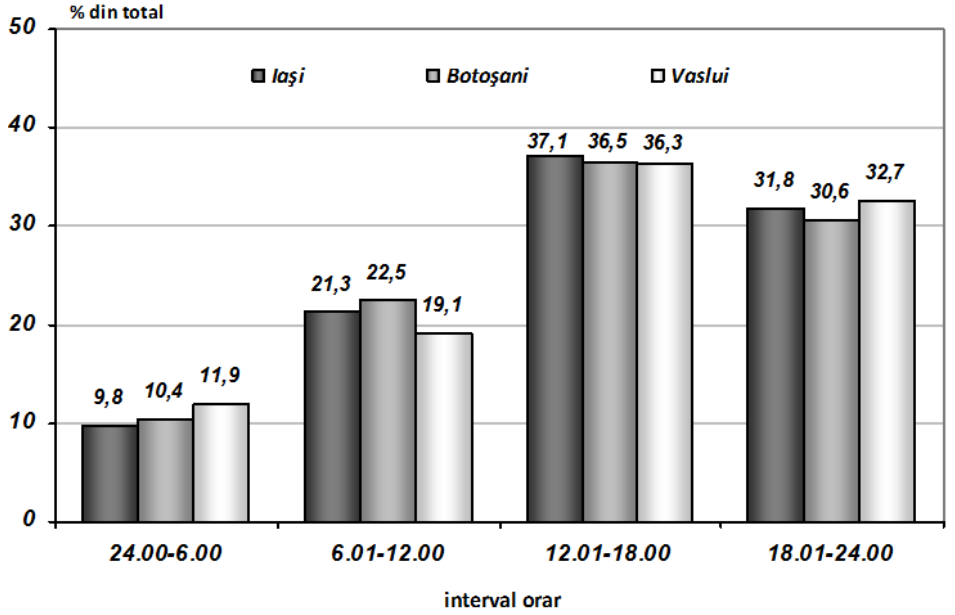

Fig. 3. Repartiţia în relaţie cu intervalul orar al prezentării în U.P.U. a cazurilor de accidente rutiere, comparativ în judeţele Iaşi, Botoşani şi Vaslui, în perioada 2005 - 2010

Referitor la intervalul orar al prezentării pacienților accidentați în traficul rutier, am constatat faptul că cele mai multe cazuri au fost întâlnite între orele 12.01 18.00, cu un maxim de 37,1% la Iași, maximul intervalului orar de 18.01-24.00 fiind constatat la Vaslui (32,7%); 6.01-12.00, la Iaşi (22,5%); 24.01-6.00 cu maxim tot la Vaslui (11,9%) (fig. 3).

Deci, distribuția cazurilor pe intervale de câte şase ore, începând cu ora zero, a evidențiat proporții similare cu cele din literatura de specialitate (2, 3, 6, 7) şi a fost semnificativ diferită față de o distribuție simetrică, aşteptată ($p<0,001$). Aceste rezultate pot fi explicate prin intensitatea crescută a traficului rutier în județul Iaşi, în perioada de zi (ocupațional, pietonal), iar pentru primele ore ale dimineții, cât şi ale amurgului, prin efectuarea navetei între cele trei județe.

5. Distribuția leziunilor în funcție de localizare a arătat faptul că traumatismele cranio-cerebrale (TCC) şi cele craniofaciale (TCF) au fost cel mai frecvent întâlnite, în toate cele trei județe (Iaşi – 829 cazuri; Botoşani – 598; Vaslui - 500), dar şi leziunile de tip fracturi ale gambei, contuziile toracelui şi ale membrelor superioare au fost situate în primele locuri (tab. III).

TABELUL III

Distribuția leziunilor, în funcție de localizare, ale cazurilor de accidente rutiere, comparativ în cele trei județe studiate

Tipul leziunii	Jud. Iaşi		Jud. Botoşani		Jud. Vaslui	
	Nr.	%	Nr.	%	Nr.	%
TCC, TCF	829	27,2	598	50,4	500	44,0
Comoții cerebrale, leziuni intracraniene	145	4,8	81	6,8	119	10,5
Traumatisme vertebrale	217	7,1	95	8,0	100	8,8
Fracturi viscero-neurocraniene	53	1,7	52	4,4	53	4,7
Fracturi pelvis	171	5,6	33	2,8	41	3,6
Fracturi femur	284	9,3	47	4,0	57	5,0
Fracturi gambă	510	16,7	98	8,3	85	7,5
Fracturi picior	90	3,0	2	0,2	6	0,5
Fracturi claviculă, braț, umăr	335	11,0	83	7,0	78	6,9
Fracturi antebraț, mână	271	8,9	38	3,2	58	5,1
Fracturi coaste	138	4,5	83	7,0	13	1,1
Contuzii torace	506	16,6	240	20,2	183	16,1
Contuzii abdomen, lombe, bazin	471	15,4	188	15,8	162	14,2
Contuzii şold, coapse	192	6,3	82	6,9	89	7,8
Contuzii genunchi	361	11,8	109	9,2	298	26,2
Contuzii gambă, picior	321	10,5	82	6,9	272	23,9
Contuzii membre superioare	498	16,3	151	12,7	253	22,3
Luxații, entorse, întinderi	365	12,0	77	6,5	36	3,2
Plăgi deschise	343	11,2	200	16,8	297	26,1
Excoriații multiple	177	5,8	47	4,0	247	21,7
Leziuni acute organe interne abdominale	57	1,9	22	1,9	25	2,2

CONCLUZII

Accidentele rutiere, în județele Iaşi, Botoşani şi Vaslui, prin numărul mare de cazuri, se încadrează în aşa-numita *epidemie traumatică rutieră*, având în comun un profil al pacientului care se calchează pe următoarele caracteristici: adult tânăr, frecvent bărbat, care se prezintă în U.P.U cel mai adesea în luna august, în weekend, în intervalul orar 12-18, cu politraumatism, frecvent TCC, fractură de gambă, contuzie toracică şi / sau abdominală.

Cunoaşterea impactului traumatismelor prin accidente rutiere va facilita adaptarea U.P.U. la dinamica anuală a cazurilor, va permite o mai rapidă orientare a personalului medical în fața urgențelor medicale şi chirurgicale şi, pornind de la datele obținute, se pot implementa programe eficiente de prevenție primară a accidentelor rutiere, în cele județe studiate.

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EVALUAREA DISHOMEOSTAZIEI SISTEMULUI STOMATOGNAT LA VÂRSTNICI

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ASSESSMENT OF THE DYSHOMEOSTASIS OF THE STOMATOGNATHIC SYSTEM IN THE ELDERLY (Abstract) : Old age (over 65) is considered to be a fragile stage, a stage of involution and regression and very often raises multiple psychological and clinical problems. Just like the other stages of life, old age is characterized by certain psychological features, a certain psychical state that depends of the nervous central system's state and of past experiences of life as well. The effects of aging on the stomatognat system can be identified at all the system's elements ; it is sometimes difficult to differentiate them from the physiological or pathological phenomena and their mechanisms. After analyzing the dishomeostasis of the stomatognat system from a gerontologic perspective it resulted that the stomatologic geroindex established in relation to the dental chronology is dominated by the prevalence of mono or bi maxillary total edentation to which stomatologic treatment is associated. In order to be a success, from a therapeutic, functional and aesthetic point of view, specific techniques must be used on elderly patients. These techniques have to be integrated into the aging process and must be related to the elderly patient's energy to adjust to changes, an energy that decreases with the passing of time. **Key words**: DISHOMEOSTASIS, ELDER PATIENT, STOMATOGNAT SYSTEM, GERONTOSTOMATOLOGY

Gerontologia, domeniul al cunoştinţelor despre procesul de îmbătrânire umană a acumulat experienţă în contextul dezvoltării doctrinelor fiziologice şi mai târziu în dezvoltarea cunoştinţelor biologiei şi medicinei cu multă vreme înainte de epoca noastră. Observaţiile au permis să se strângă informaţii din viaţa socială, privind bătrâneţea, ca vârstă a înţelepciunii, vârsta bilanţurilor, cu tendinţă de împăcare cu lumea, de detaşare treptată de magma ei fierbinte.

Bătrâneţea şi problematica sa a fost semnalată din înscrisurile antice cu mult înainte de perioada sumeriană şi aprofundată pe linia filozofică, apoi pe ramurile sale ştiinţifice, pînă azi, pe măsură ce cunoaşterea a luat locul intuiţiei sau speculaţiilor filozofice. Însă toate aceste observaţii sunt străbătute de aceleaşi idei că : *îmbătrînirea* şi *moartea* nu sunt decât accidente, viaţa fiind eternă. Îmbătrânirea a fost considerată o pedeapsă a spiritului în astrologia şi magia timpului.

Efectele îmbătrânirii la nivelul sistemului stomatognat, atinge toate elementele sistemului, uneori greu de diferenţiat între fenomenele fiziologice şi cele patologice, cât şi a mecanismelor lor exacte.

Gerontostomatologia s-a impus astfel ca o disciplină de sine stătătoare cu concept, strategie şi metodologie deja precizate în contextul medicinei stomatologice, orientată pe integrarea bio-psiho-socială.

Abordarea sistemului stomatognat, impune probleme specifice şi particularităţi care se corelează cu aspectele involutive generale şi locoregionale.

De la Hippocrat încoace, evaluarea comparativă organism uman- sistem stomatognat a cunoscut numeroase precizări care iniţial au fost constatate ca simptomatologie, ulterior au fost explicate pe baze ştiinţifice din ce în ce mai perfecţionate tehnologic.Cu toate acestea o abordare analitică aşa cum este realizată încă de medicina modernă, nu a permis studii deosebit de performante din punctul de vedere al sistematicii biologice, iar studiile sinergetice sunt încă limitate de exigenţele etice şi financiare care influenţează aplicarea lor în practica medicală şi cu atât mai mult în stomatologie.

Evaluarea vârstnicului a prezentat în contextul dezvoltării gerontostomatologiei noi faţete ale particularităţilor morfofuncţionale cu implicaţii directe în abordarea terapeutică a cazurilor.

Cu toate noutăţile importante în domeniile gerontologiei şi geriatriei, homeostazia vârstnicilor rămâne o problemă cu multe necunoscute.

Sistemul stomatognat sub aspectul abordării homeostazice a fost raportat în premieră de şcoala ieşeană de stomatologie, care a precizat corelaţiile intra şi extra sistemice ce conduc la homeostazie sau dishomeostazie în contextul teoriei "etiopatogenice integrative" (1,2).

OBIECTIVELE LUCRĂRII

Măsura în care formele clasice ale dishomeostaziei sistemului stomatognat la vârstnici se succed sau se interpătrund, şi care sunt metodele cele mai eficiente de evaluare a sistemului stomatognat la vârstnici, avându-se în vedere particularităţile de management impuse de această categorie de vârstnici.

Cunoscându-se faptul că vârstnicului îi sunt caracteristice limite largi de variabilitate în privinţa simptomatologiei sistemului stomatognat, în contextul homeostatic, se impune o conduită sistematică de investigare şi terapeutică pe considerente gerontostomatologice.

Gerontostomatologia a şi precizat o serie de aspecte manageriale incluse în contextul evaluării sistemului stomatognat la vârstnici.

MATERIAL ŞI METODĂ

Homeostazia şi dishomeostazia sistemului stomatognat sunt achiziţii recente ale medicinei stomatologice care au generat şi mai generează controverse pe tărâmul semantic, conceptual şi chiar în contextul pragmatic al acestei specialităţi.

Homeostazia şi dishomeostazia sunt două concepte importante pentru organismul uman şi implicit sistemul stomatognat considerat ca subsistem.

Homeostazia reprezintă în esenţă *–înţelepciunea organismului* (7) ce poate fi interpretată în raport de factorii de referinţă, căile de realizare, componentele sistemelor homeostazice şi factorul cronologic.Formele homeostaziei organismului viu sunt : cronobiologice ; genetică şi imunocelulară ; morfologică şi funcţională ; nervoasă ; neuropsihică. (6).

Cunoaşterea etapelor dishomeostaziei(*de susceptibilitate, preclinică, de debut, manifestă, de decompensare*) care *caracterizează orice altă maladie*, permite aprecierea evolutivă şi prognostică a dinamicii sistemului stomatognat, raportată la conceptul adaptabilităţii presupus de procesul homeostazic.

Studiile privind îmbătrânirea sub diferitele ei aspecte-*senescenţă- senectute- senilitate* - precizează următoarele aspecte : în raport cu evoluţia ontogenetică, îmbătrânirea este un proces dinamic complex cu debut latent şi la vârste fragede ; fenomenul îmbătrânirii nu este linear şi identic la populaţia de aceeaşi vârstă, şi se caracterizează printr-o variabilitate ce îmbină fenomenele de homeoreză cu cele de boală ; variabilitatea gerontologică poate fi stabilită pe trei nivele în raport de geroindex şi poate exprima o

TABELUL I
Repartiţia pe vârste şi sexe a pacienţilor examinaţi

VÂRSTA	SEX FEMININ	SEX MASCULIN	LOTURILE DE STUDIU
54-64 ANI	13	18	LOTUL I
65-74 ANI	11	12	LOTUL II
75-84 ANI	2	5	LOTUL II
PESTE 85 ANI	1	1	LOTUL II

îmbătrânire fiziologică, îmbătrînire nefiziologică şi îmbătrănirea patologică. După aspectul geriatric există o polipatologie somato-psihică ce evoluează pe căile homeostaziei-dishomeostaziei.

Aspectele îmbătrănirii generale se interrelaţionează fără dubiu cu involuţia sistemului stomatognat, care este guvernat de aceleaşi legi biologice.

În contextul aprecierii sistemului stomatognat al vârstnicilor se impune reconsiderarea criteriilor de evaluare şi terapeutică în raport de particularităţile gerontologice şi geriatrice stipulate de studiile nosologice şi particularităţile gerontostomatologice care sunt precizate în multe cercetări.

Categoria vârstnicilor cuprinde o mare variabilitate de criterii : domiciliari/ instituţionalizaţi ; valizi / invalizi / handicapaţi.

Această variabilitate se materializează şi în contextul stomatologic, fiind pacienţi cu diferite forme de patologie a sistemului stomatognat.

Au fost examinaţi 63 pacienţi de vârste cuprinse în contextul gerontologic între 54 şi peste 85 de ani, cu diagnosticul stării generale precizat în contextul explorării comportamentale indicate de stomatolog, sau medicul generalist.

Cei 63 de pacienţi examinaţi au fost împărţiţi în două loturi :

Lotul martor I - reprezentat de pacienţi cu vârste cuprinse între 54 şi 64 ani-31 pacienţi-49,20%.

Lotul de studiu II - reprezentat de pacienţi cu vârste cuprinse între 65 şi peste 85 de ani-32 pacienţi-50,80%.

Scopul examinării a constat în decelarea simptomatologiei ce poate aparţine factorilor suprasistemici, intrasistemici şi reflectă interrelaţia lor.

În contextual evaluării pacientului gerontostomatologia a precizat coordonatele manageriale sistematizate în raport de cerinţele vârstnicului şi conceptual gnatoprotetic cu orientare sistemică integrativă.

Factorii suprasistemici sunt factorii psihici, neurologici, endocrine, boli de sistem, boli metabolice ce duc la elementele sistemului (dinte, parodonţiu, muşchi, ATM, oase) dând naştere la sindromul disfuncţional al sistemului stomatognat.

Sistemul stomatognat cuprinde factorii intrasistemici-malocluzii, asimetrii funcţionale, asimetrii morfologice, obiceiuri vicioase şi patologie specifică.

REZULTATE ŞI DISCUŢII

Evaluarea dishomeostaziei sistemului stomatognat a parcurs etapele esenţiale preconizate de conceptual gnatoprotetic, fiind orientată pe examinarea semiologică sistemică : anamneză, stare generală, stare locală (extraorală, intraorală), examinări complementare în vederea precizării geroindexului (general, loco-regional, local) şi a necesităţilor terapeutice.

Examinarea prodiagnostică cu scop final, instituirea unei reechilibrări terapeutice în contextual sistemic au fost permanent comparativ orientate pe criteriile biologic, biomecanic, socio-economic, la care s-a adăugat încă o axă constituită din deducerea aspectelor generale şi implicit stomatognate : va-

riabilitate, fragilitate, dependenţă.

Din punct de vedere gerontostomatologic această axă se raportează la aspectele esenţiale ale simptomatologiei vârstnicului.

Evaluarea anamnestică s-a axat pe precizări importante vizând datele generale comparate cu cele actuale, condiţii de mediu, familiale, nutriţionale, anamneza în raport cu simptomatologia dominantă şi de etapă terapeutică prezentă la momentul evaluării, anamneza familială şi aspectele comparative, loco-regionale şi locale prezente.

Examinarea generală a stării s-a realizat în contextual interdisciplinarităţii-cabinet gerontostomatologic → cabinet de familie → cabinet de specialitate → medic geriatrie.

În privinţa colaborării gerontostomatologie-geriatrie, datele atestă receptivitatea existentă pentru această linie, dar şi necesitatea perfectărilor de cadru.

În privinţa documentelor obiective pe care un pacient le poate prezenta în contextul gerontostomatologic şi care pot să ateste trecutul medical dar şi starea prezentă, cea mai ergonomică metodă s-a dovedit colaborarea cu medical curant generalist sau de specialitate.

Din punct de vedere al stării generale somato-psihice la pacienţii examinaţi s-a constatat următoarele afecţiuni generale manifeste :

- 14 cazuri-afecţiuni cardiovasculare : 22,22%,
- 5 cazuri afecţiuni gastro-entero-hepatice : 7,93%,
- 3 cazuri cu afecţiuni bronho-pulmonare : 4,76%,
- 2 cazuri cu afecţiuni reumatismale cronice : 3,17%,
- 4 cu afecţiuni renale : 6,34%.

Diagnosticul a fost efectuat în clinici medicale.

Modificările cervico-faciale şi intraorale la examenul loco-regional au evidenţiat semnele caracteristice afecţiunilor asociate şi cu aspectele de senescenţă patognomonice.

Simptomatologia constatată în evaluarea gerontostomatologică este edificatoare pentru diagnosticul de stare generală influenţată pe filiera suprasistemică.

Modificările constatate merg pe două axe :

- Manifestări comune mai multor afecţiuni generale (modificări de culoare-cianoză, paloare, congestie la nivelul tegumentelor şi mucoaselor) şi manifestări caracteristice patognomonice pentru unele afecţiuni generale ;
- Manifestări gerontologice conexe involuţiei bioreutice.

Importanţa semiologică a modificărilor de la nivelul sistemului stomatognat pentru analiza prodiagnostică a stării generale a fost semnalată de numeroşi autori. Relevante ni se par aspectele evolutive ale acestor modificări (agravarea sau reducerea intensităţii lor fiind concordantă cu stadiul de decompensare al afecţiunii generale dar şi cu vârsta, sexul şi predispoziţia constatată pe baza datelor anamnestice).

Aspectele de dishomeostazie au inclus şi modificările psihosomatice prezentate pe parcursul examinării proterapeutice, în scopul decelării particularităţilor de adaptare la varianta terapeutică preconizată.În condiţiile actuale reacţiile psihosomatice sunt dominate mai puţin de criteriul biologic, au prevalat aspecte socio-economice care domină existenţa jurnalieră a fiecărui vârstnic impunând o conduită frustrantă uneori (lipsa condiţiilor de reabilitare generală într-un context psihosocial neprotectiv).

Înţelepciunea vârstei recunoscută de-a lungul evoluţiei speciei a dovedit un model comportamental decisiv în asigurarea cooperării prodiagnostice.

Explicarea necesităţilor de investigare complexă a indus un comportament mai cooperant pentru pacienţii din lotul studiat în comparaţie cu cel martor, dominat de problematica recentei pensionări asociată cu sentimente mai acute de frustare şi inutilitate.

Dishomeostazia sistemului stomatognat în contextul gerontostomatologic a prezentat următoarele aspecte : prezenţa modificărilor involutive caracteristice vârstnicilor asociată cu afectarea stării generale şi afecţiunile pre-

dominante specifice cardiovasculare care prevalează în raport cu cele bronhopulmonare, şi alte tipuri de afecţiuni.

Afecţiunile gerontologice specifice involuţiei se asociază cu patologia de predispoziţie declanşată din perioada de maturitate şi presenescenţă, fapt ce impune o conduită profilactică mult mai bine supravegheată cu un cadru adecvat.

Geroindexul general stabilit este predominant supraunitar, semnalizând îmbătrânirea accelerată, în timp ce valorile subunitare-îmbătrânire întârziată, sunt rar întîlnite.

Analizând dishomeostazia sistemului stomatognat în contextul gerontologic constatăm geroindexul stomatologic stabilit în raport de coordonatele vârstelor biologică / cronologică dentară, dominată de prevalenţa edentaţiei totale uni sau bimaxilare, la care se asociază şi tratamente stomatologice.

O particularitate a evaluării gerontostomatologice este aceea că prevalează tratamentele iatrogenice din perioada matură sau de prenescenţă, ce au o influenţă majoră dishomeostazică.

Din punct de vedere al involuţiei biologice, adaptabilitatea se diminuează iar rezistenţa scăzută a elementelor stomatognate se asociază cu necesitatea trecerii bruşte de la o stare de edentaţie redusă la extinsă sau subtotală cu rol agravant pentru starea generală influenţată.Evaluarea prodiagnostică este dominată de conduita terapeutică, ceia ce necesită tranziţii şi temporizare pe conceptele protezării imediate sau precoce pentru a evita dishomeostazia.

Analizând dishomeostazia sistemului stomatognat în contextul gerontologic constatăm geroindexul stomatologic stabilit în raport de coordonatele vârstelor biologică / cronologică dentară, dominată de prevalenţa edentaţiei totale uni sau bimaxilare, la care se asociază şi tratamente stomatologice.

CONCLUZII

Evaluarea gerontostomatologică va trebui să se raporteze mai intens în contextul interdisciplinarităţii medicină generală stomatologie, în beneficiul pacienţilor vârstnici.

Evaluarea gerontostomatologică se impune a fi realizată în raport de căile şi formele de manifestare ale dishomeostaziei sistemului stomatognat, motivelor prezentării pacienţilor vârstnici li se adaugă comparativ şi doleanţele lor în raport de care se va adapta şi conduita terapeutică.

O evaluare atentă a parametrilor de adaptare a sistemului stomatognat ar permite o adaptare optimală, funcţională şi estetică pe parcursul tratamentului.

La vărstnici, problemele de recuperare funcţională şi estetică fiind supuse limitelor energiei de adaptare trebuie statutate din perioada evaluării sistemului stomatognat.

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DETERMINĂRI CANTITATIVE, PRIN E-TEST, PENTRU EVALUAREA REZISTENŢEI *NEISSERIA GONORRHOEAE*

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E-TEST QUANTITATIVE DETERMINATION FOR EVALUATING *NEISSERIA GONORRHOEAE* RESISTANCE (Abstract): Thanks of underreported and of difficulties of isolation, antibiotic susceptibility profile of *N. gonorrhoeae* strains circulating is not sufficiently known in our country as well in the Suceava county. In addition, WHO' experts recommended the establishment of MIC (minimum inhibitory concentration) values using E-test strips, completing the database at the European level. **Aim**: To determine the type of resistance of *N. gonorrhoeae* strains by E-test in patients with gonorrhoea in Suceava county, in the 2009 -2010 period. **Material and method**: We tested the sensitivity of 32 strains of *N. gonorrhoeae* isolated using classical algorithm and E-test strips according with CLSI 2008 *(Clinical and Laboratory Standard Institute*) standard. We tested the sensitivity for penicillin, amoxicillin, augmentin, clarytromycin, tetracycline, ceftriaxone, ciprofloxacin, and spectinomycin. Production of β-lactamases was performed using API-NH test (*Neisseria-Haemophylus-Biomerieux*). **Results**: 96.9% strains were sensitive for ceftriaxone and spectinomycin, each 10 strains (31.2%) were resistat for penicillin and tetracycline, 34.5% strains were sensitive for amoxicillin, 37.5% sensitive for ciprofloxacin, and 13/32 strains (40.6%) were sensitive for augmentine. 7 strains were ß–lactamases positive and sensitive to all antibiotics, excepting penicillin and tetracyclin. Our results, especially the low rate of sensitivity for penicillin and tetracycline (68.8%) were similar with other from Asia, America or Africa, including Iaşi region. **Conclusion**: Our results demonstrated for first time in the studied aria, using E-test strips, the level of resistance of *N. gonorrhoeae* offering useful informations for clinicians in order to treat the patients with ceftrixone and spectinomycine as empirical treatment, and for other antibiotics, according with antibiogram results. **Key words**: *NEISSERIA GONORRHOEAE*, E-TEST, MIC, SENSITIVITY, RESISTANCE

În prezent, în România, nu se cunosc morbiditatea, incidenţa, prevalenţa sau spectrul de sensibilitate la antibiotice şi circulaţia tulpinilor rezistente de *Neisseria gonorrhoeae*. În general, incidenţa gonoreei este subraportată în România, inclusiv în judeţul Suceava, în principal prin lipsa de adresabilitate a pacienţilor care preferă anonimatul sau/şi tratarea infecţiilor cu transmitere sexuală prin eludarea sistemului de supraveghere al bolilor transmisibile. Datorită subraportării şi dificultăţilor de izolare binecunoscute, profilul de sensibilitate la antibiotice al tulpinilor circulante de *N. gonorrhoeae* nu este suficient de bine cunoscut. În plus, majoritatea autorilor recomandă stabilirea CMI, prin utilizarea benzilor E-test, în vederea completării bazei de date la nivel european (1,2).

Studiul a avut ca **obiectiv** stabilirea tipu-

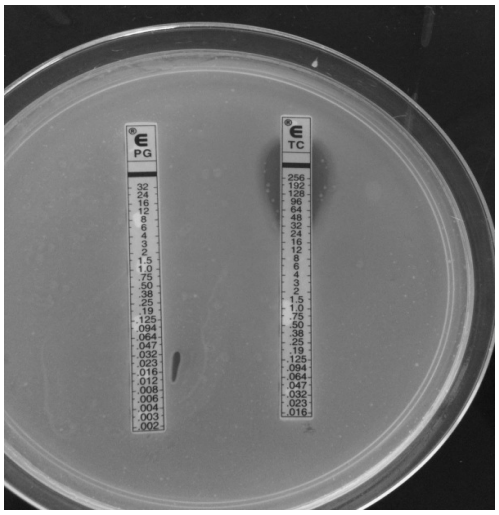

Fig. 1. E-test pe mediu GC agar bază completat cu 1% IsoVitaleX, fară hemoglobină pentru penicilină şi tetraciclină

lui de rezistenţă a tulpinilor de *N. gonorrhoeae*, prin E-test, la bolnavi cu gonoree din judeţul Suceava, în perioada 2009-2010.

MATERIAL ŞI METODE

În vederea selectării tulpinilor incluse în studiu, prelevarea, transportul şi conservarea probelor până la prelucrare s-au realizat ţinând cont de sensibilitatea gonococului la factorii de mediu ; tampoanele utilizate pentru recoltarea secreţiilor endo-cervicale, la femei şi a secreţiei matinale, la bărbaţi au fost însămânţate pe medii preîncălzite la 37°C, cu pH de 7,2-7,8. Am realizat frotiuri colorate Gram şi cu albastru de metilen care au fost examinate la microscopul cu obiectiv cu imersie pentru 32 probe (de la 6 femei şi 26 bărbaţi) recoltate în perioada octombrie 2009-august 2010. Am însămânţat produsele patologice pe medii nutritive (GC bază + 1% hemoglobină + 1% supliment nutritiv IsoVtalex, VITOX cu supliment selectiv VCN), plăcile fiind incubate în atmosferă de 5% CO_2 pentru 24-72 h la 37°C, 70% umiditate. Identificarea s-a realizat conform algoritmului clasic, cu truse comerciale API-NH (*Neisseria-Haemophylus - Biomerieux*), care au posibilitatea detectării concomitente şi a producerii de ß-lactamază. După identificare, tulpinile au fost stocate prin congelare la -25°C în bulion tripticază soia şi 20% glicerol (3). Am determinat valorile concentraţiilor minime inhibitorii (CMI) prin metoda E-test, tehnică recomandată de O.M.S ; suspensia utilizată a fost standardizată din cultura obţinută după 18 h cu etalonul 0,5 McFarland. Benzile E-test utilizate au fost : penicilină (CMI : 0,002-32 μg/mL), amoxicilină (CMI : 0,016-256 μg/mL), augmentin (CMI : 0.016-256 μg/mL) claritromicină (CMI : 0,016-256 μg/mL), tetraciclină (CMI μg/mL : 0,016-256), ceftriaxonă (CMI μg/mL : 0,002-32), ciprofloxacină (CMI μg/mL : 0,002-32), respectiv spectinomicină (CMI μg/mL : 0,064-1024) ; am repartizat câte două benzi pe fiecare placă (ex. penicilină- tetraciclină ; fig. 1).

Pentru stabilirea rezultatelor am utilizat criteriile de interpretare şi control, conform CLSI *(Clincal and Laboratory Standard Institute*) 2008 (tab. I) (4). Pentru asigurarea calităţii am utilizat tulpinile de referinţă de *N. gonorrhoeae* ATCC 49226, *Staphylo-coccus epidermidis* ATCC 12228 şi *Proteus mirabilis* ATCC 43071 pentru verificarea calităţii nutritive a mediilor de cultură şi a rezultatelor antibiogramei ; prelucrarea datelor, pentru cazurile de gonoree depistate şi confirmate s-a realizat utilizând programele *EpIData* şi *EpiInfo*.

REZULTATE

Am izolat 32 tulpini de *N. gonorrhoeae* de la 26 bărbaţi (81%) şi 6 femei (19%), respectiv de la cazuri suspecte de gonoree, pe baza definiţiei de caz (clinic şi epidemio-logic) ; 5 cazuri au fost confirmate numai prin frotiu.

Valorile CMI pentru penicilină, ampicilină, augmentin, tetraciclină, claritromicină, ceftriaxonă, ciprofloxacin, au fost transformate în procente (rate) de rezistenţă (tab.

TABELUL I

Valorile CMI, conform CLSI 2008 (4)

Antibiotic	Tulpină sensibilă CMI (µg/mL)	Tulpină intermediar sensibilă CMI (µg/mL)	Tulpină rezistentă CMI (µg/mL)
Penicilină	0,06	0,12-1	> 2
Ceftriaxonă	0,25	Nu există limite	Nu există limite
Ciprofloxacin	0,06	0,12-05	> 1
Spectinomicină	32	64	128
Tetraciclina	0,25	0,5-1	2-32

II) (3). În vederea exprimării rezultatelor obţinute este necesar să precizăm următoarele : producerea de penicinilază de către *N. gonorrhoeae* (PPNG - *Penicilinase Producing Ng*) este specifică tulpinilor β-lactamază pozitive ; rezistenţa cromosomală a *N. gonorrhoeae* (CMRNG – *Chromosomal Resistence Ng*) se defineşte pentru tulpinile care au CMI > 2 µg/mL şi sunt â-lactamază negative, iar CMI pentru tetraciclină este cuprinsă între 2-8 µg/mL ; rezistenţa la tetraciclină a *N. gonorrhoeae* (TRNG – *Tetracicline Resistante Ng*) se defineşte astfel : tulpinile au CMI > 16 µg/mL şi sunt β-lactamază negative (tab. I).

N. gonorrhoeae cu rezistenţa cromosomală la tetraciclină (*tetR*) are CMI pentru tetraciclină cuprinsă între 2-8 µg/mL şi CMI a penicilinei < 1 µg/mL ; rezistenţa la quinolone a *N. gonorrhoeae* (QRNG-*Quinolone Resistante Ng*) se defineşte pentru tulpinile care au CMI pentru ciprofloxacin > 1µg/mL ; în ceea ce priveşte ceftriaxona, valoarea CMI > 0,125µg/mL defineşte rezis-

TABELUL II

Rezultatele testării cu benzi E-test pentru tulpini de *Neisseria gonorrhoeae* izolate în perioada 2009-2010

Antibiotic	Rezistent % (pozitiv/total)	Intermediar % (pozitiv/total)	Sensibil % (pozitiv/total)
Ceftriaxona	**0 (0/32)**	**3,1 (1/32)**	**96,9 (31/32)**
Penicilina	**53,1 (17/32)**	**15,6 (5/32)**	31,2 (10/32)
Tetraciclina	**59,4 (19/32)**	**9,4 (3/32)**	31,2 (10/32)
Amoxicilina	**43,7 (14/32)**	**21,9 (7/32)**	34,4 (11/32)
Augmentin	**40,6 (13/32)**	**18,8 (6/32)**	40,6 (13/32)
Claritromicina	**34,4 (11/32)**	**40,6 (13/32)**	**25 (8/32)**
Ciprofloxacina	**34,4 (11/32)**	**28,1 (9/32)**	37,5 (12/32)
Spectinomicină	**0 (0/32)**	**3,1 (1/32)**	96,9 (31/32)

* Criterii de interpretare conform CLSI 2008 (4)

TABELUL III

Valorile CMI 50 şi CMI 90 şi limitele CMI de sensibilitate pentru antibioticele testate prin E-test la 32 izolate de *Neisseria gonorrhoeae*

Antibiotic	CMI (µg/mL) Limite	CMI (µg/mL) 50%	CMI (µg/mL) 90%
Ceftriaxona	**0,02-0,25**	**0,08**	0,25
Penicilina	**0,03-16**	**0,03**	16
Tetraciclina	**0,25-4**	**2**	32
Amoxicilina	**0,04-16**	**0,25**	16
Augmentin	**0,02-32**	**0,25**	8
Claritromicina	**0,016-12**	**0,38**	12
Ciprofloxacina	**0,02-32**	**0,06**	32
Spectinomicină	**0,064-64**	8	64

TABELUL IV

Rata de rezistenţă la antibiotice şi gradele de comparaţie cu alte state Europene, Japonia şi Australia pentru tulpini de *Neisseria gonorrhoeae* testate prin metoda E-test* (7-11)

	Ceftriaxona %	QRNG %	CMRNG %	PPNG %	TRNG %	Tet R %
Austria	0	53,1	3,1	**15,6**	1	46,9
Belgia	0	46,9	10,9	4,7	**12,5**	**35,9**
Danemarca	0	46	11,2	1	5,1	43,9
Anglia	0	12	8	1	6	20
Franța	0	**32,7**	10.9	**15**	6,7	57,4
Grecia	0	7,6	3,8	8,9	6,3	40,5
Italia	4,8	**33,3**	9,5	9,5	0	42,9
Olanda	0	16	0	8,6	8,6	33,3
Portugalia	0	17,6	0	**11,8**	35,3	**35,3**
Scoția	0	30,3	**17,2**	7,1	1	25,3
Spania	0	15,2	2,2	6,5	6,5	43,5
Suedia	1	48	7,3	9,6	8,3	**40,6**
Japonia	30,3	78,3	59,3	1	2,2	68,8
Australia	-	**38**	**34**	-	**12**	-
România (jud.Suceava)	0	**34,4**	**25**	**21,8**	**12,5**	**37,5**

* Rezistenta înaltă a fost corelată cu rezistenţa mediată plasmidic iar rezistenţa moderată/intermediară a fost corelată cu rezistenţa mediată cromosomal. Criteriile utilizate sunt cele ale CLSI (6)

tenţa pentru tulpinile de *N. gonorrhoeae*, iar rezistenţa la spectinomicină este definită pentru tulpinile care au CMI > 128 μg/mL (5).

Rezultatele testării prin utilizarea benzilor E-test, pentru cele 32 de tulpini de gonococ studiate sunt prezentate în tabelul II, iar criteriile de interpretare, conform CLSI 2008 sunt redate în tabelul III.

Dintre tulpinile studiate, şapte au fost β-lactamază pozitiv. Un număr de 17 tulpini au fost rezistente la penicilină (17/32), iar 19 dintre ele, la tetraciclină (19/32) (tab. II). La testarea sensibilităţii pentru penicilină, 7 tulpini (21,8%) au avut rezistenţă de nivel înalt, corelată cu rezistenţa plasmidică PPNG, iar 8 tulpini (25%) au fost definite CMRNG, deoarece mecanismul rezistenţă a fost mediat cromosomal (CMI >1 μg/mL) (tab. IV).

19 tulpini au fost rezistente la tetraciclină (tab. II) : 1l tulpini (37,5%) au avut rezistenţă înaltă, mediată cromosomal *tetR* (CMI >8 μg/mL), iar 4 tulpini (12,5%) au fost TRNG (CMI > 16 μg/mL), cu rezistenţă mediată plasmidic (CMI >1 μg/mL) (tab. IV). CMI 90 cu valoarea 32 μg/mL a indicat o rezistenţă de nivel înalt (tab. III).

TABELUL V

Valorile CMI 50 şi CMI 90 şi limitele CMI* de sensibilitate la antibioticele testate pentru *Neisseria gonorrhoeae* în Europa de Vest (12)

	CMI (µg/mL)	**CMI (µg/mL**	**CMI (µg/mL**
Antibiotic	**Limite**	**50%**	**90%**
Ceftriaxona	0,02-0,25	0,002	0,015
Penicilina	0,03-3,4	0,125	1
Tetraciclina	0,25-4	2	32
Ciprofloxacina	0,02-4	0,015	32
Spectinomiciina	0,064-64	8	64

*Criterii de interpretare conform CLSI (2008)

În ceea ce priveşte rezultatele testării pentru ciprofloxacin: 11 tulpini (34,4%) au fost rezistente (tab. II), fiind QRNG (CMI > 1 μg/mL) (tab. IV), iar 9 tulpini (28,1%) au prezentat o reducere a sensibilităţii la ciprofloxacin. CMI 90 cu valoarea 32 μg/mL a indicat o rezistenţă de nivel înalt, iar pentru CMI 50 am obţinut valoarea de 0,06 μg/mL (tab. III). O singură tulpină (3,1%) a prezentat o reducere a sensibilităţii la testarea pentru ceftriaxonă (tab. II). Deoarece concentraţia care defineşte reducerea sensibilităţii este o valoare CMI > 0,125 μg/mL (tab. I), nicio tulpină nu a fost rezistentă la ceftriaxonă. Rezultate similare s-au obţinut pentru spectinomicină: o singură tulpină (3,1%) a prezentat reducerea a sensibilităţii (tab. II). Nu am izolat nicio tulpină rezistentă la spectinomicină (respectiv cu CMI > 128 μg/mL). CMI 90 cu valoarea 64 μg/mL, indică o rezistenţă de nivel înalt, ceea ce corespunde unui CMI 50 de 8 μg/mL (tab. III).

DISCUŢII

Multirezistenţa a fost definită pentru următoarele asocieri: QRNG - PPNG; QRNG – TRNG; QRNG, PPNG şi TRNG. În timp ce pentru penicilină, tetraciclină, ceftriaxonă şi ciprofloxacin sunt criterii clare în ghidul pentru interpretarea sensibilităţii (4,6), pentru amoxicilină, augmentin şi claritromicină acestea nu sunt bine definite. Toate cele 7 tulpini ß-lactamază pozitive au fost rezistente la penicilină; producerea de β-lactamază nu a fost corelată cu reducerea sensibilităţii la alte antibiotice, exceptând tetraciclina.

Un număr mare de tulpini (20/32), respectiv 62,5%, au prezentat o reducere a sensibilităţii la ciprofloxacin. Scăderea sensibilităţii la antibiotice, în special pentru penicilină şi tetraciclină (68,7%) este comparabilă cu scăderea sensibilităţii tulpinilor raportată în Asia (74,3% pentru penicilină şi 98,4%, pentru tetraciclină); America (90% la penicilină şi 100% la tetraciclină); Africa (100% la penicilină şi 91,7% la tetraciclină) (7); Rusia (76% la penicilină şi 92% la tetraciclină) (8, 9, 10). Pentru zona de NE a ţării, în Iaşi, studii recente au determinat că 100% dintre tulpini sunt rezistente la penicilină şi 92,6%, la tetraciclină (11).

CMI 50 pentru penicilină a fost de 0,03 μg/mL, iar CMI 90, 16 μg/mL; valorile CMI 90 pentru tetraciclină (32 μg/mL), respectiv CMI 50 (2 μg/mL) sunt comparabile cu ţările din vestul Europei (tab. V); reamintim că CMI 50 şi CMI 90 (tab. III) sunt concentraţiile care inhibă 50%, respectiv 90% dintre tulpinile testate (concentraţia cea mai mică de antibiotic inhibitorie pentru un anumit procent din tulpinile testate).

Determinarea CMI este o necesitate practică, deoarece permite nu numai decizii terapeutice mai subtile, ci facilitează analiză epidemiologică a rezistenţei la agenţii antimicrobieni. Aceste valori permit orientarea rapidă către cel mai indicat medicament şi alegerea antibioticoterapiei de primă intenţie.

CONCLUZII

În zona noastră penicilina, tetraciclina, ciprofloxacina, claritromicina, amoxicilina şi augmentinul, nu pot fi folosite în tratamentul gonoreei, decât după rezultatul testării la antibiotice.

Continuarea la nivel regional şi naţional a supravegherii sensibilităţii la antibiotice este crucială în detecttaea emergenţei noilor fenotipuri de rezistenţă, dublată de monitorizarea variaţiei *pattern*-urilor de sensibilitate, în vederea adaptării schemelor terapeutice.

Evoluţia sensibilităţii la cefalosporine trebuie monitorizată obligatoriu, fiind singura clasă de antibiotice disponibilă în momentul de faţă pentru terapia sindromică în uretrita gonococică, pentru surprinderea apariţiei tulpinilor rezistente.

În continuare sunt necesare studii microbiologice extinse, inclusiv prin utilizarea markerilor de epidemiologie moleculară, pentru stabilirea mecanismelor de rezistenţa şi a modului lor de transmitere.

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NOUTĂŢI

Urolitiaza pediatrică : îngrijirea chirurgicală curentă

Copiii reprezintă 1% din totalul pacienţilor cu urolitiază dar 100% din aceşti copii sunt consideraţi cu risc înalt de recidivă a formării de calculi astfel ncăt este crucial pentru ei sa primească o terapie care îi va feri de formarea de calculi în viitor. În plus, este necesară o supraveghere metabolică pentru a asigura p prevenţie sau o întârziere a recurenţelor. Cea mai adecvată terapie depinde de localizare, mărime, şi compoziţia calculilor, precum şi de anatomia tractului urinar, În centrele specializate ste accesibil întregul arsenal delitotripsie prin undă de şoc extracorporeală (ESWL), ureterorenoscopie (URS) şi nefrolitotomie percutană (PCNL) ; toate au la copil aceeaşi eficacitate şi siguranţă ca şi la adult (Straub M, Gschwend J, Zorn C, Pediatric urolithiasis : the current surgical management, *Pediatr Nephrol* 2010 ; 25 : 1239-1244).

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SEMNIFICAŢIA IMUNOHISTOCHIMICĂ A CK5/6 ŞI P63 ÎN LEZIUNILE PREMALIGNE ŞI ÎN ADENOCARCINOMUL DE PROSTATĂ

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IMMUNOHISTOCHEMIC STAINING FOR CK5/6 AND P63 SIGNIFICANCE IN PROSTATE PREMALIGNANT LESIONS AND ADENOCARCINOMA (Abstract): The morphologic information from usual hematoxiline-eosine staining are sometimes insufficient in certifying or excluding the adenocarcinoma of the prostate, cases who must be study by immunohistochemistry. **Aim**: The investigation of staining for CK5/6 and P63 in cases diagnosed with prostate adenocarcinoma associated with high grade prostatic intraepithelial neoplasia. **Material and methods**: The study was realized on 56 cases analyzed in Pathology Laboratory of Focşani Emergency Hospital. **Results**: We registered a positive correct diagnosis for atypical adenomatous hyperplasia in 86% of cases and for prostate adenocarcinoma grade 1 in 14% of cases. **Conclusions**: CK5/6 and P63 are useful markers for elucidating a positive false diagnosis for prostate adenocarcinoma grade 1. **Key words:** PROSTATE ADENOCARCINOMA, HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA, ATYPICAL ADENOMATOUS HYPERPLASIA, CK5/6, P63

Adenocarcinomul de prostată (ACP) reprezintă peste 90% din cancerele epiteliale prostatice. Forma bine diferenţiată este dificil de diferenţiat de o serie de leziuni benigne pe criterii morfologice (1). Neoplazia intraepitelială de prostată de grad înalt (HPIN) este leziunea premalignă definită ca leziune proliferativă intraepitelială atipică la nivelul acinilor şi ductelor (2). Distincţia majoră între aceste două leziuni este prezenţa stratului celular bazal în HPIN, care poate fi demonstrat folosind anticorpi specifici pentru celule bazale (3). Hiperplazia adenomatoasă atipică (AAH) este definită ca o proliferare atipică a acinilor mici din prostată, ce poate fi diagnosticată eronat ca ACP de grad 1 datorită aspectelor citologice şi arhitecturale.

CK5/6 este o citocheratină superioară ca specificitate în diferenţierea glandelor benigne de glandele maligne. Celulele bazale din acinii prostatici benigni exprimă CK5/6 (4). Gena P63 este un membru al familiei P53 şi a fost folosită pentru a evidenţia celulele bazale din epiteliul prostatic normal (5). P63 are o mare specificitate datorită fixării nucleare, în timp ce CK5/6 are un mare potenţial pentru reacţie nespecifică, având fixare citoplasmatică (6).

Scopul acestui studiu a fost să investigăm expresia fixării CK5/6 şi P63 în cazurile diagnosticate cu ACP asociat cu HPIN şi să

demonstrăm utilitatea imunohistochimiei (IHC) cu markeri pentru celule bazale ce contribuie la acurateţea diagnosticului pozitiv de AAH şi la elucidarea diagnosticului fals pozitiv de ACP grad 1.

Lucrarea a fost structurată conform următoarelor obiective : 1. selectarea unui număr de 56 cazuri diagnosticate cu ACP asociat cu HPIN ; 2. investigarea expresiei fixării CK5/6 şi P63 în cazurile studiate, în scopul diferenţierii ACP grad 1 de AAH ; 3. analizarea utilităţii IHC cu markeri pentru celule bazale în cazurile studiate.

MATERIAL ŞI METODĂ

1. Criterii de includere

Au fost incluse în studiu cazurile de prostatectomii radicale diagnosticate cu ACP asociat cu HPIN, înregistrate în Laboratorul de Anatomie Patologică al Spitalului Judeţean de Urgenţă Focşani în perioada octombrie 2006- noiembrie 2008.

2. Lotul de studiu

Din totalul cazurilor de 240 de prostatectomii radicale înregistrate în Laboratorul de Anatomie Patologică, au îndeplinit criteriile de includere un număr de 56 cazuri.

3. Analiza datelor

Pentru prelucrarea statistică a datelor din cadrul acestei cercetări s-a folosit softul statistic specializat *SPSS 13.0* şi Excel XP. S-au utilizat statistici descriptive, χ^2, corelaţii calculate la un prag de semnificaţie de 95%.

4. Tehnica de lucru

Am realizat un studiu IHC pe un număr de 56 cazuri de prostatectomii radicale diagnosticate cu ACP asociat cu HPIN, înregistrate în Laboratorul de Anatomie Patologică al Spitalului Judeţean de Urgenţă Focşani.

Studiul IHC a fost realizat pe baza folosirii a doi markeri pentru celule bazale prostatice : CK5/6 şi P63. Blocurile de parafină din materialul bioptic de studiat au fost secţionate la microtom la 2μ, secţiunile fiind apoi prelucrate pentru a fi colorate IHC. Pentru cercetarea IHC s-a folosit metoda avidină-biotină-peroxidază pe secţiunile obţinute din blocurile incluzionate la parafină. Reacţia pentru CK5/6 a fost realizată utilizând anticorpul Anti-Human citokeratin 5/6 monoclonal mouse clona D5/16B4, cod M7237(Dako, Danemarca). Reacţia pentru P63 a fost realizată utilizând anticorpul Anti-Human P63 protein monoclonal mouse, clona 4A4, cod M7247(Dako, Danemarca). Pentru ambii anticorpi s-a folosit ca anticorp secundar Biotinylated Goat Anti-Polyvalent (Ultra Vision Large Volume Detection System Anti-Polyvalent, Lab Vision Corporation), iar pentru evidenţierea antigenului s-a aplicat DAB (Liquid DAB Substrate Chromogen System cod K3466, Dako Danemarca).

Expresiile CK5/6 şi P63 la cazurile luate în studiu au fost analizate în grupuri morfologice ţinând cont de continuitatea fixării markerilor la nivelul celulelor bazale prostatice.

Astfel :

1. ACP ca şi control negativ a fost caracterizat prin absenţa pozitivării la CK5/6 şi P63, fiind considerată ca reacţie negativă (-).
2. AAH şi HPIN : cazurile în care pozitivarea la CK5/6 şi P63 a fost discontinuă au fost încadrate ca având reacţie uşor pozitivă (+), iar cazurile în care pozitivarea la CK5/6 şi P63 a fost continuă au fost considerate ca având reacţie moderat pozitivă (++).
3. Glandele benigne adiacente ca şi control pozitiv au fost caracterizate prin pozitivitate.

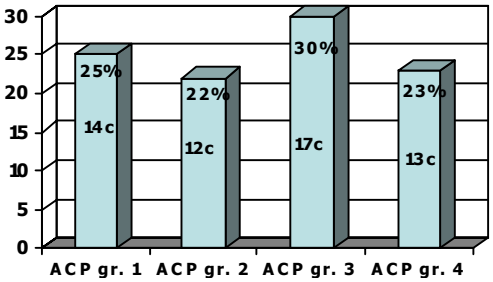

Fig. 1. Distribuţia cazurilor în raport de gradul Gleason şi criterii morfologice

varea la CK5/6 şi P63, care a fost etichetată ca reacţie intens pozitivă (+++).

Fixarea cu P63 a fost mai intensă decât fixarea cu CK5/6 şi a fost mai uşor de interpretat, fiind o fixare nucleară faţă de fixarea citoplasmatică a CK5/6. Au fost folosiţi 2 markeri pentru celule bazale prostatice pentru siguranţa diagnosticului pozitiv.

REZULTATE ŞI DISCUŢII

1. Structura lotului pe grupe de vârstă

Din analiza lotului de studiu de 56 cazuri diagnosticate cu ACP asociat cu HPIN s-a obţinut următoarea distribuţie a cazurilor pe grupe de vârstă: 28% (16/56) la grupa de vârstă 60-69 ani, 52% (29/56) la grupa de vârstă 70-79 ani şi 20% (11/56) la grupa de vârstă 80-89 ani. Rezultatele noastre evidenţiază o pondere crescută a cazurilor până la 80 de ani de 80% (45/56) care necesită IHC, comparativ cu ponderea scăzută a cazurilor peste 80 de ani de 20% (11/56) care necesită IHC. Evaluările comparative relevă: cazurile care necesită IHC sunt semnificativ statistic mai frecvente până la 80 de ani ($\chi^2=38,89$; GL=1; p=0,001) Acest aspect este explicat prin faptul că frecvenţa ACP grad 1 scade odată cu vârsta (7).

2. Structura lotului de studiu în funcţie de gradul Gleason pe criterii morfologice

În urma examinării fragmentelor tisulare colorate cu HE, încadrarea în raport cu gradul Gleason a fost următoarea: grad Gleason 1=25% (14/56); grad Gleason 2=22% (12/56); grad Gleason 3=30% (17/56); grad Gleason 4=23% (13/56) (fig. 1).

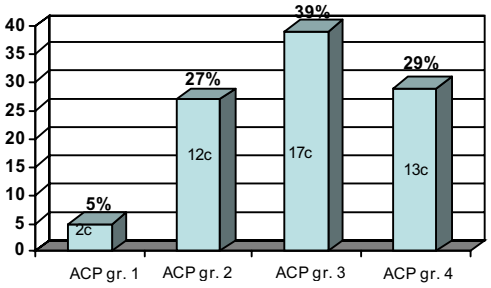

Fig. 2. Distribuţia cazurilor în raport de gradul Gleason şi criterii IHC

3. Structura lotului de studiu în funcţie de gradul Gleason după efectuarea IHC

Distribuţia cazurilor în raport cu gradul Gleason în urma efectuării IHC cu CK5/6 şi P63 a fost următoarea: grad Gleason 1=5% (2/44); grad Gleason 2=27% (12/44); grad Gleason 3=39% (17/44); grad Gleason 4=29% (13/44) (fig. 2).

Se constată că cel mai frecvent întâlnit este gradul Gleason 3 (39%), urmat de gradul Gleason 4 (29%) şi apoi de gradul Gleason 2 (27%). Gradul Gleason 1 este cel mai rar întâlnit (5%). Rezultatele noastre sunt similare cu datele din literatură (8).

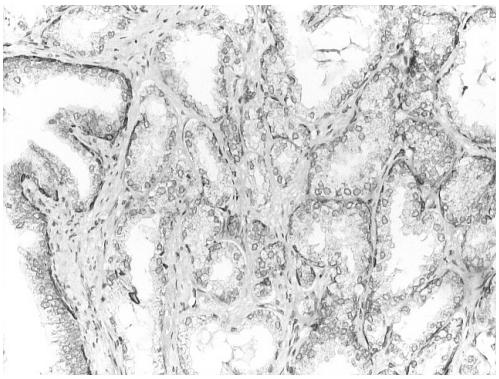

Fig. 3. HPIN asociat cu AAH, x10 CK5/6 fixare pozitivă discontinuă, reacţie uşor pozitivă(+)

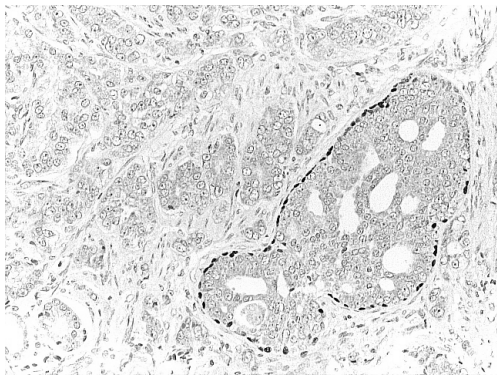

Fig. 4. HPIN asociat cu ACP grad 1, x10, P63 fixare negativă, reacţie negativă (-)

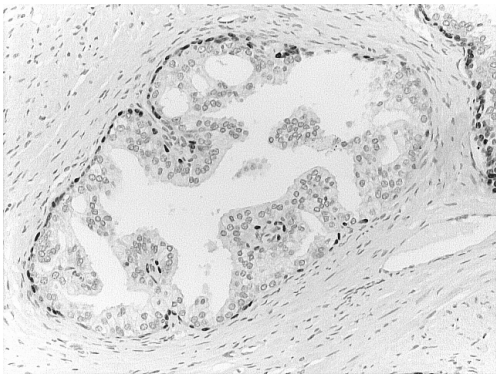

Fig. 5. HPIN, x10, P63 fixare pozitivă discontinuă, reacţie uşor pozitivă (+)

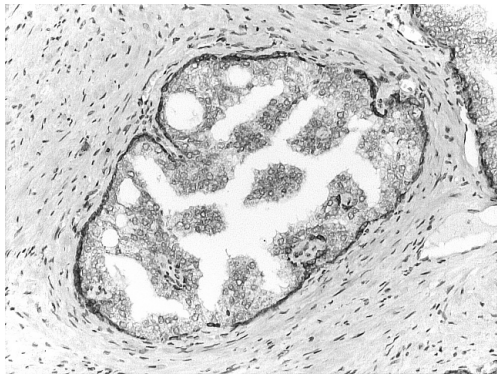

Fig. 6. HPIN, x10, CK5/6 fixare pozitivă continuă, reacţie moderat pozitivă (++)

4. Structura lotului de studiu în funcţie de reacţia fixării IHC cu CK5/6 şi P63

Examenul IHC a permis evidenţierea următoarelor aspecte în distribuţia cazurilor. Din cele 25% (14/56) cazuri diagnosticate ACP grad 1 prin coloraţie HE, 86% (12/14) au prezentat fixare pozitivă discontinuă fiind încadrate ca reacţie uşor pozitivă (+) şi diagnosticate AAH (fig. 3), iar celelalte 14% (2/14) au prezentat absenţa fixării markerilor, fiind încadrate ca reacţie negativă (-) şi diagnosticate ACP grad 1 (fig. 4). Restul cazurilor, reprezentând 75% (42/56) diagnosticate ACP grad 2,3,4 prin coloraţie HE, au relevat absenţa fixării markerilor fiind încadrate ca reacţie negativă (-).

În urma efectuării IHC, distribuţia cazurilor pe lotul de studiu a fost următoarea: 5% (2/44) au fost ACP grad 1, iar 95% (42/44) au fost ACP grad 2, 3 şi 4.

În ceea ce priveşte HPIN, 59% (33/56) au evidenţiat fixare pozitivă discontinuă (fig. 5), fiind încadrate ca reacţie uşor pozitivă (+), iar 41% (23/56) au prezentat fixare pozitivă continuă (fig. 6), fiind încadrate ca reacţie moderat pozitivă (++). Rezultatele noastre se pot compara cu cele relatate de Abrahams et al. 2003, care a raportat pentru HPIN o fixare pozitivă discontinuă a markerilor în 55% din cazuri şi o fixare pozitivă continuă a markerilor în 45% din cazuri (9).

Pentru control pozitiv glandele benigne adiacente au evidenţiat fixarea continuă a markerilor, fiind încadrate ca reacţie intens pozitivă (+++).

Din prelucrarea datelor statistice efectuate asupra celor două leziuni studiate, se pot face următoarele aprecieri.

1. Modificarea cazurilor ACP grad 1 după efectuarea IHC

În urma efectuării IHC cu markeri pentru celule bazale prostatice CK5/6 şi P63, cazurile diagnosticate cu ACP grad 1 au scăzut de la 25% (14/56) înainte de IHC la 5% (2/44) după IHC. Considerăm că este o diferenţă semnificativă statistic ($\chi^2 = 6,22$; GL = 1; p = 0,013) pentru confirmarea utilităţii IHC în acurateţea diagnosticului pozitiv de ACP grad 1.

2. Relaţia dintre ACP grad 1 şi AAH după efectuarea IHC

În urma efectuării IHC cu CK5/6 şi P63 s-a elucidat diagnosticul corect pozitiv de AAH în 86% din cazuri (12/14) şi de ACP grad 1 în 14% din cazuri (2/14). Rezultatele noastre demonstrează că este o diferenţă semnificativă statistic ($\chi^2 = 11,57$; GL = 1; p = 0,007) pentru confirmarea utilităţii markerilor CK5/6 şi P63 în diferenţierea ACP grad 1 de AAH, conform datelor din literatură (10).

Rezultatele noastre se pot compara cu cele relatate de Molinie et al., care a stabilit

un diagnostic corect pozitiv prin IHC în 91% din cazuri (11).

CONCLUZII

În prezentul studiu am explorat utilitatea IHC cu CK5/6 şi P63 în scopul elucidării diagnosticului fals pozitiv de ACP grad 1. Markerii pentru celule bazale prostatice se găsesc în celulele bazale ale glandelor benigne, dar sunt absente în ACP.

În studiul nostru grupul de cazuri ACP grad 1 asociate cu HPIN a avut aspecte morfologice de grad Gleason 1.

Folosirea IHC a condus la un diagnostic corect pozitiv de AAH în 86% din cazuri şi de ACP grad 1 în 14% din cazuri. Rezultatele noastre arată că un procent semnificativ de cazuri cu modificări morfologice de ACP grad 1 poate reprezenta AAH.

Considerăm că elucidarea diagnosticului pozitiv de AAH în 86% din cazuri este un argument pentru necesitatea utilizării IHC cu markeri pentru celule bazale în acurateţea diagnosticului pozitiv de AAH şi în elucidarea diagnosticului fals pozitiv de ACP grad 1.

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STUDIU DESCRIPTIV PRIVIND EVALUAREA STATUSULUI BIOCHIMIC PE UN LOT DE 464 DE PERSOANE DIN MEDIU URBAN ŞI RURAL DIN JUDEŢUL BACĂU

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BIOCHEMICAL STATUS ASSESSMENT IN 464 PERSONS FORM BACĂU COUNTY (Abstract) : The purpose of the study was to assess the biochemical status in a populational lot of 464 persons from Bacău County (urban and rural), through a minimal set of laboratory tests (glycemia, cholesterol, HDL-cholesterol, triglyceride). **Results** : The average values from the rural lot are closed to the minimal limits of the values interval. The highest values (peaks) of glycemia in the rural lot are in the over 65 y.o. group, followed by great values registered in the 41-65 y.o. group. For the urban lot, the highest value is in the 41-65 y.o. group, that might be explained by the etiopathogenesis role of stress in the diabetes at young ages. Also, comparing the peaks in these two lots (rural vs. urban), we can notice that in the rural lot, the peaks are much higher than in the urban lot. A possible explanation might be a tighter monitoring of diabetes in urban vs. rural or a proper diet and lifestyle behavior in the urban lot.The averages values of cholesterol in the rural lot, for all groups of ages, are below the maximal admitted value (220 mg/dL) and the peaks are in the 41-65 y.o. group and over 65 y.o., while in the urban lot the peaks are in the over 65 y.o. group. The minimal values of HDL cholesterol fraction which are inverse correlated with the risk of cardiovascular diseases emergence, are found in the extreme ages groups : 20-40 y.o. and over 65 y.o. In the urban lot, the average values for the three age groups are closed and shows a low risk of cardiovascular diseases emergence (values up to 55 mg/dL). Regarding the levels of triglycerides, even if are below the maximal admitted values, in the rural lot, the peaks values are dispersed and are very high in the extreme ages groups : 20-40 y.o. and over 65 y.o., while in the urban lot peaks values are in the 41-65 y.o. group. **Key words** : BIOCHEMICAL STATUS, MAXIMAL ADMITTED VALUE, AVERAGE VALUE

MONOCLONAL ANTIBODIES – PAST, PRESENT AND FUTURE

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MONOCLONAL ANTIBODIES – PAST, PRESENT AND FUTURE (Abstract): Monoclonal antibodies (mAbs) are monovalent antibodies, produced artificially from a single B-lymphocyte clone. They have a higher specificity binding to the same epitope. The mAbs era started in 1975 with the monoclonal antibody technique. This field is rapidly growing, around 60 therapeutic monoclonal antibodies being now available on the market for organ transplantation or to treat several diseases. Over the years, the technological developments and improvements have generated chimeric, humanized and human mAbs. Reopro (Abciximab) was approved in 1994 as the first chimeric mAb for the prevention of blood clots, Zenapax (Daclizumab) was approved in 1997 as the first humanized mAb to diminish kidney transplant rejection, Humira (Adalimumab) was approved as the first human mAb obtained through phage display library in 2002 for the treatment of rheumatoid arthritis, and Vectibix (Panitumumab) was approved as the first human mAb obtained through transgenic mouse technology in 2006 for the treatment of colorectal cancer. The present commercial success of mAbs is due to the new technological achievement which allowed lower administration costs, greater clinical efficacy and lower side effects. A series of fundamental technologies enabled the development of monoclonal antibodies derivatives such as: antibody fragments, bispecific antibodies, antibody drug conjugates, fusion proteins. All the technological and scientific acquisitions over the last years have made mAbs available to an ever-increasing number of patients for whom the quality of lives has improved. **Keywords:** MONOCLONAL ANTIBODIES, MABs, HYBRIDOMA TECHNIQUE, PHAGE DISPLAY TECHNOLOGY.

The idea that an antibody can target like a "magic bullet" only one specific antigen was and still is considered a major scientific breakthrough, of tremendous therapeutic value.

Monoclonal antibodies (mAbs) are antibodies produced artificially, with a higher specificity, from a single B-lymphocyte clone. They are monovalent antibodies, which bind to the same epitope.

The mAbs era started in 1975 with the monoclonal antibody technique, created by Georges Köhler, César Milstein and Niels Kaj Jerne by using mouse x mouse hybrid-

oma. Further, in 1984 they received the Nobel Prize in Physiology or Medicine for this discovery.

In 1986 (11 years later), the US Food and Drug Administration (FDA) approved the first therapeutic monoclonal antibody, Muromonab-CD3 (Orthoclone OKT3) to prevent the rejection of transplanted kidneys. By 2017, FDA has approved around 60 therapeutic mAbs (1, 2). In 2015 FDA approved 10 new therapeutic monoclonal antibodies, being the historical high since first approval (3).

All monoclonal antibodies currently approved by the regulatory authorities such as Food and Drug Administration (FDA) or European Medicines Agency (EMEA) are used in humans for treating various diseases such as: cancer, chronic inflammatory diseases, infectious diseases, autoimmune diseases and cardiovascular diseases or in organ transplantation. For these diseases mAbs have revolutionized treatment practices and also improved patient quality of life.

The mAbs field is rapidly growing, humanized and human monoclonal antibodies being now the fastest growing group of biotechnology-derived molecules in clinical trials. The global value of the monoclonal antibodies market is approximately $20 billion per year, with an expected annual rate of growth of 15% (4, 5).

The lack of early mAbs commercial success was due to the high costs of its administration; therefore, in order to become more commercially viable, greater clinical efficacy and fewer side-effects are still needed for monoclonal antibodies.

Antibody structure

Antibodies are Y-shaped molecules belonging to five major classes of immunoglobulin (Ig): IgA, IgD, IgE, IgG, and IgM. The IgG class forms the framework for the development of therapeutic antibodies.

The antibody molecule has two identical light (L) chains and two identical heavy (H) chains, held together by hydrogen bonds and disulfide linkages (fig. 1) (6).

The first sequence of both chains form the variable domain (V_H and V_L), each of them containing hypervariable sequences, known as complementarity determining regions (CDRs). The remaining amino-acid sequences of each light chain form the constant domain (C_L), and the remaining sequences of each heavy chain form the constant domain (C_{H1}, C_{H2}, C_{H3}). The variable regions are the antigen-binding regions via CDRs and the constant regions are responsible for effector recognition and binding (6).

The antibody molecule can be cleaved by proteolytic digestion into different fragments: Fab (antigen binding fragment), Fc (crystallizable fragment) and Fv (variable fragment).

The amino-acid sequences from the constant regions of each heavy chain found in the Fc fragment are the same in individuals within the same species but different among the species.

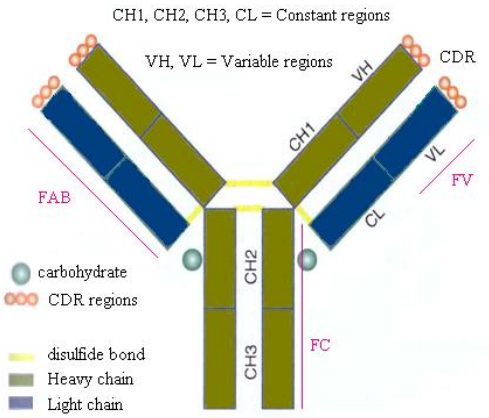

Fig. 1. Antibody structure

Monoclonal antibody technologies

A series of fundamental technologies allowed the development of monoclonal antibodies and its derivatives such as: antibody fragments, bispecific antibodies, antibody drug conjugates, fusion proteins etc.

The hybridoma technique created the first monoclonal antibody approved for therapeutic use in 1986, Orthoclone-OKT3 (muromonab), a fully murine antibody, used in preventing kidney transplant rejection. This technique involves the obtaining of B-lymphocytes from the spleen of an immunized animal fused then with an immortal myeloma cell line in order to obtain hybridoma cells that are cultured *in vitro* in selective medium (6).

Muromonab is rarely used due to its side-effects (human anti-mouse antibody response-HAMA), which explain the lack of initial clinical and commercial success of monoclonal antibodies.

The technical difficulties in large scale production, the high production costs, low yields, together with poor penetration in solid tumors, potential viral safety problems, the mAbs short half-life, the HAMA response led to technological developments and improvements. All these improvements embodied in strategies to generate chimeric, humanized and human mAbs (fig. 2) (6).

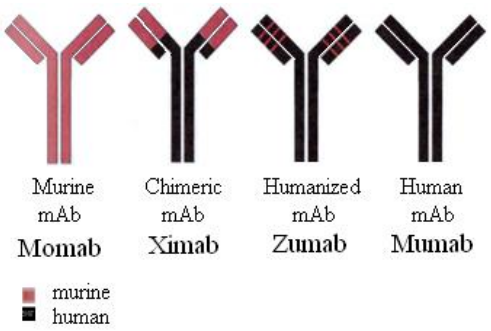

Fig. 2. Generations of therapeutic mAbs

Chimeric antibodies can be obtained by depletion of amino-acid sequence from the constant region of mouse antibody and replacing it with an amino-acid sequence of the Fc region of a sequence. Chimeric antibodies have a constant part from human peptide sequences, but the variable part of the Fab fragments contains mouse protein. Reopro (Abciximab) was approved as the first chimeric mAb in 1994 for prevention of blood clots in angioplasty. Abciximab is originally produced in mammalian cell cultures as chimeric antibody and then cleaved enzymatically (6, 7).

Chimeric antibodies are about 70% human. The specific action of the chimeric antibody is identical to that of the murine antibody, but the *in vivo* half-life and other pharmacokinetic functions are human, being determined by the Fc fragment. Unfortunately, there is an important immune response less likely than murine antibodies.

Humanized antibodies are about 95% human. These mAbs are largely human except for the hypervariable sequences (CDR) which is still of murine origin. The technologies for antibody humanization became available during the mid-1980s and are continuously improved. During the 1990s and 2000s several humanization methods such as CDR grafting, resurfacing, super humanization, human string content optimization, phage, ribosome or yeast display have been developed in order to achieve full biocompatibility (7).

Zenapax (Daclizumab) was approved in 1997 as the first humanized mAb to diminish kidney transplant rejection. Daclizumab was generated by selecting human framework regions to maximize resemblance to the murine antibody sequence.

Human antibodies are 100% human and fully biocompatible. In order to obtain

these mAbs several technologies such as phage display and transgenic mice technology are available.

Humira (Adalimumab), the first human mAb obtained through phage display library, reached the market in 2002 for the treatment of rheumatoid arthritis and Crohn's diseases. The entire technology is done *in vitro* and involves the isolation of mRNA from human B-lymphocytes in order to amplify all the V_H and V_L regions via PCR, followed by the insertion of the genes into M13 bacteriophage vector in order to transfect *E. coli* to generate a large phage library of antibodies. Each phage exposes a protein and its encoding DNA which occurred jointly on its surface (7).

Vectibix (Panitumumab), the first human mAb obtained through transgenic mouse technology reached the market in 2006 for the treatment of colorectal cancer. The technology implies the replacement of the endogenous murine IgG genes with human IgG genes in transgenic mice (XenoMouse® - Abgenix, Inc., Fremont, CA), hence mouse B-lymphocytes will synthesize human antibodies and hybridomas can be produced (6, 7).

About 60 mAbs are approved by the FDA or EMEA for therapeutic use and are now marketed, as shown in Table I.

TABLE I

Therapeutic monoclonal antibodies approved in the European Union or United States

International Common Denomination (ICD)	Trade name	Characteristics	Therapeutic indication	Approval year
Muromonab-CD3	Orthoclone OKT3	Murine IgG2a	Allograft rejection	1986
Abciximab	Reopro	Chimeric IgG1 Fab	Prevention of blood clots in angioplasty	1994
Rituximab	MabThera, Rituxan	Chimeric IgG1	Non-Hodgkin's lymphoma	1997
Basiliximab	Simulect	Chimeric IgG1	Prevention of kidney transplant rejection	1998
Palivizumab	Synagis	Humanized IgG1	Prevention of RSV infection	1998
Infliximab	Remicade	Chimeric IgG1	Crohn disease	1998
Trastuzumab	Herceptin	Humanized IgG1	Breast cancer	1998
Adalimumab	Humira	Human IgG1	Rheumatoid arthritis	2002
Ibritumomab tiuxetan	Zevalin	Murine IgG1	Non-Hodgkin lymphoma	2002
Omalizumab	Xolair	Humanized IgG1	Asthma	2003
Cetuximab	Erbitux	Chimeric IgG1	Colorectal cancer	2004
Bevacizumab	Avastin	Humanized IgG1	Colorectal cancer	2004
Natalizumab	Tysabri	Humanized IgG4	Multiple sclerosis	2004
Tocilizumab	Actemra	Humanized IgG1	Castleman disease	2005
Ranibizumab	Lucentis	Humanized IgG1 Fab	Macular degeneration	2006
Panitumumab	Vectibix	Human IgG2	Colorectal cancer	2006
Eculizumab	Soliris	Humanized IgG2/4	Paroxysmal nocturnal	2007

International Common Denomination (ICD)	Trade name	Characteristics	Therapeutic indication	Approval year
			hemoglobinuria	
Certolizumab pegol	Cimzia	Humanized Fab Pegylated	Crohn disease	2008
Golimumab	Simponi	Human IgG1	Rheumatoid and psoriatic arthritis, Ankylosing spondylitis	2009
Canakinumab	Ilaris	Human IgG1	Muckle-Wells syndrome	2009
Ustekinumab	Stelara	Human IgG1	Psoriasis	2009
Tocilizumab	RoActemra	Humanized IgG1	Rheumatoid arthritis	2009
Ofatumumab	Arzerra	Human IgG1	Chronic lymphocytic leukemia	2009
Denosumab	Prolia	Human IgG2	Bone loss	2010
Belimumab	Benlysta	Human IgG1	Systemic lupus erythematosus	2011
Ipilimumab	Yervoy	Human IgG1	Metastatic melanoma	2011
Brentuximab vedotin	Adcetris	Chimeric IgG1	Hodgkin lymphoma	2011
Pertuzumab	Perjeta	Humanized IgG1	Breast cancer	2012
Mogamulizumab	Poteligeo	Humanized IgG1	T cell leukemia-lymphoma	2012
Trastuzumab emtansine	Kadcyla	Humanized IgG1	Breast cancer	2013
Obinutuzumab	Gazyva	Humanized IgG1	Chronic lymphocytic leukemia	2013
Alemtuzumab	Lemtrada	Humanized IgG1	Multiple sclerosis	2013
Vedolizumab	Entyvio	Humanized IgG1	Crohn disease, Ulcerative colitis	2014
Ramucirumab	Cyramza	Human IgG1	Gastric cancer	2014
Pembrolizumab	Keytruda	Human IgG4	Metastatic melanoma	2014
Siltuximab	Sylvant	Chimeric IgG1	Castleman disease	2014
Nivolumab	Opdivo	Human IgG4	Malignant melanoma	2015
Secukinumab	Cosentyx	Human IgG1	Psoriasis	2015
Dinutuximab	Unituxin	Chimeric IgG1	Neuroblastoma	2015
Alirocumab	Praluent	Human IgG1	Hypercholesterolemia	2015
Idarucizumab	Praxbind	Humanized Fab	Reversal of dabigatran-induced anticoagulation	2015
Evolocumab	Repatha	Human IgG2	Hypercholesterolemia	2015
Daratumumab	Darzalex	Human IgG1	Multiple myeloma	2015
Mepolizumab	Nucala	Humanized IgG1	Severe asthma	2015
Necitumumab	Portrazza	Human IgG1	Metastatic melanoma	2015
Elotuzumab	Empliciti	Humanized IgG1	Multiple myeloma	2015
Obiltoxaximab	Anthim	Chimeric IgG1	Anthrax infection	2016
Ixekizumab	Taltz	Humanized IgG4	Psoriasis	2016
Reslizumab	Cinqair	Humanized IgG4	Severe asthma	2016

International Common Denomination (ICD)	Trade name	Characteristics	Therapeutic indication	Approval year
Daclizumab	Zinbryta	Humanized IgG1	Multiple sclerosis	2016
Adalimumab-Atto	Amjevita	Human IgG1	Rheumatoid arthritis, Ankylosing spondylitis, Psoriasis, Crohn disease	2016
Atezolizumab	Tecentriq	Humanized IgG1	Urothelial carcinoma	2016
Olaratumab	Lartruvo	Human IgG1	Soft tissue sarcoma	2016
Bezlotoxumab	Zinplava	Human IgG1	Reduce recurrence of *Clostridium difficile* infection	2016
Brodalumab	Siliq	Human IgG2	Psoriasis	2017
Sarilumab	Kevzara	Human IgG1	Rheumatoid arthritis	2017
Ocrelizumab	Ocrevus	Humanized IgG1	Multiple sclerosis	2017
Dupilumab	Dupixent	Human IG4	Severe atopic dermatitis	2017
Durvalumab	Imfinzi	Human IgG1	Urothelial carcinoma	2017
Avelumab	Bavencio	Human IgG1	Merkel cell carcinoma	2017

Production of mAbs at industrial level

The clinical and commercial success of monoclonal antibodies has led to the need of developing strategies to translate small-scale production into large-scale production.

An industrial production process involves the obtaining of a mAb (through immunization of an animal or by molecular biology methods), the identification of the coding DNA sequence and the construction of a clone which is further cultivated. All these steps require about 1-2 years. Commercial production of mAbs usually uses different expression systems such as mammalian cells of mouse (type NS0 or SP2/0 cell lines), Chinese hamster ovary (CHO cell line) and prokaryote (*Escherichia coli*, only in the production of mAbs fragments) (5).

Recently, many other different expression systems for monoclonal antibodies have been tested, such as human embryonic retina cells (Per.C6®), avian EBx cell line, rat myeloma YB2/0 cell line, transgenic animals, transgenic plants (*Nicotiana tabacum*) (5).

For mammalian cell cultivation are used highly complex media (which contain, among others, amino-acids, trace elements, vitamins, nucleosides etc), the production processes being carried out in stainless-steel stirred-tank bioreactors, working volume ranges from 1000 to 25000 L. The cultivation process usually takes place at 37^0C, pH 7.15, 30-60% dissolved oxygen levels for 12-14 days of culture (5).

Today large-scale production processes enable mAbs yields of 3-5 g/L, some companies reaching up to 10-13 g/L of mAbs in fed-batch processes or up to 25 g/L via modified perfusion processes (8).

Future perspective

Nowadays, many of monoclonal antibody therapeutic candidates (more than 200) are in clinical trials at different companies. The present commercial success of mAbs is due to the new technological achievement which allowed lower administration cost, greater clinical efficacy and lower side-effects.

Various optimization strategies can be used to improve the effector functions of

mAbs. IgG1, IgG4 and IgG2 antibody isotype were engineered to reduce instability of the mAbs and to reduce the binding properties to cellular Fc receptors (4). Glycoengineered antibodies obtained by engineering of the glycosylation profile can enhance the antibody-dependent cellular cytotoxicity (ADCC) activity (9). As well, the modification of the protein backbone of mAbs can enhance or reduce the binding properties to Fc receptors.

Immunoconjugates and bispecific antibodies are some of the promising methodological approaches. The use of antibody drug conjugates (a toxin, a chemotherapeutic agent, a radioactive isotope linked to a mAb) allows a specific tumor targeting of mAbs and provide highly potent drugs (4, 10). Also, the use of the bispecific antibodies, generated by genetic engineering techniques, enable simultaneous targeting of two distinct target antigens, likewise the delivery of mAbs into the brain in order to increase brain penetration (4, 10).

Even if the discovery and development of therapeutic mAbs is a very elaborate process, there is a great interest from scientists to improve clinical efficacy and to discover additional effects for mAbs.

CONCLUSIONS

Our study revealed that monoclonal antibodies, due to their specificity and flexibility, are an attractive therapeutic class to be watched in the future.

There is a major interest from pharmaceutical companies to develop monoclonal antibodies for clinical use though the commercial production of mAbs is a lengthy process, complex from technical point of view and a resource consuming one.

All the technological and scientific acquisitions over the last few years have made mAbs available to an ever-increasing number of patients for whom the quality of lives has improved.

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EVALUATION OF THE INFLUENCE OF LOCAL ADMINISTRATION OF VITAMIN D ON THE RATE OF ORTHODONTIC TOOTH MOVEMENT

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EVALUATION OF THE INFLUENCE OF LOCAL ADMINISTRATION OF VITAMIN D ON THE RATE OF ORTHODONTIC TOOTH MOVEMENT (Abstract): Accelerated orthodontic tooth movement is currently the subject of numerous scientific studies that aimed to improve the duration of orthodontic treatment. Several approaches had been performed in order to reduce the period in which a patient is subjected to orthodontic treatment. This are biological, surgical and physical approaches. **Aim:** This study has two purposes: first to assess the influence of local administration of vitamin D3 on orthodontic tooth movement and second to evaluate if there is any secondary effect of locally administration of vitamin D3 on dental roots. **Material and methods:** Every dental arch has been divided in two: one control quadrant and one experimental quadrant. The control canine received only conventional orthodontic treatment in comparison with the experimental canine who benefited from the association between orthodontic therapy and intraligamentary administration of vitamin D3. **Results:** 6 dental arches were studied. The average rate of tooth movement was greater in experimental canines compared to control canines. We recorded an average of 70 % more tooth movement for the experimental teeth compared to control ones. The differences between the two quadrants (control and experimental) are statistically significant. **Conclusions:** Locally administration of vitamin D3 seemed to increase the rate of orthodontic tooth movement. No root resorption was found three months after the first administration of vitamin D3 evaluated on cone-beam CT exam (cone-beam computed tomography). **Keywords:** ACCELERATED ORTHODONTIC TOOTH MOVEMENT, BONE TURNOVER, VITAMIN D, BONE REMODELLING.

Accelerated orthodontic tooth movement is currently the subject of numerous scientific studies that aimed to improve the duration of orthodontic treatment. Several approaches had been performed in order to reduce the period in which a pa-

tient is subjected to orthodontic treatment. Based on scientific data available in literature, there are mainly three approaches, which associated with conventional orthodontic treatment, reduce the treatment period and increase the rate of tooth movement: biological, physical and surgical approaches (1, 2, 3).

Given the magnitude of mechanical force applied to a tooth in order to obtain tooth movement, Schwarz is the one who defined the "optimal orthodontic force" concept (4). According to this, the force is optimal when it entails a tissue pressure approximately equal to periodontal ligament capillaries blood pressure, allowing blood flow in the periodontal ligament. The forces below this level do not cause any dental movement, whereas the forces above the optimal value lead to areas of cellular necrosis because of creating avascular areas.

Several researchers have shown their interest in optimizing the response of periodontal tissues during orthodontic treatment while maintaining on optimum level of mechanical forces. The physical, biological and surgical approaches have in common the fact that they increase or stimulate the biological mechanisms responsible for the regeneration of bone around the tooth (1, 2, 5).

Surgical approaches (osteotomy, cortectomy, surgical reduction of the interseptal bone, piezoincision) associated to orthodontic treatment in order to optimize its duration, is based on the principle according to which the bone turnover is accelerated in the fractures and osteotomies (1, 5, 6, 7). The "accelerating regional phenomenon" was particularly attributed to surgical induced regeneration of the bone in case of cortectomies created in association with orthodontic treatment in order to enhance the rate of orthodontic tooth movement (1,5-7).

Given the role of cytokines, parathyroid hormone, active vitamin D3 (1,25 dihydroxycholecalciferol) in bone regeneration, several studies have been conducted in which exogenous biological molecules have been locally administrated in order to stimulate orthodontic tooth movement (8-12). Vitamin D3 intraligamentary administered increased the rate of dental movement in mice when canine bilateral distalization was performed using low forces (11).

The studies on the effect of intraligamentary administration of vitamin D3 on the dental movement performed in human beings are few and inconclusive.

This study has two purposes: first to assess the influence of local administration of vitamin D3 (1.25 dihydroxycholecalciferol) on orthodontic tooth movement and second to evaluate if there is any secondary effect on dental roots after intra-ligamentary administration of vitamin D3.

MATERIAL AND METHODS

Patients were selected based on the following **inclusion criteria:**

- Presence of abnormal teeth alignment that requires bilateral extractions of the first premolars;
- Need of bilateral distalization of the maxillary or mandibular canines;
- General good health;
- Absence of chronic disorders or chronic drug intake;
- No history of orthodontic treatment;
- Presence of vital teeth, with healthy periodontium, without root resorption.

After patients' selection, a written consent for participation in the study was submitted. Only four patients agreed and were included in the study.

Six arches (four maxillary and two mandibular) were studied. Three patients were diagnosed with malocclusion and space deficit, requiring extractions of the four first premolars and one of the patients was diagnosed with bilateral anodontia of the lateral incisor, requiring the distalization of the canines (tab. I).

TABLE I
Illustration of patients' data.

	Age	Sex	Arch	Diagnostic	Duration of alignment and levelling (months)	Dental movement (mm)		
						Control canine	Experimental canine	Difference
1	18	M	Maxillary	DDM	4	1.40	2.40	1.00
2	34	F	Maxillary	Anodontia IL	5	0.00	1.20	1.20
3	15	M	Maxillary	DDM	3	1.01	1.40	0.39
4	15	M	Mandible	DDM	2	1.00	1.21	0.21
5	13	F	Maxillary	DDM	2	1.80	2.60	0.80
6	13	F	Mandible	DDM	3	0.80	1.39	0.59
Average	**18**				**3.17**	**1.00**	**1.70**	**0.70 mm**

After diagnostic and treatment plan recording (performing clinical and paraclinically examinations: intra-oral pictures, study model, panoramic x-ray, profile radiographic cephalometric), fixed orthodontic appliance was fitted. (Roth 0.22). All patients received instructions regarding good oral hygiene. It was prohibited to take drugs during the follow-up period. In case of pain associated to dental movement, only the administration of paracetamol was allowed. According to the treatment plan, extractions of the first premolars were performed after the trans palatal arch positioning. Seven days after, the fixed orthodontic appliance was applied to all teeth. The maxillary and mandibular arch was divided in two quadrants: one control and on experimental quadrant. Canine distalization was achieved using a closed Niti spring of 12 mm which generated a force of 150gm (TruFlex™ NiTi Closed Coil Springs, 12 mm, 150 gm Medium, Ortho Technology, Tampa, Florida, USA).The distalization force for each canine was measured using a dynamometer (Falcon Medical, Italy). Experimental canines benefitted from intra-ligamentary administration of vitamin D3 (Decostriol®, Mibe Jena, Germany). The vector for the administration of vitamin D3 was dymethylsulphoxyde, lip soluble solvent (DMSO, Milan, Italy). An amount of 0.2 mL of vitamin D3 (42 pg/mL) was administered, once a week, three weeks. Three cone-beam CT examinations were carried out for each patient: before the canine distalization, one month after and three months after the first administration of vitamin D3. Intra-oral pictures were also taken on a monthly basis and every week during the follow-up period.

Dental movement was measured on CBCT (Planmeca Romexis Viewer v 3.2.7). A conventional axial plane passing from the enamel-cement junction of the second premolars was identified in order to perform required measurements (fig. 1). On this axial plane, a conventional horizontal line passing through the middle of the pulp chamber of the second right premolar was used as reference to measure the sagittal

positions of the control and experimental canines one month before and after the distalization treatment (fig. 1). The difference between the initial measurements and the measurements made one month after indicates the amount of tooth movement. Clinical aspects of maxillary arch of a patient are shown in figure 2.

Secondary effect on the root of the canines (cement resorption) was evaluated on cone-beam CT 3 months after the first administration analyzing the axial, transversal and sagittal sections of each tooth included in the study.

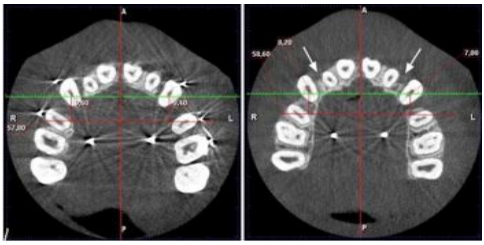

Fig. 1. Cone-beam CT of the maxillary arch.

Fig. 2. Clinical picture of the patient's maxillary arch.

Dental movement was analyzed by comparing the averages using the test Wilconson-Mann Whitney. A difference of a $p<0.05$ was considered statistically significant. The study protocol was approved by the Committee of Ethics and Research within our university.

RESULTS

On the average, the treatment with vitamin D3 induces a dental movement which is higher by 0.70 mm to control tooth (table I). Dental movement is 1.70 mm for test group and 1.00mm for control group (tab. I). In percentages, this means a dental movement 70% higher in the experimental group.

The parametric test Wilcoxon-Mann Whitney suggests there is no significant statistical difference between the group of men and that of women (table II). On the average, dental movement is more important at maxillary arch compared to mandible. It is 1.90 mm at maxillary, against 1.30 mm for the mandible (tab. II). However, the Wilcoxon-Mann-Whitney test shows that this difference does not testify a difference statistically significant in the two groups. Dental movement does not seem associated to the duration of the alignment (tab. II). Therefore, the movement within the treated group is 1.91 mm for an alignment period of 2 months, and 2.40 mm for an alignment period of 4 months, whereas it is 1.40 mm for a period of 3 months and 1.20 mm for an alignment period of 5 months. The Wilcoxon test on the dental movement differences between the two groups, test and control suggests that dental movement is systematically more important in the test group in relation to the control groups. It could be noted that the test of the Wilcoxon ranges shows the fact that the results are statistically significant, suggesting the existence of a distribution statistically significant in the two groups (p-value=0.0313). The differences between the two groups are statistically significant and could lead to the conclusion that an intra-ligamentary administration of the vitamin D3 seemed to increase the rate of tooth movement. In addition, no root resorption was observed three months after the first injection of vitamin D3.

TABLE II.

Perspective on the sex influence, dental arch of interest and duration of alignment and levelling for the dental movement of the control and experimental teeth: *Test statistically impossible because of an insufficient batch (only one arch studied for an alignment period of 4 and 5 months).

			Average	Type of distance	Minimum	Maximum	p value
Sex	M	control	1.33	0.50	0.80	1.80	0.1904 (>0.05)
		test	2.13	0.65	1.39	2.60	
	F	control	0.67	0.58	0.00	1.01	
		test	1.27	0.11	1.20	1.40	
Arch of interest	Maxillary	control	1.05	0.77	0.00	1.80	0.4875 (>0.05)
		test	1.90	0.70	1.20	2.60	
	Mandible	control	0.90	0.14	0.80	1.00	
		test	1.30	0.13	1.21	1.39	
Duration of alignment and levelling	2 months	control	1.40	0.57	1.00	1.80	*
		test	1.91	0.98	1.21	2.60	
	3 months	control	0.91	0.15	0.80	1.01	
		test	1.40	0.01	1.39	1.40	
	4 months	control	1.40	-	1.40	1.40	
		test	2.40	-	2.40	2.40	
	5 months	control	0.00	-	0.00	0.00	
		test	1.20	-	1.20	1.20	

DISCUSSION

Vitamin D3 was mentioned in literature as the most important bio-modulator of bone tissue (8,13) which justifies our study to assess its role on the rate of orthodontic tooth movement in human beings. Collins and Sinclair reported a 60% increase of the rate of orthodontic tooth movement in experimental animals after intra-ligamentary administration of vitamin D3 (11). This is explained by the effect of vitamin D3 on osteoclasts cells (13).

Intra-ligamentary administration was selected given the effect of vitamin D3 on bone tissue metabolism (11) at a physiological dosage (15). The reference blood value of vitamin D3 is 30-80 pg/mL. A lower quantity (0.2 mL) of vitamin D3 administered by intra-ligamentary path was performed in accordance with Malamed instructions: administration of a low quantity of substances using low pressure to limit the pain of the patient (16,17). According to the data existing in literature, the optimal orthodontic pressure to move maxillary canine is 150-260 cN (152.9-261.01 gm) and 150-200 cN (152.9-203.874 gm) for mandibular canine (18).

Dental movement measures were made on CBCT in order to observe 3D movements of the roots, not only those of the dental crown. Moreover, cone beam CT examination have proven to be very effective in identifying initial root resorptions (19,20,21). In this study, no root resorption was observed three months after the first administration of vitamin D3. For the first time, this study brings information about the effect of vitamin D3 on the roots during a follow-up period of three months by using the 3D scanner.

CONCLUSIONS

Exogenous administration of active vitamin D3 seems to be involved in stimulating orthodontic tooth movement. Three months after local administration of vitamin D3 no root resorption was observed.

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ALCOHOL CONSUMPTION WITH FAMILY PATTERN RELATED TO STRONG PERSONALITIES: A CASE-CONTROL STUDY IN MARRIED ROMANIAN STUDENTS

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ALCOHOL CONSUMPTION WITH FAMILY PATTERN RELATED TO STRONG PERSONALITIES: A CASE-CONTROL STUDY OF ROMANIAN MARRIED STUDENTS (Abstract): In the study conducted the aim was to investigate strong personalities (SP) related to alcohol consumption in married students from Romania. **Material of study** consisted in 2 samples: a case-sample (23 alcohol consumer students with a family pattern of weekly consumption, 47.8% male and 52.2% female, aged 21-29 years) and a control-sample (42 no-alcohol consumer students without a family pattern of consumption, 26.2% male and 73.8% female, aged 21-29 years), selected from a sample of 176 married students. **Method**: an observational inquiry (case-control) consisted in applying 2 questionnaires: Health Questionnaire (60 items, 7: Q43-Q49 -alcohol consumption) and Strong Personalities Questionnaire (88 items, alpha-Crohnbach index-0.823). Statistical analysis was performed by the aid of SPSS 20 Program. **Results**: For alcohol consumer students with weekly pattern of consumption the main reason of alcohol consumption was curiosity (60.9%) and the most frequent consumed alcohol was beer (38.6%, 500-3000 ml/week). Personalities` profiles revealed V-hyperthymic, III-hyperperseverant and X-emotive strong personalities (SP) as being symptomatic (over 50% symptomatic level-SL) for both samples, with a difference (control-case) for X-emotive SP (71.7% students – 57.1% students, respectively). I-demonstrative, VII-cyclothymic and IV-unruly SP presented a difference between case (symptomatic: 61.8%; 61.8% and 61.8% students, respectively) and control (symptomatic: 36.8%; 37.5% and 45% students, respectively) samples. A statistically significant difference case-control samples was demonstrated for I-Demonstrative (item S29: chi square χ^2=10.65; Sig.0.002; gamma correlation γ=0.73; Sig.0.002) and for X-emotive (item S25: χ^2=8.76; γ=-0.66; Sig.0.003) SP. In **conclusion**, a relation SP-alcohol consumption in married students is suggested.
Keywords: ALCOHOL CONSUMPTION, PERSONALITY, MARRIED STUDENTS.

Alcohol consumption continues to be a risk behavior of young people in Romania (1) and Europe (2). Alcohol culture and family structure were demonstrated as important predictors for alcohol consumption (3). Personality structure could be a condition for alcohol consumption behavior that could determine a psychological outcome (4). In this study I aimed to investigate strong personalities of young married students related to their alcohol consumption behavior and their family pattern of alcohol consumption.

MATERIAL AND METHODS

This study was performed on two samples: a case-sample (23 alcohol consumer students with family pattern of weekly consumption, 47.8% male and 52.2% female, aged 21-29 years – 91.2%) and a control-sample (42 no-alcohol consumers with no family pattern of consumption, 26.2% male and 73.8% female, aged 21-29 years – 90.4%). The two samples were selected from 176 married students (31.2% male and 68.8% female, aged 19-30 years).

The method - an observational inquiry (case-control) applying 2 questionnaires: Health Questionnaire (60 items, 7: Q43-Q49 - alcohol consumption) and Strong Personalities Questionnaire (SPQ-88 items, Crohnbach`s alpha index = 0,823) (5). The Q43-Q49 items assess: reason, frequency, relation to sexual inhibitions, to school/work difficulties, type and quantity, social environment and family pattern of alcohol consumption. Ten strong personalities (SP) are highlighted by SPQ: I-demonstrative, II–hyperexact, III-hyperperseverant, IV–unruly, V–hyperthymic, VI–dysthymic, VII-cyclothymic, VIII–exalted, IX–anxious, X-emotive. The application of both questionnaires was: individual, anonymous, 1 hour and a half long; and asking for free written consent. Statistical analysis (chi square-χ^2 and gamma correlation-γ) was performed by SPSS 20 Program.

RESULTS

In the case-sample: reasons of **alcohol consumption** were curiosity (60.9%) and releasing themselves from emotional and sexual inhibitions (43.5%); alcohol consumers with weekly pattern of consumption consumed alcohol 3-5 times/week (100%); they consumed 500-3000 ml beer/week (38.6%),200-1000 ml wine/week (30.4%) and 50-500 ml vodka/week (17.3%); they drank alcohol with a group of friends (52.2%) and with the family (26.1%).

A comparative analysis of SP between case (A) and control (B) samples demonstrated 3 SP at high symptomatic level (SL) for both samples: X-emotive, V-hyperthymic and III-hyperperseverant (tab. I, II). A difference case-control samples regarding SL was registered for 3 SP: **I-demonstrative** at SL 58.1% (33.3% students–A) and at SL 41.5% (23.6% students-B), **IV-unruly** at SL 62.5% (28.5% students-A) and at SL 50% (17.5% students–B) and **IX-anxious** at SL 75% (23.5% students-A) and at SL 37.5% (24.3% students-B) (tab. I, II). An analysis of **total symptoms over 50%** SL presence for each SP demonstrated the 3 mentioned SP with high students` frequency at high SL as being symptomatic for both samples: **V-hyperthymic** (71.4% students-A / 67.5% students-B), **III-hyperpeseverant** (71.39% students-A / 56.41% students-B) and **X-emotive** (71.7% students-B / 57.1% students-A). **I-demonstrative** (61.89% students-A / 36.83 students-B) and **IV-unruly** (61.8% students-A / 45% students-B) maintained the difference between the 2 case-control samples. **VII-Cyclothymic** SP registered a difference of total symptoms presence over 50% between case (61.8% students) and control (37.5% students) samples (tab. I, II). SP profiles (fig.1) present comparatively for case-control samples the mentioned aspects.

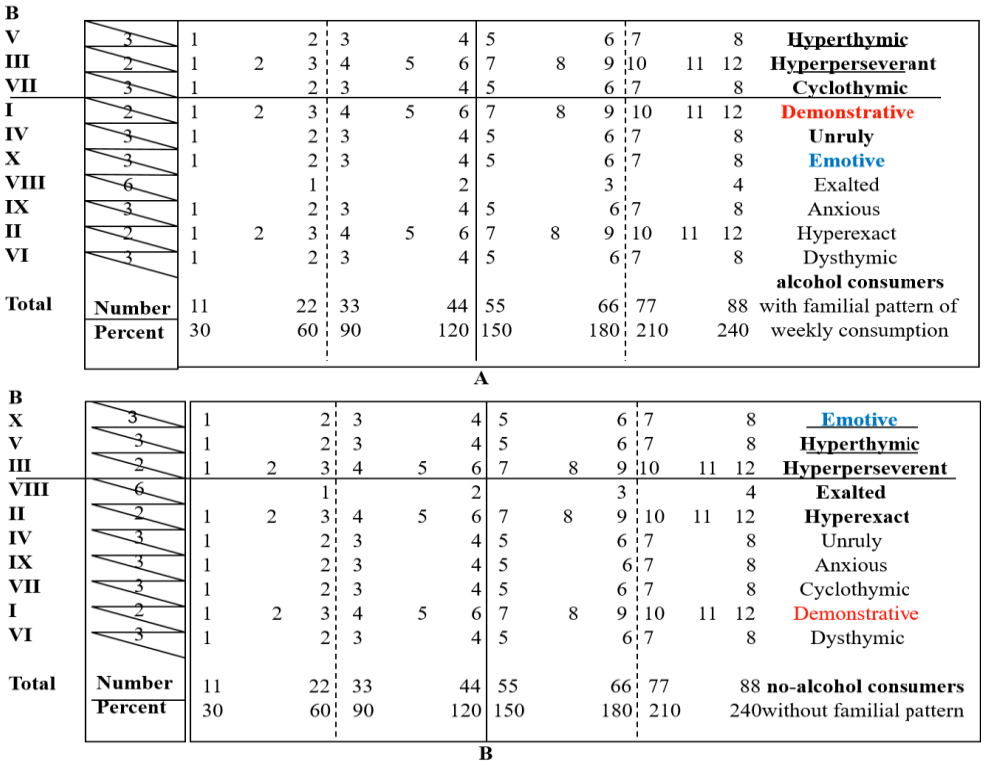

Fig. 1. SP profiles of case (A) and control sample (B)

TABLE I.

Symptomatic levels (SL, s-symptoms) and student's frequency (%) for 3 SP presented comparatively between case-A and Control-B samples

	SL(%)	I-A	I-B	II-A	II-B	III–A	III-B
1s	8.3	0	0	14.2	2.85	0	0
2s	16.6	0	0	4.76	2.85	0	0
3s	24.9	0	7.89	14.28	0	4.76	7.69
4s	33.2	4.76	18.42	9.52	2.85	0	12.82
5s	41.5	9.52	**23.68**	9.52	**25.71**	9.52	10.28
6s	49.8	23.8	13.15	**23.8**	14.28	14.28	12.82
7s	58.1	**33.33**	21.05	14.28	14.28	**28.57**	**20.51**
8s	66.4	14.28	2.63	0	17.14	9.52	17.94
9s	74.7	9.52	5.26	4.76	0	33.3	5.12
10s	83	0	2.63	0	14.28	0	10.28
11s	91.3	4.76	5.26	4.76	5.71	0	2.56
12s	99.6	0	0	0	0	0	0
T	> 50	**61.89**	**36.83**	23.8%	51.41	**71.39**	**56.41**

TABLE II.

Symptomatic levels (SL, s-symptoms) and student's frequency (%) for 7 SP presented comparatively between case-A and Control-B samples

	SL (%)	IV-A	IV-B	V-A	V-B	VI-A	VI-B	VII-A	VII-B	VIII-A	VIII-B	IX-A	IX-B	X-A	X-B
1s	12.5	4.7	0	0	0	5.2	12.8	0	0			17.6	5.4	4.7	5.1
2s	25	4.7	20	4.7	7.5	21	**25.6**	4.4	12.5	9.5	21.0	17.6	16.2	9.5	7.6
3s	37.5	14.2	17.5	4.7	7.5	**36.8**	23.0	0	**30**			5.8	**24.3**	19.0	2.5
4s	50	14.2	**17.5**	19	17.5	15.7	20.5	**33.3**	20	**42.8**	26.3	17.6	13.5	9.5	12.8
5s	62.5	**28.5**	25	14.2	12.5	10.5	10.2	19.0	20			5.8	18.9	19.0	7.6
6s	75%	9.5	10	**28.5**	17.5	10.5	7.6	19.0	2.5	28.5	**42.1**	**23.5**	18.9	4.7	17.9
7s	87.5	14.2	5	19	**27.5**	0	0	9.5	10			11.7	2.7	**23.8**	**28.2**
8s	100	9.5	5	9.5	10	0	0	14.2	5	19.0	10.5		0	9.5	17.9
T	> 50	**61.8**	**45**	**71.4**	**67.5**	21	17.9	**61.8**	**37.5**	47.6	52.6	41.1	40.5	**57.1**	**71.7**

A statistically significant difference case-control samples was demonstrated for I-demonstrative (S29: χ^2=10.65; Sig.0.002; γ=0.73; Sig.0.002) and X-emotive (S25: χ^2=8.76; Sig.0.004; γ=-0.66; Sig.0.003) SP.

DISCUSSION

Novelty seeking (curiosity) and decrease of sexual inhibitions relate to later positive reinforcement of alcohol drinking motives in the literature (6) and they are the main reasons for alcohol consumption in the conducted study. Strong personalities` profiles are more symptomatic in case (I-demonstrative, IV-unruly and VII-cyclothymic) than in control samples (where X-emotive SP is more symptomatic). In the research literature, weekly alcohol consumption behavior relates to: responsibility and impulsivity among males (7), different levels of self-esteem in men and women (8), extraversion in weekend consumption and low agreeableness in weekday alcohol use (9). In the performed study I-demonstrative and X-emotive SP relate significantly to alcohol consumption pattern. Reduced emotional stress of married alcohol consumers is demonstrated in this study through X-emotive nature in SP profiles and it also sustains disinhibition as a later positive reinforcement drinking motive (6). A limit of this study is that it is an observational one.

CONCLUSIONS

A relation of strong personalities to alcohol consumption with weekly family pattern in married students is suggested.

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NEWS

ANGIOGENESIS IN BREAST CANCER AND ITS CORRELATION WITH ESTROGEN, PROGESTERONE RECEPTORS AND OTHER PROGNOSTIC FACTORS

There has been global increase in incidence of breast cancer over the last several decades in Asian countries than in United States and Europe. A prognostic factor is any measurement available at the time of surgery that correlates with disease-free or overall survival in the absence of systemic adjuvant therapy and, as a result, is able to correlate with the natural history of the disease. In contrast, a predictive factor is any measurement associated with response to a given therapy. Lymph node status, Tumour size, Lymphovascular invasion, Proliferation markers, Ethnicity, and Age are the prognostic factors while ER/PR status, HER2/neu, Urokinase-type plasminogen activator (uPA) and its inhibitor, plasminogen activator inhibitor type 1 (PAI-1), Genetic profiling are prognostic as well as predictive factor. Angiogenesis is required for invasive tumour growth, metastasis; and constitutes an important role in the control of cancer progression. Its inhibition may be a valuable new approach to cancer therapy. Angiogenesis provide increased availability of oxygen and nutrients to the tumour as well as the most important route of exit from the primary tumour into the blood stream. Assessment of intratumoural microvessel density using immunohistochemistry with panendothelial markers like factor VIII–related antigen, CD31, CD34, and the activated endothelial cell marker CD105 is a commonly used technique for quantifying tumour angiogenesis in breast cancer. The presence of estrogen and progesterone receptors in an invasive breast carcinoma is both prognostic and predictive. Its prognostic effect is difficult to evaluate in that it must be assessed in the absence of adjuvant tamoxifen. In the era of targeted therapy, some authors use antiangiogenic therapy to reduce the tumour progression and tumour metastasis. The most important clinical implication of tumour angiogenesis is the development of a novel strategy of anticancer therapy targeting tumour vessels instead of cancer cells. Antiangiogenic therapy aims to inhibit the growth of tumour, and current evidence suggests that it works best in combination with conventional cytotoxic chemotherapy. Evaluation of estrogen and progesterone receptors status is a routine procedure in the management of breast cancer. Receptor status is useful not only for defining prognosis in breast cancer but also for selection of patients for therapy. In conclusion, the information about prognostic factors in breast cancer patients may lead to better ways to identify those patients at high risk who might benefit from adjuvant therapies (Bharti JN, Rani P, Kamal V, et al. Angiogenesis in Breast Cancer and its Correlation with Estrogen, Progesterone Receptors and other Prognostic Factors. Journal of Clinical and Diagnostic Research. 2015, Vol-9(1): 5-7).

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SEXUALITY, BODY IMAGE AND PSYCHOLOGICAL WELL-BEING OF PATIENTS WITH MASTECTOMY AFTER BREAST CANCER

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SEXUALITY, BODY IMAGE AND PSYCHOLOGICAL WELL-BEING IN OF PATIENTS WITH MASTECTOMY AFTER BREAST CANCER (Abstract). **The aim** of the study is to identify the factors which influence the psychological state of well-being, the variables which determine satisfaction with sexual life and the effects of a positive assessment of the body image. **Material and methods:** 31 women, aged between 39-69 years old (M = 57 ± 7.8), mastectomized after breast cancer answered a survey regarding body image, sexual activity and quality of their sex life. The age when they underwent the intervention was between 33 and 67 (M = 48.30 ± 7.5). 45.6% of the surveyed patients are not part of a couple (they are single, divorced or widows). **Results:** 55% of subjects are unhappy with their body image, when looking in the mirror. 48.39% no longer have any sexual activity, or that they are very unhappy with it. Weakly positive correlations have been recorded between satisfaction with one's physical appearance and the frequency of sexual intercourse (r = .44*, p = .020), as well as the belief in one's own attractiveness (r = .41, p = .033). Strongly positive correlations have been highlighted between the level of libido and sexual satisfaction (r = .98**, p = .00), as well as between the comfort and relaxation during sexual acts and sexual satisfaction (r = .92**, p = .00). **Conclusions:** the quality of life is strongly influenced by the level of satisfaction regarding the body image, the relationship with the significant other and the quality of sexual activity. The "level of education" variable was correlated positively with all variables regarding the body image and sexual life. **Keywords:** MASTECTOMY, QUALITY OF LIFE, BODY IMAGE, SEXUAL SATISFACTION, PSYCHOLOGICAL DISTRESS.

Breast cancer is the most frequent form of cancer diagnosed in women (23% of all neoplastic cases) and it represents the main cause of death by cancer; 14% of fatalities are due to neoplasia (1) and breast surgery impacts psychosocial life, reflecting on the patient's femininity, on body image projection and, indirectly, on behavior and social integration (2).

The psychological impact of mastectomy is generated by stress and the feeling of disfigurement caused by losing the breast, to which one may add anxiety generated by the pathology threatening the vital prognosis (3).

Psychological health, a satisfactory relationship with one's partner and sexual life are important factors in the quality of life for a woman affected by breast cancer and mastectomy. Under these circumstances, at an overall assessment, the loss of a breast represents only a relatively unimportant

problem, by comparison with the negative effects produced by this loss. Sexual health refers to interest, dysfunction and satisfaction within intimate relationships.

The parameters which may measure sexual satisfaction in women are linked to the number of sexual acts, the level of sexual desire, the subjective experience of arousal, vaginal lubrication, genital sensations, number of orgasms etc., generally referring to the comfort perceived by the patient during the sexual act (4). Many of these indicators, referring to vaginal dryness, emotional well-being, the quality of relations with the partner and sexual problems, may be important factors in medical and psychological counseling.

Some studies show that there is a positive correlation between sexual difficulties and variables such as: age, marital status, postoperative chronic fatigue and the therapy method (hormonal therapy, chemotherapy or radiotherapy) (5).

A study conducted on 41 mastectomized women found that, though most of them showed a high rate of adaptation to circumstances, there were obvious signs of psychological suffering, such as suicidal thoughts or anti-health behaviors (use of alcohol and tranquilizers). Premorbid data, age and social support are factors that may signal the necessity for quick psychotherapeutic intervention, especially during the period preceding surgery, when distress is stronger (6). The same authors found that, in partners of mastectomized patients, though the rate of adaptation was relatively high, as in their female partners, a high rate of distress was identified in a subgroup; the causes were sexual restrictions within the couple, the female partner's body image after the surgical intervention and frequent visits to the hospital (7).

MATERIAL AND METHODS

From January to May 2015, 31 women diagnosed with breast cancer and mastectomized answered a survey regarding aspects of the quality of life. The research was approved by the Research Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iaşi. The surveys were filled in after the initial training of the patients and after they signed the informed consent form.

The survey included information about socio-demographic data, family history (hereditary collateral antecedents), co morbidities, data about the illness (diagnosis, age at surgery), treatment and type of surgical intervention, ways of information about the possibility of breast reconstruction, the source of information and reasons why breast reconstruction surgery was not resorted to up to the time of the survey.

The items also covered femininity, bodily self-perception, confidence felt around people of the same or the opposite sex, satisfaction with one's physical appearance, satisfaction regarding the relationship with the significant other (frequency of sexual intercourse, libido, comfort and relaxation during sexual acts and the level of sexual satisfaction). Socio-demographic variables taken into account included age, background, level of education, marital status, number of children; medical variables taken into account: level of depression, data about the surgical intervention and type of therapy (hormonal therapy, chemotherapy, radiotherapy and targeted therapy).

Answers for the items targeting physical aspects and the relationship with the significant other were quantified by an ordinal scale with four values, where 1 – very dissatisfied, 2 – a little dissatisfied, 3 – satisfied, 4 – very satisfied. Items targeting

femininity and self-confidence were also quantified by a scale with four values, where 1 – to a very small extent, 2 – to a small extent, 3 – to a large extent and 4 – to a very large extent.

The data obtained have been processed by means of the SPSS 22.0 statistical software.

RESULTS

Demographic data show that most patients (93.1%) come from an urban background; 6.9% come from a rural background. Ages range between 39-69 years old, with an average age of 57.22 ± 7.85. Patients who answered the survey had undergone surgical intervention 1-21 years prior to it. Their age when undergoing removal surgery for a malignant breast tumor was between 33 and 67 years old, with an average age of 48.30 ± 7.54.

Regarding the level of education, 50% of patients have university degrees, 30% have high school education, 6.67% have secondary education and 13.32% have primary or no formal education. In terms of marital status, 6.45% are unmarried, 54.84% are married, 25.81% are divorced and 12.90% are widows. A total of 45.6% of the investigated patients are not part of a couple. The "number of children" variable has been taken into account and it has turned out that 12.90% of the patients do not have children, 41.94% have one child, 38.71% have two children and 6.45% have three children.

Medical data. Approximately half of the investigated patients (46.67%) have various associated conditions (other oncological pathologies, arterial hypertension, diabetes etc.), 46.67% have declared that they have no associated conditions and 6.67% claim not to be aware of the existence of other conditions, apart from the one they have undergone surgery for. 50% of the patients declare they have no family history concerning pathologies of oncological character, 30% have such family history (parents, siblings or extended family) and 16.67% claim not to know about the existence of such diagnoses in close relatives.

The subjects have also been questioned about the reasons why they did not resort to breast reconstruction. The patients bring forth financial reasons (20.69%), fear of illness recurrence (20.69%), the wish to avoid a new surgical intervention (37.93%) or the disinterest in physical appearance (27.59%). 6.9% claim not to have known that such surgical intervention can be performed in Romania.

Survey results. The items were aimed at identifying the level of satisfaction with the following aspects connected to femininity, body image, and relationship with the significant other and sexual satisfaction: the postoperative scar; her appearance when she looks in the mirror; the way she looks from a physical point of view; her confidence around other women; her confidence around men; the relationship with the significant other; the desire to make love / to have sex (level of libido); the frequency of sexual intercourse; her sexual satisfaction; her comfort and relaxation during sexual intercourse; the belief that she is attractive while naked; the sense of femininity.

The answers were assessed on a scale of 1 to 4 (where 1 = very dissatisfied, 2 = a little dissatisfied, 3 = satisfied and 4 = very satisfied) and the results obtained are detailed below. Concerning satisfaction with the postoperative scar (M = 2.73 ± 1.11), of all patients surveyed, 19.23% are very dissatisfied, 19.23% claim to be a little dissatisfied, 30.77% are satisfied and

30.77% claim to be very satisfied with the aspect of the postoperative scar.

Under the item questioning their appearance when they look in the mirror, 22.22% of all patients declare they are very dissatisfied, 25.93% are a little dissatisfied, 33.33% are satisfied and 18.52% are very satisfied.

In what concerns satisfaction with the self-perception of the body image, over 35% are very dissatisfied or a little dissatisfied with their physical appearance, 25% are satisfied and 39.29% are very satisfied with their physical appearance.

A minority of the surveyed women (7.69%) did not wish to answer the item referring to confidence around people of the opposite sex (tab. I).

TABLE I

Confidence felt around women and men

Item	very dissatisfied	a little dissatisfied	satisfied	very satisfied
Confidence felt around other women	7.41%	29.63%	40.74%	22.22%
Confidence felt around men	7.69%	23.08%	42.31%	19.23%

Under the item aimed at identifying satisfaction with the frequency of sexual intercourse, 25.81% of the patients surveyed have declared they no longer have sexual activity, 22.58% are very dissatisfied, 12.90% are satisfied and 29.03% are very satisfied with the frequency of their sexual intercourse.

In terms of the desire to make love, 21.43% of the patients surveyed did not wish to answer the question, 25% declared themselves to be very dissatisfied, 14.29% are a little dissatisfied, 28.57% are satisfied and 10.71% of respondents have claimed to be very satisfied with their level of libido. The items were also aimed at the sexual activity of mastectomized women and the quality of their sexual intercourse (comfort, relaxation, satisfaction). The values obtained are shown below (tab. II).

TABLE II

Satisfaction with sexual intercourse

Item	Did not answer	Very dissatisfied	A little dissatisfied	Satisfied	Very satisfied
Sexual satisfaction	21.43%	21.43%	17.86%	25%	14.29%
Comfort and relaxation during sexual intercourse	22.22%	18.52%	11.11%	33.33%	14.81%

Regarding the item aimed at satisfaction with the self-perceived attractiveness while naked, only 18.51% of the patients claim to be satisfied or very satisfied with it. The rest of the women state that they are very dissatisfied (11.11%) or a little dissatisfied (44.44%). 3.7% of the surveyed women did not answer this item. In what concerns femininity, 17.86% mention they are very dissatisfied, 25% are a little dissatisfied, 35.71% are satisfied and 21.43% claim to be very satisfied with their femininity.

This research has also included a *correlational study*, aimed at identifying variables which influence the level of satisfaction with the body image, the relationship

with the partner and femininity. The study emphasizes the fact that patients' age, marital status and number of years passed since the surgery, do not influence the relationship with their partner, sexual activity, the level of assessment of the body image or femininity.

Positive correlations were identified between all other research items. Weak positive correlations were recorded between satisfaction with one's physical appearance and the frequency of sexual intercourse (r = .44*, p = .020), as well as the confidence about one's own attractiveness (r = .41, p = .033), in that, the more satisfied the patient feels with her physical appearance and the more attractive she feels, the more the frequency of her sexual acts increases.

Positive correlations were obtained between all aspects investigated and the level of education. The higher the level of studies, the higher the satisfaction with the self-image, one's physical appearance, one's confidence around other people and in relationship with the significant other. The level of education seems to have a positive influence on physical and relational self-evaluation.

Strongly positive correlations have been highlighted between the level of libido and sexual satisfaction (r = .98**, p = .000), as well as between the comfort and relaxation during sexual acts and sexual satisfaction (r = .92**, p = .000) (tab. III).

TABLE III

Spearman's Correlation Coefficient

Scale	education	xviii	xix	xx	xxv	xxvi	xxvii	xxviii	xxix
Education	1.000	.21	**.52****	**.42***	**.48****	**.48****	**.51****	**.52****	**.59****
	.	.138	.002	.016	.003	.004	.002	.003	.000
Your appearance when you look at yourself in the mirror (xviii)	.21	1.00	**.69****	**.74****	**.44****	.23	.23	.27	**.41***
	.138	.	.000	.000	.010	.118	.121	.083	.017
Your confidence around women (xix)	**.52****	**.69****	1.00	**.78****	**.47****	**.59****	**.52****	**.56****	**.69****
	.002	.000	.	.000	.006	.001	.002	.001	.000
Your confidence around men (xx)	**.42***	**.74****	**.78****	1.00	**.55****	**.60****	**.50****	**.51****	**.57****
	.016	.000	.000	.	.002	.000	.004	.004	.001
The frequency of sexual relations (xxv)	**.48****	**.44****	**.47****	**.55****	1.00	**.66****	**.67****	**.64****	**.63****
	.003	.010	.006	.002	.	.000	.000	.000	.000
The desire to make love/level of libido (xxvi)	**.48****	.23	**.59****	**.60****	**.66****	1.00	**.92****	**.92****	**.75****
	.004	.118	.001	.000	.000	.	.000	.000	.000
Sexual satisfaction (xxvii)	**.51****	.23	**.52****	**.50****	**.67****	**.92****	1.00	**.98****	**.69****
	.002	.121	.002	.004	.000	.000	.	.000	.000
Comfort and relaxation during sexual acts (xxviii)	**.52****	.27	**.56****	**.51****	**.64****	**.92****	**.98****	1.00	**.70****
	.003	.083	.001	.004	.000	.000	.000	.	.000
Do you feel attractive while naked? (xxix)	**.59****	**.41***	**.69****	**.57****	**.63****	**.75****	**.69****	**.70****	1.00
	.000	.017	.000	.001	.000	.000	.000	.000	.

***. Correlation is significant at the 0.01 level (1-tailed).*

**. Correlation is significant at the 0.05 level (1-tailed).*

DISCUSSION

The data obtained during this study prove that satisfaction with the body image has a significant positive influence on the quality of sexual activity (the frequency of sexual acts, sexual satisfaction, the comfort and relaxation experienced during sexual intercourse) and on relationships with the people around (patients with high scores in body image assessment have also obtained high scores in the items referring to the confidence felt around women or men).

Though a significant percentage of the subjects did not give answers to items referring to sexual life, this may be explained by the fact that 45.6% of the investigated patients are not part of a couple (they are single, divorced or widows).

Despite the fact that studies about the relation between the body image and the level of education are more frequent in what regards children and teenagers, it is important to note that a considerable influence of the level of education on all aspects of the research has been identified. The level of education seems to have a positive influence on bodily self-esteem, confidence and relating to others.

The body image is a subjective concept, depending on the person's psychological universe. The body image is defined as a person's mental image of the physical body, the attitude toward the physical self, health status, normal functioning and sexuality (4). It is manifesting at the level of the feminine gender by one's desire to be attractive and by femininity. The psychological well-being is thus determined by the body self-evaluation, which, in its turn, has positive effects on sexuality (8).

Many studies have identified the fact that mastectomized patients, with or without chemotherapy, have been reporting problems related to their sexual activity. Sexual problems and sexual functioning issues seem to persist over time, even many years after the breast removal surgery (9, 10).

The vicious circle of the body image is recharged by the increase in mental distress, which, in its turn, will affect the self-image and the desire to engage in sexual activity. In this situation, the multidisciplinary intervention for preoperative physical and mental preparation, as well as postoperative psychological and medical therapy, should take into account the quality of mental life in order to determine the best type of individual and couple therapy (11, 12). Other studies researching quality of life on mastectomized patients for breast cancer, with instruments that measure both physical and emotional impact, have been shown that females' activities are negatively influenced for more than half of the study groups, no matter the stage of the cancer evolution (metastatic or not) (13, 14).

CONCLUSIONS

In our study we found out that the quality of life for mastectomized patients is strongly influenced by the level of satisfaction with the body image, the relationship with the significant other and the quality of sexual activity. The age at which the surgical intervention was performed and the length of time passed since the surgery do not influence the quality of life. We showed highly significant correlations between satisfaction and body image and the quality of sexual activity (the frequency of sexual intercourse, sexual satisfaction, level of libido). The "level of education" variable positively correlates with all variables referring to body image and sex life. The study shows that preoperative psychological intervention and psychological

support following the surgery are important for both the patient's and the couple's quality of life.

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ANTIBIOTIC RESISTANCE SPECTRUM OF NON FERMENTING GRAM NEGATIVE BACILLI ISOLATED IN THE ORTHOPEDIC TRAUMATOLOGY CLINIC OF „SF. SPIRIDON" CLINICAL EMERGENCY HOSPITAL IAŞI

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ANTIBIOTIC RESISTANCE SPECTRUM OF NON FERMENTING GRAM NEGATIVE BACILLI ISOLATED IN THE ORTHOPEDIC TRAUMATOLOGY CLINIC OF „SF. SPIRIDON" CLINICAL EMERGENCY HOSPITAL IAŞI (Abstract) **Aim:** The retro-prospective analysis of antibiotic sensitivity of non-fermenting gram negative bacilli strains circulating in the Orthopedics-Traumatology Clinic from "Sf. Spiridon" Emergency Clinical Hospital in view of determining the trend of the resistance phenomenon and indicating the most useful treatment for the infections caused by these strains. **Material and methods:** The retrospective component was conducted from 01.01.2003 to 31.12.2012, and the result of the diffusimetric antibiograms was taken from the hospital's informatics system; the prospective component of the study involved the collection of pathological products from the patients admitted during January - December 2013, who showed clinical suspicion of infection, in compliance with the general collection norms for the products destined for the bacteriological exam. **Results:** From the total 167 strains of *Pseudomonas aeruginosa* isolated and identified from the patients, 48 (28.74%) were sensitive to at least one antibiotic from each tested class, 29 (17.39%) were resistant to a single antibiotic and the rest of 90 (53.89%) showed multiple resistance. We noticed a statistically significant difference between the number of strains sensitive to at least one antibiotic from each tested class and those with multiple resistance ($p<0.05$). For the strains of *Acinetobacter baumanii* combined resistance was identified for 121 (87.04%), out of which 55 (39.56%) were resistant to two classes of antibiotics and the other (47.48%) to all three classes. The most frequently met was the association of resistance to quinolones and aminoglycosides, namely for a number of 49 strains (35.25%); only 3.59% of them were simultaneously sensitive to the three classes of antibiotics. **Conclusion:** The already high percentages and the rising trends of antibiotic resistance of non-fermenting gram-negative bacteria described in this study confirm the continuous decrease of the efficiency of antimicrobial agents and underline the necessity of a global strategy which aims at all health sectors regarding the rational use of antibiotics, on the one hand, and the continuation of studies concerning the surveillance of the antimicrobial resistance phenomenon, on the other hand. **Key words**: NON-FERMENTING GRAM NEGATIVE BACILLI, ANTIBIOTIC RESISTANCE, COLISTIN.

The involvement of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* gram negative bacilli in nosocomial infections is continuously rising, being accompanied by an increased mortality rate (1, 2). Due to the special structural characteristics of their external membrane, these bacteria show antibiotic multi-resistance (3).

The infections caused by *P. aeruginosa* show a high risk especially for patients in critical condition or with compromised immunity (4). The colonization rate with pyocyanic bacillus grows for patients with prolonged hospitalization and/or those who received broad-spectrum anti-infections chemotherapy. In the last ten years, *A. baumanii* has become one of the most frightening pathogen because the treatment of infections caused by this microorganism is limited to a handful of antibiotics due to the various antimicrobial resistance mechanisms it displays. Multi-drug resistant strains (at least three antibiotic classes) are more and more frequently reported at world level (5). The infections determined by these strains are associated with high morbidity and mortality rates (6). Unfortunately, the carbapenem resistance is continuously rising and no longer constitutes the "saving" therapy in *A. baumanii* infections (7). The purpose of this study was to make a retro-prospective analysis of antibiotic sensitivity of non-fermenting gram negative bacilli circulating in the Orthopedics - Traumatology Clinic of "Sf. Spiridon" Emergency Clinical Hospital Iaşi, in view of determining the trend of the resistance phenomenon and indicating the most useful treatment for the infections caused by these strains.

MATERIAL AND METHODS

The retrospective study was conducted during 01.01.2003-31.12.2012, and the result of the diffusimetric antibiograms was taken from the hospital's informatics system; the prospective component of the study involved the takeoff of pathological products from the patients admitted from 01.01.2013 to 31.12.2013, who showed clinical suspicion of infection, in compliance with the general collection norms for products destined to bacteriological exam. We excluded from the data base strains from the same bacterial species and with an identical resistance phenotype to anti-infectious chemotherapy drugs isolated from the same patient. The strains were isolated and identified in the Microbiology Laboratory of "Sf. Spiridon" Emergency Clinical Hospital and in the Microbiology Laboratory within "Gr. T. Popa" University of Medicine and Pharmacy Iaşi. The test of antibiotic sensitivity was conducted using two methods: diffusimetric - Kirby-Bauer and of MIC (minimal inhibitory concentration) determination using the E-test (for *P. aeruginosa* strains, isolated in 2013), and the interpretation of sensitivity was made in a standardized manner, in compliance with the CLSI standard (*Clinical and Laboratory Standards Institute*) for antibiotic testing in force at the moment (8).

RESULTS

In the study period we isolated and identified 167 strains of *P. aeruginosa* and 139 of *A.baumanii.*

Testing sensitivity to aminoglycosides. The mean value of the frequency of resistant *P. aeruginosa* strains was of 45.85%±26.5 for amikacin (AMK), namely of 57.41%±22.9, for gentamicin (GEN). Gentamicin resistance of *A. baumanii* strains was extremely high, eight years in a row reaching 100%, the mean resistance to this antibiotic for the whole duration of the

study being of 87.85%±26.83. The mean resistance to amikacin was 93.74%±11.61, and out of the six years of testing in four was observed a 100% resistance (tab. I).

TABLE I

Aminoglycosides resistance depending on the year of study

		years of study											
ANTIBIOTIC		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Mean ± SD
AMK (%)	***P. aeruginosa***	58.33	17.85	21.42	0.00	50.00	66.66	70.00	NT	75.00	30.00	69.23	45.85 ± 26.5
	A. baumanii	NT	93.8	100.0	100.0	100.0	100.0	NT	NT	NT	NT	33.3	93,74±11,61
GEN (%)	***P. aeruginosa***	41.66	32.14	21.42	27.27	66.66	66.66	70.00	63.63	87.50	70.00	84.61	57.41±22.9
	A. baumanii	86.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	77.8	66.7	87,85±26,83

In the case of *P. aeruginosa* strains, the tendency calculated in the period 2003-2013 was on the rise for aminoglycosides (y=5,8226x+22,478 for gentamicin and y=4,107x+23,259 for amikacin), even if these frequencies decreased in the last two years.

Testing sensitivity to quinolones. GNB (Gram negative bacilli) were tested, depending on the year, both for 2nd generation quinolones, such as pefloxacin (PFX) and ciprofloxacin (CIP), and for 3rd generation quinolones, such as levofloxacin (LVX) (tab. II).

TABLE II

Quinolone resistance depending on the year of study

		Years of study											
ANTIBIOTIC		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Mean ± SD
CIP (%)	***P. aeruginosa***	41.66	96.42	85.71	9.09	75.00	55.55	70.00	54.54	62.50	50.00	84.61	62.28±24.38
	A. baumanii	NT	NT	NT	100.0	100.0	100.0	100.0	100.0	100.0	NT	75.0	96,42±9,44
LVX (%)	***P. aeruginosa***	NT	25.00	14.28	9.09	50.00	55.55	70.00	45.45	75.00	60.00	84.61	48.89±25.68
	A. baumanii	NT	81.3	72.7	66.7	100.0	100.0	100.0	100.0	100.0	NT	75.0	88,41±14,32
PFX (%)	***P. aeruginosa***	50.00	92.85	85.71	72.72	100.00	NT	NT	NT	NT	NT	NT	80.25±19.68
	A. baumanii	80.0	100.0	100.0	100.0	100.0	100.0	NT	NT	NT	NT	NT	96,66±8,16

The highest frequency of *P. aeruginosa* strains resistant to ciprofloxacin was noticed in 2004 (96.42%), followed by 2005 (85.71%). Even if in the following years these frequencies decreased, the trend calculated for the eleven years of study was slightly increasing (y=0.4122x+59.807), as compared to LVX (y=7.1932x+9.3353). Even if for pefloxacin both strains were tested only for 5 years (2003-2007) one can notice from table II that the resistance was on a continuous rise, reaching 100%, reason for which the strains were no longer tested for this antibiotic.

Testing sensitivity to carbapenems. The percentage of *P. aeruginosa* strains resistant to carbapenems varied depending on the year and the tested antibiotic. The high-

est frequency of strains resistant to imipenem (IPM) was noticed in 2013 (84.61%), the lowest one being in 2003 (8.33%) showing a clear trend of increase of the resistance to this antibiotic. If, in the first three years of study, the frequency of *P. aeruginosa* strains resistant to etanercept (ETP) varied between 27.27% (2006) and 32.14% (2003), in 2007 it rose to 75%, reaching values of 100% in 2008-2011. Out of the few classes of antibiotics to which the *A. baumanii* is still sensitive, the carbapenems have rather good results. But, even in their case, the mean resistance of strains was of 52.60%±28.67 to IPM and of 98.70%±3.18 for ETP (tab. III).

TABLE III

Carbapenems resistance depending on the year of study

		Years of study											
ANTIBIOTIC		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Mean ± SD
IPM (%)	***P. aeruginosa***	8.33	14.28	35.71	45.45	58.33	55.55	80.00	45.45	37.50	40.00	84.61	45.92±23.51
	A. baumanii	13.3	0.0	27.3	50.0	66.7	85.7	78.6	70.0	78.6	66.7	41.7	52,60±28,67
ETP (%)	***P. aeruginosa***	NT	32.14	28.57	27.27	75.00	100.00	100.00	90.90	100.00	NT	NT	69.23±34.08
	A. baumanii	NT	NT	NT	100.0	100.0	100.0	100.0	100.0	92.9	NT	NT	98.70±3.18

Starting with 2009, the *P. aeruginosa* and *A. baumanii* strains were tested for colistin as well, the sensitivity to this antibiotic being of 100%, in 2009-2013. For 12 strains of *P. aeruginosa* isolated in 2013 we determined the MIC, by the E-test, for colistin; this varied between 0.5-1.5 mg/ml. Regardless of the MIC value to colistin, out of the 12 strains 10 were MDR (multi-drug resistant) and only 2 were also sensitive to amikacin, beside colistin.

Cross resistance. Given the fact that all the tested strains were sensitive to polymyxin E (colistin), cross resistance was observed for the following five classes of antibiotics: aminoglycosides, 3rd generation cephalosporins (ceftazidime), quinolones, penicillins associated with β-lactamase (TZP-tazobactam) and carba-penems.

Of the total 167 strains of *P. aeruginosa* isolated and identified from patients in the eleven years of the study, 48 (28.74%) of them were sensitive to at least one antibiotic from each testes class, 29 (17.39%) were resistant to a single antibiotic and the rest of 90 (53.89%) showed multiple resistance. There is a statistically significant difference between the number of strains which are sensitive to at least one antibiotic from each tested class and the number of strains with multiple resistance ($t=-4.251$; $p<0.05$).

From the strains with multiple resistance, 21 (12.57%) were tested for two classes of antibiotics, 12 (7.18%) for three classes, 18 (10.77%) for 4 classes and most of them, 39 (23.35%), for all five classes. Of the 29 strains which showed resistance to a single class of antibiotics, most of them were resistant to carbapenems (15 strains) and the fewest were resistant to ceftazidime. The most frequent association of two antibiotics, to which *P. aeruginosa* proved resistant, was represented by the association of TZP+ceftazidime (6 strains) and quinolones + aminoglycosides (5 strains), respectively.

In the case of strains resistant to four classes of antibiotics, 11 strains out of 18 associated cross resistance to: TZP + fluoro-

quinolone+ aminoglycosides + carbapenems, 6 strains to: TZP + fluoroquinolones + aminoglycosides + ceftazidime, and another strain to fluoroquinolones + aminoglycosides + carbapenems + ceftazidime. Cross resistance to all five classes of antibiotics was noticed starting with 2004 (4 strains) and continued until 2013 (13 strains).

In the case of *A. baumanii* strains, only 5 (3.59%) of them were sensitive simultaneously to the three classes of antibiotics tested (quinolones, carbapenems, aminoglycosides), the rest being resistant to at least one antibiotic from each class.

Combined resistance could be observed with 121 strains (87.04%), out of which 55 (39.56%) were resistant to two classes of antibiotics and the rest of 66 (47.48%) to all three classes. The most frequently met was the association of resistance to quinolones + aminoglycosides, namely 49 strains (35.25%) (tab. IV).

Resistance to a single class of antibiotics could be observed only for 13 strains (9.35%), the relation between fluoroquilones and aminoglycosides being fairly equal. We couldn't identify any strain resistant only to carbapenems.

TABLE IV
Antibiotics resistance pattern for *A. baumanii* strains

Resistance *Pattern*	Europe 2012 (%)	Our study (%)
Sensitive to all tested classes	27.1	3.59
Resistance to a single class of antibiotics		
TOTAL	**4.9**	**9.35**
Quinolones	3.4	4.31
Aminoglycosides	0.9	5.03
Carbapenems	0.6	0
Resistance to two classes of antibiotics		
TOTAL	**17**	**39.56**
Quinolones + Aminoglycosides	8.2	35.25
Quinolones + Carbapenems	8.5	2.87
Aminoglycosides + Carbapenems	0.3	1.43
Resistance to three classes of antibiotics		
Aminoglycosides + Carbapenems + Quinolones	**51**	**47.48**

Of the strains which associated resistance to all the three classes of antibiotics, the most numerous were isolated in 2009 and 2011. Even if in 2013 we identified only 4 resistant strains, simultaneously for all 3 classes, the general linear trend is rising ($y=0.8182x+1.0909$).

DISCUSSION

P. aeruginosa is resistant to most antimicrobial agents due to its selective ability of the outer membrane. The antibiotic classes to which *P. aeruginosa* is also sensitive are: fluoroquinolones (ciprofloxacin + levofloxacin), aminoglycosides (gentamicin, amikacin and tobramycin), certain β-lactamases (piperacillin + tazobactam, ceftazidime, cefepime, imipenem, doripenem and meropenem) and poly-myxins (polymyxin B and colistin).

All presented results indicate the fact that there is an increase of the circulation of

strains which became resistant to the most frequently used antibiotics. Starting with 2005, EARSS (European Antimicrobial Resistance Surveillance System) started to centralize data regarding antibiotic resistance of *P. aeruginosa* strains. In the case of carbapenems we took into account imipenem resistance, the mean frequency of resistant strains being of 45.92%±23.51 (9). According to the data reported by EARSS, carbapenems resistance for Romania was of 34.20%±26.16, without a statistically significant difference (t=1.65, GL=10, p=0.129). Furthermore, in 2012, in Europe was reported a rate of strains resistant to this class of antibiotics of 17.1%, varying from 3.2% in Malta, to 51.2% in Romania (9). In the same year, IPM resistance in the case of strains isolated in the Orthopedics - Traumatology Clinic was 40%.

In 2012, the resistance reported for fluoroquinolones was of 21% in Europe, varying from 0% in Malta, to 56.3% in Slovakia (9); in our study, CIP resistance was of 50% and LVX resistance of 60%. The mean frequency of *P. aeruginosa* strains resistant to fluoroquinolones in the period 2005-2013 was of 44.16%±20.87 for Romania, and in our study of 62.28%± 24.38 for CIP and 48.89%±25.68 for LVX, with statistically significant differences only in the case of CIP (t=2.465, GL=10, p=0.033). The increased CIP resistance is explained by its frequent use in the hospital environment (10).

For aminoglycosides, the mean frequency of resistant strains was 44.75%±16.1 for Romania, and in our study it was very close, (45.85%±26) for AMK and 57.41%± 22.9 for GEN, with no statistically significant differences. In 2012, in Europe this level of resistance was, on the whole, of 18.4%, varying from 0%, in Iceland to 48.8% in Romania, once again "leading" the top. In the same year, isolated strains in our study had a resistance of 30% to amikacin and 70% to gentamicin.

After analyzing the data reported by Romania to EARSS for 2005-2012 (9) we noticed a general increasing trend of resistance to aminoglycosides, fluoroquinolones, the most important rising tendency being in the case of carbapenems.

Aminoglycosides generally have a good activity against the most important GNB. According to our study, 45.85% of the strains were resistant to amikacin. This percentage is much higher as compared to the one reported by Carvallo et al. (11) (namely 14%). Miller et al. (12), state that the resistance pattern to aminoglycosides is affected by the selective pressure of antibiotics, with higher variations among various regions, a phenomenon related to the "abuse" of antibiotics therapy.

In 2012, the percentage of multiple resistance strains (to more than three classes of antibiotics) of *P. aeruginosa* varied from 0% in Malta, 1.7% in Great Britain and 1.8% in Denmark, to 46.3% in Romania, which ranked first in Europe (9). The emergence of MDR strains of *P. aeruginosa* is continuously rising all over the world and is a major problem in the management of these infections (13), a fact also corroborated by the analysis of the data reported by Romania to EARSS.

From the total of *P. aeruginosa* strains, only 48 were sensitive to all classes of antibiotics, 29 were resistant to a single class and the rest of 90 (53.89%) were resistant to at least two classes and classified as MDR. These data are very similar with those in the study conducted by Strateva et al.(13) (49.8%).

A. baumanii was included in *EARS-Net*

(European Antimicrobial Resistance Surveillance Network) only in 2012, when several member states noticed an increase of antibiotic resistance of this microorganism. The percentage of strains sensitive to all antibiotics was, on average, in Europe, of 27.1% (9), varying from 4.2% in Romania, to 100% in Netherlands. According to our study, on the whole duration of the eleven years, the percentage of strains sensitive to all tested classes was only of 3.59%, the rest of 96.41% being resistant to at least one class of antibiotics and for 2012, out of the 18 strains which were isolated, none was sensitive to all antibiotics and 50% (9) strains were resistant simultaneously to fluoroquinolones, aminoglycosides and carbapenems, being sensitive only to colistin.

Moreover, according to the same *EARS-Net* report, fluoroquinolones resistance was of 90%, aminoglycosides resistance of 54.2% and carbapenems resistance of 81.3%. These data are partially similar to the presented data, since the aminoglycosides resistance signaled by us was much higher (86.8% to amikacin and 92.8% to gentamicin), with statistically significant differences ($p<0.001$). In the case of carbapenems, the resistance was much lower to imipenem (52.8% *vs.* 81.3%), a statistically significant difference ($p<0.001$). On the other hand, in the case of fluoroquinolones, the percentage of resistant strains was approximately equal (88.41% - LVX and 96.66% PFX *vs.* 90%), with no statistically significant difference.

In Europe, aminoglycosides resistance varied from 1.7% in Netherlands to 83.1% in Italy, for carbapenems varied from 0% in Norway to 87.8% in Italy and for fluoroquinolones from 0% in Netherlands and Norway to 93.1% in Greece. It seems that there are very great variations with regard to antimicrobial resistance of *A. baumanii* strains in Europe, most resistant strains being met in Southern Europe as compared to the countries from the north of Europe, most likely due to the selective pressure exercised by the anti-infectious therapies used in excess.

The global spread of *P. aeruginosa* and *A. baumanii* strains resistant to carbapenems is a major concern given the fact that carbapenems are frequently used in the treatment of such infections (14).

CONCLUSIONS

In the case of *P. aeruginosa* strains, the highest resistance was noticed for aminoglycosides and quinolones, all strains being sensitive to colistin; out of all tested strains, approximately one fourth associated cross resistance to the four classes of antibiotics.

A. baumanii strains showed an extremely high resistance to all tested antibiotic classes, the lowest being met in case of IPM testing (52.6%); all tested strains were sensitive to colistin; cross resistance to three antibiotic classes was identified at half of the strains, which is a major concern.

P. aeruginosa is found on the list of the main bacteria responsible for the infections associated with the sanitary system, being classified among the first five etiologic agents of nosocomial infections. In our country we noticed a net increase of resistance to all tested antibiotic classes, more important in the case of carbapenems, a trend also illustrated by our study.

Colistin resistance of *P. aeruginosa* and *A. baumanii* strains is still considered extremely rare, although several cases were reported, while not in our country. Unfortunately, this antibiotic which is part of the

high toxicity category remains the last therapeutic resort in the infections caused by these microorganisms, especially because the frequency of MDR strains is high.

The already high percentages and the rising trends of antibiotic resistance for Gram-negative bacteria, including non-fermenting ones, described by this study, show the continuous decrease of the efficiency of antimicrobial agents against these microorganisms and underline the necessity of a global strategy focused on all health sectors.

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THE DISCONTENT DEGREE IN RELATIONS WITH THE PROXIMAL SOCIAL ENVIRONMENT AND ADOLESCENT PERSONALITY FEATURES IN TIMIS COUNTY, ROMANIA

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THE DISCONTENT DEGREE IN RELATIONS WITH THE PROXIMAL SOCIAL ENVIRONMENT AND ADOLESCENT PERSONALITY FEATURES IN TIMIS COUNTY, ROMANIA (Abstract): In our study we investigated whether there is a relation between the degree of discontent in relations with the proximal social environment (family and peer groups) and adolescent personality features. The study was conducted on a statistical representative and homogenous sample consisting of 2908 teenagers (51.5% girls and 48.5 % boys, aged between 15 and 19 years). It was an observational (case) study and it consisted in 2 questionnaires applying: Freiburg Personality Inventory (212 items) and CORT 2004 questionnaire (116 items, 6 referring to the discontent levels created by proximal social environment). Cronbach`s alpha index was 0.802 for FPI and 0,910 for CORT 2004. Statistical analysis was performed by SPSS 16 program applying Chi square (χ^2) and gamma (γ) correlation. Results: Depression and Emotional lability correlated positively and powerfully with q15 (discontent degree of relations with present friend-peers) (γ= 0.471, Sig. 0.000, and γ= 0.383, Sig. 0.000, respectively) and q5 (discontent degree of the relation with parents) (γ= 0.380, Sig. 0.000, and γ= 0.337, Sig. 0.000, respectively). Sociability and calm correlated negative with q5 (γ=- 0.14, Sig. 0.000, and γ = - 0.35, Sig. 0.000, respectively), q15 (γ = - 0.33, Sig. 000 and γ = - 0.18, Sig. 000, respectively). In conclusion, there is direct proportional relation between the discontent degree of relations with friend-peers and parents and depression and emotional lability, and inverse proportional relation of friend-peers and parents with sociability and calm. **Keywords:** PROXIMAL SOCIAL ENVIRONMENT, FAMILY, PEERS, PERSONALITY FEATURES.

Adolescence is a developmental stage in which experiencing relationships with people around becomes important in social integration. The proximal social environment consists of family (parents, siblings), school-peers and friend-peers outside the school. The relationships system affects powerfully the developing adolescent personality, which prepares them for social competencies in adult life (1). Adolescence is a stage in which the person passes through all the previous developmental stages (recycling) (2).

MATERIAL AND METHOD

The aim of our study was to investigate the relationship between the discontent level created by proximal social environ-

ment (family and peers) and adolescent personality features. The material consisted of a statistically representative (2908 teenagers) and homogenous (51.5% girls and 48.5 % boys, aged between 15 and 19 years) sample. It was stratified in nests, the sample unit being the class group. This study was an observational one (case study) and consisted in two questionnaires: Freiburg Personality Inventory (FPI - 212 items) (3, 4) normalized for Romanian population and CORT 2004 (116 items of which 6 referred to discontent degree of relations in proximal social environment). The questionnaires were applied individually and anonymously. The application time for both questionnaires was 60 minutes. Personality items referred to: Nervousness (Nrv), Aggressiveness (Agr), Depression (D), Excitability (Exc), Sociability (Soc), Calm (Clm), Domination (Do), Inhibition (Inh), Sincerity (Snc), Extraversion (Exv), Emotional lability (Lbl) (bipolar features of the personality profile). The CORT 2004 items, which measured the discontent degree of relations in proximal social environment, were: Q1 (organized and disorganized family), Q5 (relation with parents), Q6 (relation with siblings), Q10 (financial status of the family), Q14 (relation with school-peers), and Q15 (relation with present friend-peers). Cronbach`s alpha index was 0.802 for FPI and 0,910 for CORT 2004. Statistical analysis was performed by SPSS 16 program applying Chi square ($\chi^2$) and gamma ($\gamma$) correlation. The 9 ranks of personality features` intensity were reduced to 3 (1=1,2,3; 2=4,5,6; 3=7,8,9) in order to avoid the small number of cases.

RESULTS

We registered a significant statistical difference between the degree of family disorganization (q1) regarding certain personality features (depression, excitability, sincerity, emotional lability), with Chi Square: χ^2=9.97, Sig. 0.04; χ^2=15.37, Sig. 0.004; χ^2=21.1, Sig 0.000, and χ^2=13.97, Sig. 0.007, respectively, (tab. I).

The discontent degree in the relationship with parents (q5: 1-content, 2-nor content, neither discontent, 3-discontent, 4-no parents) related strongly with 11 dimensions of the personality profiles: nervousness, aggressiveness, depression, excitability, sociability, calm, domination, inhibition, sincerity, emotional lability (tab. II). Main statistical differences between discontent degrees of relations with parents were registered for depression with χ^2 =117,512, Sig. 0.000 and for emotional lability (χ^2 =91,063, Sig. 0.000) (tab. II). The discontent degree of relation with present friend-peers (q15) had a significant statistical difference for 9 personality features of teenagers, more relevant being for depression (χ^2 =117,493, Sig. 0.000) (tab. III). The satisfaction degree of the relation with siblings (brothers and sisters) (Q6) related powerfully with 6 personality features, the highest values being registered for depression (χ^2 =72.753, Sig. 0.000) and emotional lability (χ^2 =52.782, Sig. 0.000) (tab. IV).

The discontent degree of the relationship with parents (q5) correlated with 11 dimensions of the personality profiles, followed by the discontent degree of relationship with present friend-peers (q15 – 9 personality features), and the discontent degree of the relationship with siblings (q6 – 6 personality features), correlation gamma being statistically significant (tab. V).

Depression and emotional lability proved to be the main adolescent personali-

ty features, which relate with the discontent levels of their relation with present friend-peers (q15) (γ=0.471, Sig. 0.000, and γ=0.383, Sig. 0.000, respectively) and with parents (q5) (γ=0.380, Sig. 0.000, and γ=0.337, Sig. 0.000, respectively) (tab. V).

TABLE I

Chi square (χ^2) values and significations of statistical difference of certain personality features of adolescents between the degrees of family disorganisation (q1)

	No of teens	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
q1* D	2592	9.970(a)	4	0.041
q1*Exc	2594	15.376(a)	4	0.004
q1*Snc	2593	21.109(a)	4	0.000
q1*Lbl	2594	13.977(a)	4	0.007

[a]0 cells (0%) have expected count less than 5

TABLE II

Chi square (χ^2) values and significations of statistical difference of 11 personality features of adolescents between discontent degrees of relation with parents (q5)

	No of teens	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
q5*Nrv	2591	60.361(a)	6	0.000
q5*Agr	2595	69.522(a)	6	0.000
q5*D	2593	**117.512(a)**	6	0.000
q5*Exc	2595	54.632(a)	6	0.000
q5*Soc	2593	20.455(a)	6	0.002
q5*Calm	2595	28.858(a)	6	0.000
q5*Dom	2595	25.627(a)	6	0.000
q5*Inh	2595	23.301(a)	6	0.001
q5*Snc	2594	59.399(a)	6	0.000
q5*Lbl	2595	**91.063(a)**	6	0.000

[a]0 cells (,0%) have expected count less than 5

TABLE III

Chi square (χ^2) values and significations of statistical difference of 9 personality features of adolescents between discontent degrees of relation with present friend-peers (q15)

	No of teens	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
q15*Nrv	2478	59.477(a)	6	0.000
q15*Agr	2481	59.477(a)	6	0.000
q15*D	2479	**117.493**(a)	6	0.000
q15*Exc	2481	37.240(a)	6	0.000
q15*Soc	2479	61.581(a)	6	0.000
q15*Calm	2481	22.977(a)	6	0.001
q15*Inh	2481	48.852(a)	6	0.000
q15*Snc	2480	37.740(a)	6	0.000
q15*Lbl	2481	24.528(a)	6	0.000

[a]0 cells (0%) have expected count less than 5

TABLE IV

Chi square (χ^2) values and significations of statistical difference of 6 personality features of adolescents between discontent degrees of relation with siblings (q6)

	No of teens	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
Q6*Agr	2570	45.629(a)	6	,000
Q6*D	2568	**72.753**(a)	6	,000
Q6*Exc	2570	40.943(a)	6	,000
Q6**Calm	2570	33.275(a)	6	,000
Q6*Snc	2569	33.115(a)	6	,000
Q6*Lbl	2570	**52.782**(a)	6	,000

[a]0 cells (0%) have expected count less than 5

TABLE V

Gamma coefficients (γ) established between q1 (disorganising degree of family), q5 (discontent degree of relation with parents), q15 (discontent degree of relation with present friend-peers), q6 (discontent degree of relation with siblings) and personality features of adolescents

	No of teens	Ordinal by Ordinal Gamma	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
q1* D	2592	,120	,042	2,859	,004
q1* Exc	2594	,101	,042	2,405	,016
q1*Snc	2593	,149	,041	3,556	,000
q1*Lbl	2594	,142	,042	3,367	,001
q5*Nrv	2591	,276	,036	7,606	,000
q5*Agr	2591	,298	,034	8,447	,000
q5*D	2595	**,380**	,033	10,771	,000
q5*Exc	2593	,263	,035	7,297	,000
q5*Soc	2595	-,140	,038	-3,687	,000
q5*Calm	2593	-,148	,035	-4,213	,000
q5*Dom	2595	,170	,036	4,669	,000
q5*Inh	2595	,145	,037	3,860	,000
q5*Snc	2595	,264	,035	7,260	,000
q5*Lbl	2594	**,337**	,033	9,441	,000
q15*Nrv	2478	,354	,043	8,095	,000
q15*Agr	2481	,192	,043	4,428	,000
q15*D	2479	**,471**	,037	10,934	,000
q15*Exc	2481	,253	,043	5,738	,000
q15*Soc	2479	-,330	,042	-7,364	,000
q15*Clm	2481	-,182	,042	-4,266	,000
q15*Inh	2481	,303	,042	6,734	,000
q15*Snc	2480	,210	,043	4,718	,000
q15*Exv	2481	-,206	,046	-4,339	,000
q15*Lbl	2481	**,383**	,039	8,739	,000
q6*Agr	2570	,105	,031	3,372	,001
q6*D	2568	,105	,032	3,262	,001
q6*Exc	2570	,137	,032	4,297	,000
q6*Calm	2570	-,153	,031	-4,935	,000
q6*Snc	2569	,143	,031	4,603	,000
q6*Lbl	2570	,139	,032	4,374	,000

a Not assuming the null hypothesis.

b Using the asymptotic standard error assuming the null hypothesis.

Sociability and calm correlated negative with discontent degree of relation with parents (q5) (γ=- 0.14, Sig. 0.000, and γ = - 0.35, Sig. 0.000, respectively), and discontent degree of relation with present friend-peers (q15) (γ = - 0.33, Sig. 000 and γ = - 0.18, Sig. 000, respectively). Calm correlated negatively with discontent degree of relation with brothers and sisters (q6) (γ = - 0.153, Sig. 0.000), too.

DISCUSSION

The relation with parents proved to be decisive in the process of personality forming, parents being models and modeling factors of it. Failures of this relation are visible in psychiatric pathology or in numerous psycho-pathological processes in adult life. (5).

Schools organize programs aimed at stimulating and orienting resources (motivating) with the objective of avoiding limit or burn-out situations (6).

Life satisfaction was found as protective factor against suicidal thoughts and behaviors in adolescents (7). In a study performed in Poland about the adolescents` optimism, the satisfaction of the relationships with family was significantly associated with life joy both for girls and for boys (8). In another study, the relation with others is presented like a psychological aspect of the wellbeing state (9). Our study finds a direct proportional relation of the discontent degree of relations in proximal social environment (as psychological aspect which can affect the wellbeing state) with emotional lability (positive/negative affects - the subjective aspect of the well-being state) and depression (a result of dissatisfaction in life) (9). An inverse proportional relation between discontent degrees of relations with present friend-peers or parents (social connectivity and balance) (10) and sociability and calm (subjective aspects of wellbeing) (9) was also identified in our study.

CONCLUSIONS

1. There is a relation between discontent degree of relations in proximal social environment and personality features of adolescents (11 for relations with parents, 9 for relations with present friend-peers and 6 for siblings).

2. The discontent degree of relation in family and with present friend-peers relates positively with depression and emotional lability, and negatively with sociability and calm.

3. The results of our study are similar with results in literature as they confirm the existence of a relation between the discontent degree of relations with the proximal social environment (psychological aspect of wellbeing) and personality features of adolescents (subjective aspects of wellbeing state).

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NEWS

EVALUATION OF THE EFFECTS OF DIODE (980 NM) LASER ON GINGIVAL INFLAMMATION AFTER NONSURGICAL PERIODONTAL THERAPY

Periodontitis is an inflammatory disease, for which, scaling and root planning is the common approach for non-surgical control of inflammation. The use of lasers is another approach in the first phase of periodontal treatment for control of inflammation. Diode laser has some beneficial effects such as acceleration of wound healing, promotion of angiogenesis and augmentation of growth factor release. The aim of a study realized by a group of researchers from Iran, was the evaluation of diode laser (980 nm) effect on gingival inflammation when it is used between the first and second phase of periodontal treatment, in comparison with common treatment scaling and root planning modality alone. In the study were included 21 patients with moderate to severe chronic periodontitis, divided in to control group (scaling and root planning) and test group (scaling and root planning + laser). Two months after the last scaling and laser radiation, indexes including gingival level, bleeding on probing and modified gingival index were recorded and compared with baseline. The obtained data showed two months after the beginning of the study, all indices improved in both groups. The indices were not different between two groups except for bleeding on probing which was lower in laser group. Based on overall improvement in parameters such as superiority of laser application in some indices, lack of thermal damage and gingival recession with the specific settings used in this study, the application of laser as an adjunctive treatment together with common methods is preferable (Zare D, Haerian A, Molla R, Vaziri F. Evaluation of the effects of diode (980 nm) laser on gingival inflammation after nonsurgical periodontal therapy. *J Lasers Med Sci*, 2014; 5 (1): 27-31).

Irina Grădinaru

MICROPARTICULATE DRUG DELIVERY SYSTEM CONTAINING TRAMADOL HYDROCHLORIDE FOR PAIN TREATMENT

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MICROPARTICULATE DRUG DELIVERY SYSTEM CONTAINING TRAMADOL HYDROCHLORIDE FOR PAIN TREATMENT (Abstract): The current trend of replacing conventional pharmaceutical forms is justified because most substances administered in this form give fluctuations of therapeutic concentrations and often outside the therapeutic range. In addition, these formulations offer a reduction in the dose or the number of administrations, thus increasing patient compliance. **Aim**: In the experiment, we developed an appropriate technology for the preparation of gelatin microspheres containing tramadol hydrochloride by emulsification/cross-linking method. **Materials and methods**: The formulated microspheres were characterized by product yield, size distribution, encapsulation efficiency and *in vitro r*elease of tramadol hydrochloride. Data obtained from *in vitro* release studies were fitted to various mathematical models to elucidate the transport mechanisms. The kinetic models used were zero-order, first-order, Higuchi Korsmeyer-Peppas and Hopfenberg. **Results**: Spherical microspheres were obtained, with free-flowing properties. The entrapment efficiency of tramadol hydrochloride in microparticles was 79.91% and product yield -94.92%. As the microsphere size was increased, the entrapment efficiency increased. This was 67.56, 70.03, 79.91% for formulations MT80-250, MT8-500 and, MT250-500. High entrapment efficiency was observed for MT250-500 formulation. The gelatin microspheres had particle sizes ranging from 80 to 500 µm. The drug was released for a period of 12 hours with a maximum release of 96.02%. Of the three proposed formulations, MT250-500 presented desirable properties and optimal characteristics for the therapy of pain. Release of tramadol hydrochloridi was best fitted to Korsmeyer-Peppas equation because the Akaike Information Criterion had the lowest values for this kinetic model. **Conclusions**: These results suggest the opportunity to influence the therapeutic characteristics of gelatin microspheres to obtain a suitable drug delivery system for the oral administration of tramadol hydrochloride. **Keywords:** TRAMADOL HYDROCHLORIDE, GELATIN MICROSPHERES, EXTENDED RELEASE, KINETICS MECHANISM, MATHEMATICAL MODELS.

Tramadol hydrochloryde is a synthetic opioid with dual action mechanism (the stimulation of µ receptors and the inhibition reuptake of noradrenalin and seroto-

nin) (1); clinical usefulness is mainly due to high efficiency in moderate and severe pain (trauma, colic pain, post surgery pain or that of oncological nature) but also due to the diminished dependence potential and low risk of respiratory depression, being thus efficient at patients with high cardiovascular risk or pulmonary impairment; also the dual mechanism (especially the augmentation of spinal monoaminergic system) gives it increased efficacy in neuropathic pain (2).

Weak agonist of mu opioid receptors, the compound clinically used is a racemic mixture, (-) - tramadol being an inhibitor of noradrenalin reuptake, being 10 times stronger than (+) - tramadol, which, in turn has an increased capacity to inhibit serotonin reuptake (2, 3).

Hepatic metabolism is CYP2D6-dependent, one of the metabolites (produced by O-demethylation) being pharmacodynamically active with long-term action. Since CYP2D6 is the object of an important genetic polymorphism, at slow metabolizes the analgesic effect can be suboptimum in usual dosage and in the case of fast metabolizers toxicity mediated by μ receptors can occur (4, 5).

Clearance is done by hepatic metabolization (via O-demethylation to form the active metabolite, N-demethy-lation, respectively, both are followed by glucuronide conjugation and sulfo-conjugation); tramadol and its metabolites are mainly renal cleared. Half-life of tramadol is around 5.5-6 hours, but of O-desmethyltramadol is of about 8-9 hours (2, 3, 6).

Given the need for adequate pain control throughout the day and taking into account the short half life, tramadol administration in conventional oral formulations should be made at intervals up to 6 h, up to a total cumulative dose of 400 mg daily (1).

This leads to the conclusion that a formulation with delayed release, which increases plasma concentration level in the 12 hour therapeutic interval is extremely useful, ensuring an enhanced adherence to treatment and a reduced frequency of administration, with an adequate control of pain (3, 6). An optimal formula must ensure a personalized dosage regimen targeted to the patient, independent of daily meals, flexible, with an improved control of background pain or painful flare (7).

Biodegradable polymers are widely used in the development of multi particulate drug delivery systems, and several mechanisms may be responsible for the release of the active ingredient dispersed in a matrix: erosion of the polymer, diffusion of the drug particles through the matrix and dissolution of the drug in the surrounding medium (8).

Naturally macromolecules such as gelatin are extensively used in controlled drug release to maximize the efficacy and facilitate the clinical applicability (9). The gelatin microspheres were prepared by emulsification- formaldehyde-cross-linking method.

The cross-linking reaction causes significant changes in the polymer molecule, it influences certain properties such as the reduction of imbibitions and it limits the dissolution properties of the drug influencing the failure of the microsphere.

In the experiment, we tried to develop an appropriate technology for the preparation of gelatin microspheres, obtaining a good product yield, a higher entrapment efficiency of tramadol hydrochloride and an extended-release formulation with a good efficacy and tolerability. The formulated microspheres were characterized by

product yield, size distribution, encapsulation efficiency and *in vitro* release of tramadol hydrochloride. Data obtained from *in vitro* release studies were fitted to various mathematical models to elucidate the transport mechanisms. The kinetic models used were zero-order, first-order and Higuchi. Mathematical modeling of drug release kinetics provided a basis for the study of mass transport mechanisms that are involved in the control of drug release (9).

MATERIALS AND METHODS

Materials

Tramadol hydrochloride (Hyderabad-Nacharam, India), gelatin type B 180 Bloom strength (Fluka AG), formaldehyde (Riedel de Haen), sunflower oil, isopropyl alcohol, acetone, ethyl ether. All the reagents used were of analytical grade.

Methods

Preparation of tramadol hydrochloride microspheres

Tramadol hydrochloride was dissolved in water and after the required quantity of gelatin to obtain a 25% solution was dissolved in that solution at 60 °C. Polymer drug solution was added drop wise to 100 ml of sunflower oil preheated to 60 °C, with continuous stirring at 300, 500 and 600 rpm. The emulsion was cooled and stirred at 4 °C in an ice bath for 15 minutes. The isopropyl alcohol-acetone solution was added for dehydration, in three stages (ten milliliters for each time) and the stirring was continued at room temperature. The microspheres were separated by filtration using Whatman paper and were washed with anhydrous ether to remove the sunflower oil. After washing, the microspheres were dried at 25°C and transferred to desiccators for a period of 12 hours for cross-linking with formaldehyde.

Product Yield

The product yield of the microspheres was determined using the equation (10):

$$M\ (\%) = \frac{M_0 - M_1}{M_0} \times 100$$

where, M is the mass loss, M_0 is the initial weight of drug and polymer and M_1- the weight of micro particles.

Characterization of microspheres

Particle size analysis

Determination of average particle size was measured by optical microscopy. The microspheres were dispersed in water and a smear was made on a glass slide, and the size of 200 particles was measured. The particle size distribution of the microspheres was plotted, and mean diameter was determined using the following equation (11):

$$D\ (\mu m) = \frac{\sum nd}{200}$$

where D is the mean of particle size, d is the diameter of the particle and n is the number of particles present in diameter d.

Entrapment efficiency of tramadol hydrochloride

The amount of tramadol hydrochloride encapsulated in the microspheres was determined using the formula (11):

$$E\% = \frac{Q_p\ (mg)}{Q_t\ (mg)} \times 100$$

where E is the entrapment efficiency, Qp is the quantity of drug encapsulated in microspheres, Qt is the total drug added for encapsulation.

The drug content of microspheres was

determined by dissolving a known weight of microparticles before the cross-linking process in buffer solution and analyzed after dilution at 270 nm using a UV-VIS spectrophotometer *Specord 210*.

In vitro drug release from microspheres

The *in vitro* release studies of various tramadol hydrochloride micro particles was determined using USP apparatus II (Erweka) at 37 °C ± 0.5 °C. Each sample of microspheres, equivalent to 100 mg of tramadol hydrochloride, was added to 500 ml of dissolution medium. The dissolution medium was kept at pH 1.2 for 2 hours, pH 4.5 for 2 hours and pH 7.4 for the next 8 hours. Ten millimeters of sample were withdrawn at regular time intervals and the same volume of dissolution medium was replaced. After suitable dilution, the tramadol hydrochloride content was estimated at 270 nm using a UV-VIS spectrophotometer (*Specord 210*) (12).

Drug release kinetics

The obtained experimental data from *in vitro* release studies were fitted to various kinetic equations. Knowledge of the kinetics of drug release from microspheres is important for the *in vivo-in vitro* correlation. The applied kinetic models are: zero-order ($F= k_0 \cdot t$; k_0= the zero-order release constant), first-order ($F=100 \cdot (1-Exp(-k_1 \cdot t))$; k_1= the zero-order release constant), Higuchi ($F=kH \cdot t^{0.5}$; kH= the Higuchi release constant), Korsmeyer-Peppas ($F= kKP \cdot t^n$; kKP= the release constant incorporating structural and geometric characteristics of the drug-dosage form; n= the diffusion exponent indicating the drug-release mechanism) and Hopfenberg equation ($F=100 \cdot (1-(1-kHB \cdot t)^n)$; kHB= the combined constant of the release process which considers both the erosion rate constant and the initial concentration of drug in the matrix)(13).

RESULTS AND DISCUSSION

Gelatin microspheres loaded with tramadol hydrochloride in the form of a white powder are characterized by a free flow. Observed under the microscope, they appear in the form of spherical particles.

Product yield

The obtained results indicated that as the stirring rate increased the percentage yield decreased, which indicated a loss of material due to high speed stirring. The results are shown in first table.

TABLE I
The effect of the stirring rate on product yield

Stirring speed (rpm)	Product Yield (%)
300	94,92 ± 2.24
500	90,93 ± 2.09
600	66,71 ± 3.01

All mean values are obtained by 3 readings (± SD)

Particle size analysis

The size distribution of microspheres was between 80 and 500 μm, with an average size of 190 μm (fig. 1). Gelatin microspheres loaded with tramadol hydrochloride were separated into two classes with different particle sizes, one with particles ranging from 80 to 250 μm, and another 250-500 μm. For future studies three classes of microspheres were used, different in terms of granulometry and noted: MT80-250, MT250-500, MT80-500, respectively. Formula MT80-500 consisted in not separated particles while the others two were obtained separating particles by size.

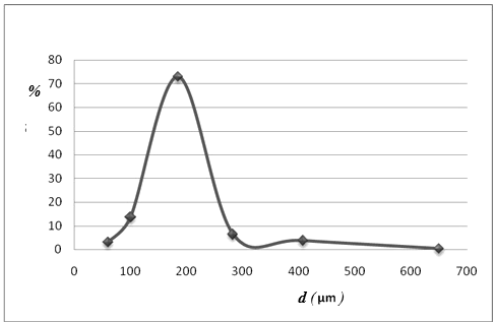

Fig. 1. Particle size distribution of microspheres loaded with tramadol hydrochloride

Entrapment efficiency of tramadol hydrochloride

The entrapment efficiency was 67.56, 79.91, 70.03% for formulations MT80-250, MT250-500 and MT80-500. As the microsphere size was increased, the entrapment efficiency increased. High entrapment efficiency was observed for MT250-500 formulation, and the lowest for MT80-250 formulation, this indicating that, during the preparation process, higher parts of drug was removed from the small particles than from the largest ones, the smaller being the particles, the higher being their exposed surface areas.

In vitro drug release from microspheres

We studied the influence of particle size on tramadol release from the microspheres. The release studies showed acceptable profile for an extended release of tramadol from cross-linked gelatin microspheres. The release rate of tramadol is shown in fig. 2 (Values represent a mean of six determinations and bars represent ± SE).

The release was biphasic with an initial higher rate of release; after the release it became slow and prolonged. The cumulative percentages of drug release after 12 hours were found to be 96.02%, 61.29% and 82.34% for formulations MT80-250, MT250-500 and MT80-500. The drug release rate was faster from MT80-250 microspheres when compared with higher particle formulations.

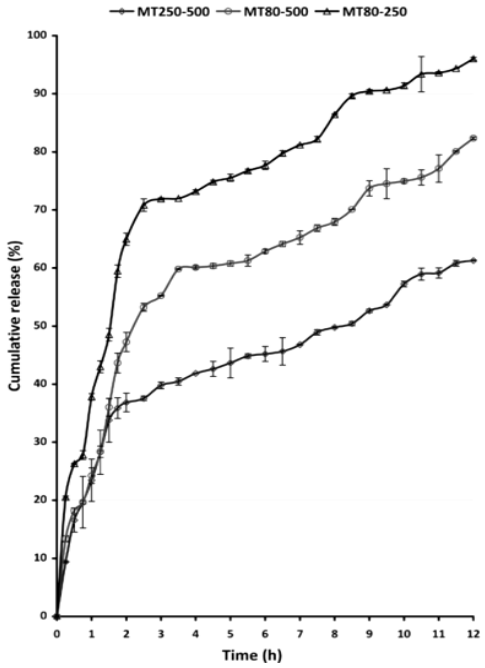

Fig. 2. The *in vitro* release of tramadol hydrochloride from gelatin microspheres

Although entrapment efficiency is directly proportional to particle size, the release rate of tramadol has been shown to be inversely proportional to particle size, due to greater surface contact with the dissolution medium.

Drug release kinetics

The models by which the release profiles were fitted in order to analyze the kinetics of tramadol release from studied formulations are shown in table II.

To discriminate between the kinetic models we used the AIC index (Akaike

Information Criterion). Considering that the lowest values indicate the best fitting, from the comparative analysis (fig. 3), it appears that for both periods (0-2 h and 2-12 h, respectively) tramadol release from the microspheres can be described by the Korsmayer-Peppas kinetic. This kinetic model is based on Fick's diffusion laws.

TABLE II
Kinetics of tramadol released from microspheres

Kinetic Models		zero-order	first-order	Higuchi	Korsmeyer-Peppas		Hopfenberg	
Kinetic Parameters		k_0	k_1	kH	kKP	n	kHB	n
Time: 0 – 2 h:								
Formula Code	**MT 250-500**	21.2	0.26	25.4	24.4	0.64	0.000	635.9
	MT 80-500	24.4	0.30	29.0	26.8	0.76	0.013	23.8
	MT 80-250	34.3	0.49	40.9	38.9	0.68	0.001	351.9
Time: 2 - 12 h								
Formula Code	**MT 250-500**	6.2	0.09	18.4	26.8	0.31	0.000	630.0
	MT 80-500	8.4	0.17	25.1	39.8	0.27	0.000	2393.1
	MT 80-250	10.3	0.32	31.0	54.3	0.22	0.000	2913.9

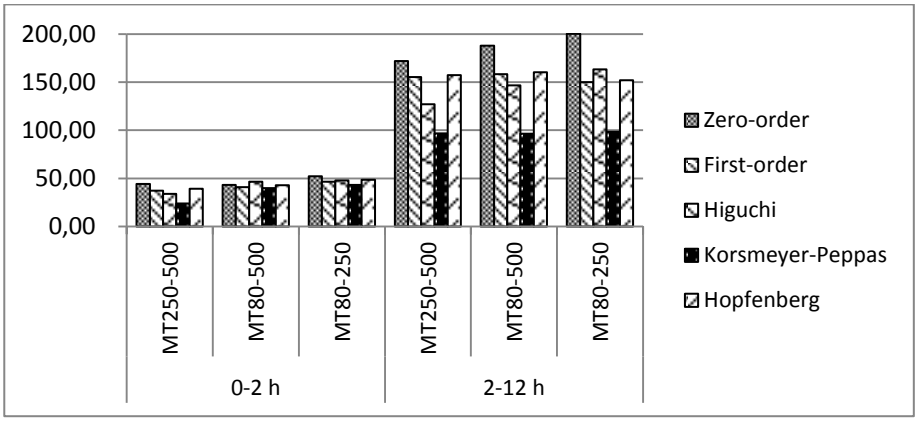

Fig. 3. AIC index values

To determine the mechanism of the kinetic processes by which the tramadol is released from the studied formulations, the *in vitro* release profiles were fitted to the Korsmeyer-Peppas model, determining the diffusion exponent 'n' and the kinetic con-

stant 'kKP' (h^{-1}). Each of the three studied profiles was subjected to the kinetic equation for the determined data over the period during which the maximum of 60% of the initial content was released (i.e.: 11.0 hours in case of MT250-500; 3.5 hours in case of MT80-500; 1.75 hours in case of MT80-250). In addition to the simple kinetic equation, we also applied two variants of the model: the equation with "T_{lag}" (h) which includes the time lag until the expression of the kinetic process; and the equation with "F_0" (%) which considers the fraction of drug that is suddenly released at the start of the kinetic process. The degree of fitting is shown by parameter values 'Rsqr_adj' (the adjusted coefficient of determination), 'MSE' (the mean square error), 'AIC' (the Akaike Information Criterion) and 'MSC' (the Model Selection Criterion) (13).The results are presented in table III.

'Rsqr_adj' parameter values (0.9616 - 0.9756) show that the kinetic model does not fully represent the factors influencing the kinetic processes, but, overall, all "Goodness of Fit" parameters show comparable kinetic processes without significant differences between the analyzed *in vitro* release profiles. In all cases, the resulting kinetic processes happen without any initial high drug quantities released (F = 0.00). The values of n = 0.65 (MT80-500) and 0.63 (MT80-250) indicate mechanisms of the releasing processes by non-Fickian (abnormal) diffusions ($0.5 < n < 1$) and the value of n = 0.34 (MT250-500) indicates a release mechanism in which diffusion does not represent the limiting process for the release kinetic ($n < 0.5$). In the latter case, a relatively short lag time occurs in the release kinetics (≈ 15 minutes) before the onset of the kinetic process.

TABLE III

Calculated parameters which highlighted the mechanism of kinetic release

Formula code:	**MT 250-500**	**MT 80-500**	**MT 80-250**
Model: Korsmeyer-Peppas **Expression:** $F=kKP\cdot t^n$ **Best-fit values:**			
kKP	25.476	27.517	38.758
n	0.340	0.656	0.635
Goodness of Fit Parameters:			
Rsqr_adj	0.9644	0.9754	0.9680
MSE	7.900	8.769	10.892
AIC	146.7	57.6	37.4
MSC	2.91	3.13	2.48
Model: Korsmeyer-Peppas with T_{lag} **Expression:** $F=kKP\cdot(t-T_{lag})^n$ **Best-fit values:**			
kKP (h^{-1})	28.135	27.514	38.745
n	0.294	0.656	0.635
T_{lag} (h)	0.230	0.000	0.000
Goodness of Fit Parameters:			
Rsqr_adj	0.9765	0.9726	0.9616
MSE	5.218	9.743	13.069
AIC	136.4	59.6	39.4
MSC	3.29	2.96	2.23
Model: Korsmeyer-Peppas with F_0 **Expression:** $F=F_0+kKP\cdot t^n$ **Best-fit values:**			
kKP (h^{-1})	25.471	27.522	38.752
n	0.340	0.656	0.635
F_0 (%)	0.000	0.000	0.000
Goodness of Fit Parameters:			
Rsqr_adj	0.9630	0.9726	0.9616
MSE	8.229	9.743	13.070
AIC	148.7	59.6	39.4
MSC	2.84	2.96	2.23

CONCLUSIONS

Tramadol hydrochloride loaded gelatin microspheres were formulated for extended release of drug after oral administration. Using a micro particulate drug delivery system with different sizes of particles can

ensure a more or less extended release of the drug. The formulated microspheres could prolong the drug release up to 12 hours.

Among the different formulations prepared in this study, M80-250 showed a better release profile, but for a more prolonged effect it can use the other two formulations.

The Korsmeyer-Peppas equation was found to be the best kinetic model to describe the release kinetics of tramadol from the microspheres.

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ANALYSIS OF PROGNOSTIC FACTORS IN COLORECTAL CARCINOMA

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ANALYSIS OF PROGNOSTIC FACTORS IN COLORECTAL CARCINOMA (Abstract): Colorectal cancer is one of the most common malignancies in development countries. The purpose of this study was to analyze the epidemiologic profiles of the disease, to examine the results of survival at five years after diagnosis and how it was influenced by pathological aspects. **Material and methods**: In collaboration with Oncologic Clinic all colorectal cancer diagnosed from January 2002 to December 2006 were included in the study. Medical records of patients were retrieved and we note: age, residence, diagnosis date, grade, and stage and histology variables. Then were analyzed prognosis and survival at 5 years of patients related to these parameters. **Results**: A total of 238 patients with colorectal cancer were identified. The average age at diagnosis was 63.3 years and more than half of cases were men (59%). By the end of the follow-up period 103 patients had died, 66.1% of them representing colon cancer. When analyzing the survival length according to tumor location at the end of the study, we found that are no significant differences between survivals in colic tumors compared to the rectum – 53.9 months for right colon, 51.4 months for left colon and 49.5 months for rectum. The majority of tumors were grade II moderately-differentiated tumors 48.7% (116 of cases), and patients with grade I had the best survival, on average of 84.52 months. Tubular forms of colorectal cancer had the best percentage of five years survival (55.81%) being also the highest rate of survival (45.24% months).**Conclusion:** Factors that contribute to a favorable prognosis in colorectal cancer are tubular microscopic form, disease diagnosed in TNM stage I and II, GI and GII grading. **Keywords:** COLORECTAL CANCER, ADEN CARCINOMA, PROGNOSTIC FACTORS.

Colorectal cancer is one of the most commonly diagnosed cancers worldwide with over 1.2 million new cancer cases and 608700 deaths estimated to occur annually. According to the last global onco-epidemiological analysis it is the second most common cancer among women, and the third most common cancer among men (1, 2).

In Romania, colorectal cancer is the most common gastrointestinal malignancy, representing the third leading cause of cancer death after lung and breast cancer. The highest incidence of colorectal cancer is in patients pertaining to the 60-69 years age group. Since in Romania, based on demographic studies, the population above 65 years old will double in the next 50 years, we have considered a more detailed analysis of the factors then can influence the development of complications and occurrence of postoperative deaths in patients

older than 80 years old that are operated on for colorectal cancer to be useful (3).

New research about colorectal cancer is determined by the continuous increase of disease incidence both undeveloped countries that already register high levels of prevalence, and in countries where colorectal cancer incidence was not regarded as a matter of concern. The increase in incidence of colorectal cancer in Romania and also in Eastern Europe countries could be attributed to lifestyle changes, presumably introduced by westernization, resulting in obesity and physical inactivity (4).

As in any neoplastic disease, the tumor stage remains to be the most important factor of prognosis in colorectal cancer and iti is the basis for the authoritative patient management guidelines that influence most patient management decision. The International Union Against Cancer (UICC)/ American Joint Committee on Cancer (AJCC) TNM classification system is the principal staging systems utilized and therapeutic decisions are most often based on this classification system (5). Great changes in the TNM staging system for colorectal cancer have occurred from the 5th edition to the 7th edition, particularly regarding the pN classification.

The diagnosis and early detection of colorectal carcinoma is one of the major goals and should become a priority for the health system. Colorectal cancer recognizes a natural evolution compatible with a long asymptomatic period, generally estimated to last over five years. This period corresponds to early stage of cancer transformation of adenomatous polyps to cancer and invasion beyond the basement membrane. A significant number of patients with colorectal carcinoma who undergo apparently curative surgery develop local recurrence or distant metastases, leading to shorter survival rate of survival.

The prognosis of colorectal carcinoma patients involves many factors such as histological type of cancer, size, location, degree of tumor invasion, loco regional metastases, and in other organs (6). Although treatments for colorectal carcinoma have developed rapidly in recent years, colorectal cancer still remains the second common cause of cancer – related death. To improve colorectal cancer prognosis, fundamental research in genetics and molecular biology and colorectal screening with the widespread practice of noninvasive techniques (virtual colonoscopy) are some new relatively new directions to be developed (7).

MATERIAL AND METHODS

The study registered 238 patients who were diagnosed with colorectal cancer hospitalized in the Filantropia Hospital of Craiova from January 2002 until December 2006. We performed this study in collaboration with the Oncology Clinic in order to identify cases of colorectal cancer, to describe the epidemiologic profiles of the disease, to examine the results of survival at five years after diagnosis and how it was influenced by pathological aspects. Processed data are from case report forms. Data on survival June 1st, 2012 – when the patients follow-up – were from the Registry of Civil Status of Craiova.

Data collection

Medical records of the 238 patients were retrieved and the following information abstracted: age, residence, diagnose date, grade, stage, topology, histology variables and treatments. There were analyzed prognosis and survival of patients examined in the batch related to these parameters. Preoperatively patients from the study

were investigated by colonoscopy. Histopathological reports were reviewed to delineate the main citopathologic features, sub- site affection, the presence of any predisposing pathology and disease stage.

In the medical records of patients were found six types of surgery: colectomy for transverse colon with colo-colic anastomosis, subtotal colectomy with ileo/colo/rectal anastomosis, amputation of the rectum, and right hemicolectomy with ileo-transverese anastomosis ileo/colo/rectal, rectosigmoidectomy resection with colo-recto anastomosis and left hemicolectomy with colorecto/colo anastomosis.

Data processing and analysis

The main statistical analysis software packages were Microsoft Excel 2003 and Epi Info 2000. The 5-years overall and specific survival rates were estimated using the Kaplan-Meier method and the differences between the categories of each variable were assessed using log-rank test.

Ethical considerations

Permissions were obtained from Filantropia Hospital of Craiova as well as from the authorities of the included hospitals after approval of the study protocol and data compilation form. Data confidentiality was maintained all through the study.

The main goals for this study were:

- to identify cases of colorectal cancer;
- to describe the epidemiologic profiles of the disease;
- to examine the results of survival at five years after diagnosis and how it was influence by location and pathological aspect.

RESULTS

A total of 238 cases of colorectal cancer were included in registry from January 2002 to December 2006 and 103 patients of these are diseased. The distribution by sex, through the studied period, shows the predominance of male (59%), giving a male: female of 1.4:1.

Patients designated as residing in an urban area constituted 63% of all patients and distribution by area of origin is urban/rural = 1.70. In any given year, between 37and 60 new cases of colorectal carcinoma were included in registry. Age and tumor site distribution of cases showed that 7.4% of all cases were under the age of 40. The age distribution showed limits ranging between 29 and 82 years with an average of 63.3 years. The maximum age incidence is between 58-74 years. Report colic cancer/rectal cancer was 161/77 = 2.19.

For an effective and comparative analysis of clinical features, treatment and prognosis in colorectal cancer, the large bowel was divided based on embryological, anatomical, clinical, pathogenesis and therapy in three segments: right colon (I) until the splenic angle, left colon (II) until the rectosigmoid junction and rectum (III) (8). According to this, the location of colorectal cancer was: right colon 34 cases (14.3%), left colon 127cases (53.4%) and rectum 77 cases (32.3%).

The vast majority of patients did not present polyps 234 (98.3%), though of the 4 patients who had polyps, 75% were over the age 40. Adenocarcinoma was the most common histopathology type of tumors 198 (91.2%)

The majority of tumors were grade II moderately-differentiated tumors 48.7% (116 of cases), followed by 23.9% (57 cases) grade III, 15.5% (37 cases) grade I and 11.8% (28 cases) grade IV. The degree of differentiation of colorectal adenocarcinoma showed a large number of patients

alive with G1 (86.48%), followed by those with G2 (61.20%), G3 (35.08%) and G4 (7.14%). Patients with G1 had the best survival, on average of 84.52 months, followed by those with G2 with an average of 59.21 months, G3 (31.34 months) and G4 (3.86 months).

TNM staging of the study group revealed 41 patients in stage I (17.2%), 86 patients in stage II (36.1%), 83 patients in stage III (34.9%) and 28 cases in stage IV (11.8%). Positive nodes were identified in 89 patients with stage III and IV, as follows: stage III - N1-47 cases and N2-36 cases; stage IV – N1-17 cases and N2- 34 cases. Metastatic colorectal cancer was diagnosed in 11.8% of cases, mostly involved peritoneum and liver.

Surgeries were performed on 217 patients. They practiced all six types of surgery listed above: colectomy for transverse colon with colo-colic anastomosis 29 cases, subtotal colectomy with ileo/colo/rectal anastomosis 19 cases, amputation of the rectum 79 cases, right hemicolectomy with ileo-transverese anastomosis ileo/colo/rectal 34 cases, recto-sigmoidectomy resection with colo-recto anastomosis 47 cases and left hemicolectomy with colo-recto/colo anastomosis 42 cases.

DISCUSSION

Colorectal cancer mortality in Romania has followed a steady increase from 14‰ to 18.3‰ for men and women in the years 1960-1990 to about 22.2‰ in 1995-2003 and decreased slightly to 19.2‰ in 2008, after the last statistical EU statistics (9). Following trends in incidence, mortality because of colorectal cancer is much higher in urban areas, without a tendency to change from one year to another.

Numbers of new cases of colorectal cancer detected are variable from one year to another, but with a growing medium and long term, despite the fact that the people of Romania that represent these patients decreases. In other words, the incidence of this cancer is increasing in our country, this fact is evidenced by a study carried out, estimating that this trend of increasing incidence will occur at least until they reach the values reported by industrialized countries. Increasing incidence of colorectal carcinoma associated with increase in both prevalence and mortality of this disease, suggests that current methods of treatment, only few cases are actually curable, with a tendency to heal (cytological forms a good degree of differentiations, in an early stage), the rest is just temporary and/or limited interventions (several months to several years) due to extension of neoplasic disease.

Results of a European study show that survival at five years in patients with colorectal cancer as a whole is especially influenced by stage and the factors that are directly or indirectly related to the primary tumor. Thus, these prognostic factors that should be considered in assessing the survival and follow-up are: stage, grading and adenopathy (10).

We note an increase in the number of cases from year to year. Thus, in 2002 there were 37 cases diagnosed, in 2003 their number increased to 41 cases, in 2004 we identify 47 cases, 53 cases in 2005 and 60 cases in 2006. The distribution by sex, giving a male: female of 1.4:1. This change in the M/F ratio is not statistically significant, although there are European studies that confirm the increased incidence of malignancies in women in particular.

In agreement with other studies (11, 12), survival rates were significantly higher in women. This association has been attributed to many underlying factors, such

as access to care, sex-specific co morbidities treatment disparities and hormonal status. The CONCORD study observed that in most countries, survival was better in patients with colon cancers than in those with rectal cancers (12). The association found between an excess risk of death and some clinical factors is consisted with the results of previous studies. Advanced stage at diagnosis (13), undifferentiated/poorly differentiated tumors (14) are factors that well known and described in the literature.

According to the present study the average at diagnosis of was 63.3 years. When compared with the reports from developed countries (15, 16), the mean age at diagnosis of colorectal cancer is lower in the Kingdom. In England, some researchers reported that between 1996 and 2004 the mean age at diagnosis of colorectal carcinoma was 68.4 years in men and 69.0 years in women (17). In Australia, the median age at diagnosis of colorectal cancer in 2008 was reported to be 70.0 years (18), similar to that is reported in the United States (19) and Sweden (20).

Since Dukes classification, all classification system of colorectal cancer were the most important predictor of survival, determining prognosis is the reason why they were created. The most used and reliable classification system is the system adopted by the AJCC TNM (American Joint Committee on Cancer) and UICC (International Union Against Cancer) (21). Undoubtedly, the most important prognostic factors for survival are the extent of tumor invasion into the wall and the presence of metastases in regional lymph nodes-the stage in which colorectal cancer was diagnosed (22). Obviously, the more advanced the stage of the cancer of the colon or rectum is, the more the life expectancy is less (23).

According to stage, patients that are still alive had a median survival of 73.61 months for stage I, 54.13 months for stage II, 43.6 months for stage III and 9.21 months for stage IV. Wilcoxon test showed the same high statistical significance differences between stage, with a p-value of less than 0.0001 (Fig. 1).

According to the TNM classification (24) pT4 is the most advanced stage of local tumor development in colorectal cancer, including both direct tumor invasion of adjacent structures (pT4) and invasion of visceral peritoneum with invasion of adjacent structures (pT4) including here free perforation into the peritoneal cavity. The most important and damaging to prognosis characteristics of colorectal tumor failing in T4 is the serous as a separate anatomopathological variable demonstrated in the multivariate analysis that this is an independent negative prognostic factor (25). In our study we find 28 patients in stage IV, 11 patients with peritoneal metastases and 19 with hepatic metastases.

When analyzing the survival length according to tumor location at the end of the study, we found that are no significant differences between survivals in colic tumors compared to the rectum – 53.9 months for right colon, 51.4 months for left colon and 49.5 months for rectum. Kaplan-Meier curves showed no significantly higher survival rated for colon compared to the rectum (fig. 2).

Gross pathological form is a parameter in formulating prognosis, tumor appearance reflecting its biological nature. Exofitic or polipoid tumor patients seem to have a better prognosis than those with ulcerated or infiltrative tumor. In our study macroscopic aspects of excision parts showed the following: infiltrative-48 cases (22.1%), ulcerative–35 cases (16.1%), vegetant–32 cases (14.7%), ulcerative and vegetant–56

cases (25.8%), infiltrative and ulcerated–21 cases (9.7 cases), infiltrating and vegetant–13 cases (6.0%), vegetant – infiltrative - ulcerated–21 cases (5.5%).

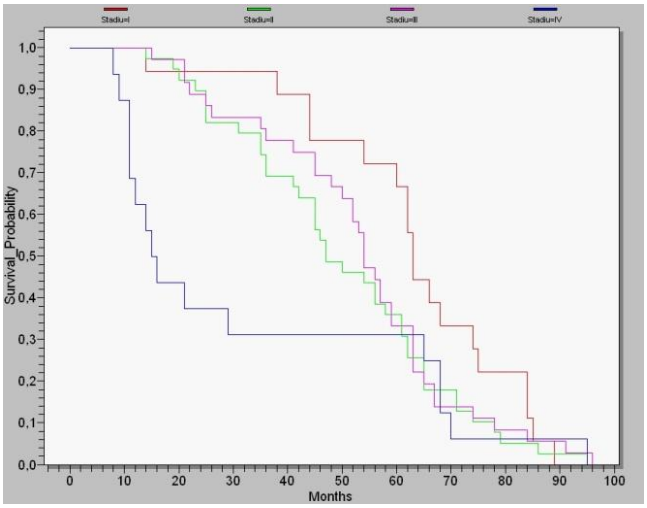

Fig. 1. Survival on July 1st 2010 according to CRC TNM stage

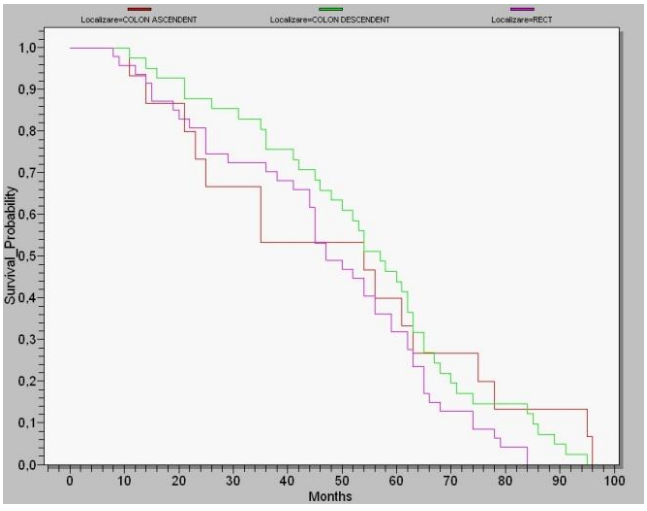

Fig. 2. Kaplan-Meier survival curves and CRC location

Concerning the microscopic examination, the highest percentage of patients alive was recorded in tubular forms (55.81%), followed by papillary forms (46.55%), rare/particular (45.83%) and mucinous (31.42%). In terms of number of cases tubular colorectal cancer had the best percentage of five years survival (55.81%) and longest higher survival (45.24%). Kaplan-Meier curves showed significantly higher survival rates for mucinous compared to tubular forms (Fig. 3).

Colon cancer, as well as rectal cancer treatment, changed over time, in our study adjuvant chemotherapy was administered

to 138 (85.71%) of patients with colon cancer and 56 (72.72%) with rectal cancer. By the end of follow-up, 103 patients had died: 68 deaths (66.1%) were due specifically to colon neoplasm and 35 (33.9%) were to rectal cancer (Fig. 4).

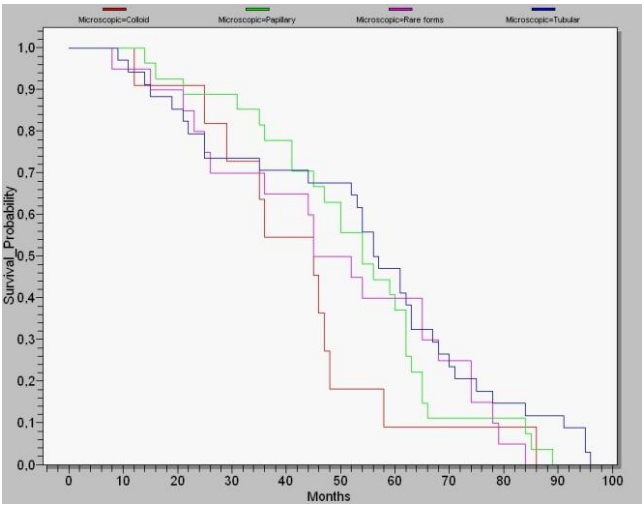

Fig. 3. Kaplan-Meier survival curves and CRC microscopic aspects

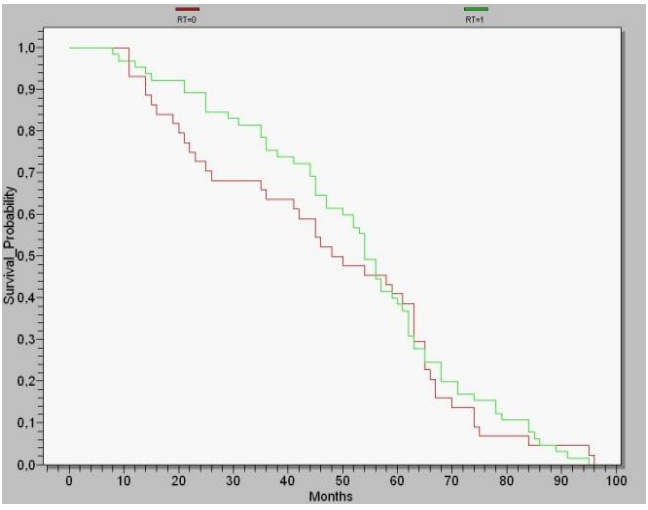

Fig. 4. Kaplan-Meier survival curves and CRC adjuvant therapy

Evaluating lymph node metastasis has become a prognostic factor for CRC, and LNR is an important component of staging. LNR has also been identified as being of significant prognostic value in breast and gastric cancer. Berger et al were the first to suggest LNR as an important prognostic factor after curative resection for CRC. It was then established as a powerful independent index of CRC that reflected the probability of positive lymph nodes based on the number of retrieved lymph nodes.

The total number of lymph-nodes resected was not found to be a significant predictor of survival under univariate analysis (P=0.44), survival for patients grades N1 and N2 was 32.3% and 43.5% at 5- years respectively (Fig. 5).

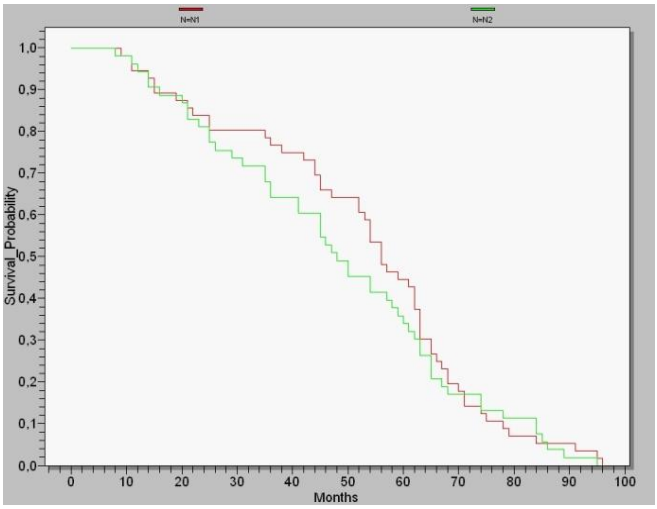

Fig. 5. Kaplan-Meier survival curve for node-positive patients stratified by N stage

CONCLUSIONS

Colorectal cancer recognizes a natural evolution compatible with a long asymptomatic with a long asymptomatic period, generally estimated to last over five years. The introduction of new techniques for screening the population, the increasing educational level of the population, the use of recent advances in laparoscopic surgery and oncology management, properly improved the result. Factors that contribute to a favorable prognosis in colorectal cancer are tubular microscopic form, disease diagnosed in TNM stage I and II, GI and GII grading.

Using routinely collected data, at no inconvenience to patients, we have identified some important areas relating to early deaths from colorectal cancer, which merit further research.

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THE EVALUATION OF PREOPERATIVE NUTRITIONAL STATUS IN PATIENTS UNDERGOING THORACIC SURGERY

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THE EVALUATION OF PREOPERATIVE NUTRITIONAL STATUS IN PATIENTS UNDERGOING THORACIC SURGERY. **Aim.** The aim of this study was to assess the preoperative nutritional status of patients undergoing thoracic surgery using different nutritional tools. **Material and methods.** We conducted a prospective study on a sample of 43 thoracic patients, including 23 with neoplasms and 20 with non-neoplastic pathology who underwent thoracic surgery procedures between July-September 2011, in the Thoracic Surgery Clinic in Iaşi. Weight and height were measured and body mass index (BMI) was calculated. WHO classification for BMI categories was used. Preoperative serum level of transthyretin (TTR) and demographic data (gender, age) were also assessed. All patients were examined by the Subjective Global Assessment (SGA) and the Nutritional Risk Screening 2002 (NRS 2002). **Results.** After performing SGA, 67.9 % of the patients were well-nourished, 21.4 % were moderately or suspected of being malnourished and 10.7 % were severely malnourished. The level of TTR was significantly lower in the moderately or severely malnourished group, compared to those considered well-nourished. According to NRS-2002, 42.9 % of the patients were considered at nutritional risk. The level of TTR of these patients was lower than the level of TTR of the patients without nutritional risk, but without statistical significance. **Conclusions.** Subjective Global Assessment (SGA) and the Nutritional Risk Screening 2002 (NRS 2002) are useful in identifying patients with nutritional risk, so that appropriate nutritional management could be initialised even before surgery. **Keywords:** PREOPERATIVE STATUS, NUTRITIONAL STATUS, THORACIC SURGERY, TRANSTHYRETIN

The prevalence of malnutrition in surgical services varies between 20-50% (1). Preoperative malnutrition is common in surgical patients and has been associated with adverse clinical outcomes (2). This suggests that identifying patients at risk and recommending perioperative nutritional support can improve nutritional status of patients undergoing surgery. Hospital malnutrition has serious adverse effects on the treatment and outcome of illness and is associated with higher infection and complication rates, increased muscle loss, impaired wound healing, longer length of hospital stay and increased morbidity and mortality (2, 3). Costs are increased in

malnourished patients whether or not a complication occurs, but they augment significantly if one happens (4). All of these factors contribute to increasing length of hospitalization, number of re-admissions in the hospital and a marked increase in patient care costs (5).

Protein-energy malnutrition is associated with a worse prognosis and increased mortality. Although several screening systems have been developed, it is still poorly recognized, and there is no consensus on which test is more reliable and feasible in clinical practice. Transthyretin (TTR) is a potential useful marker because its serum concentrations are closely related to early changes in nutritional status (6). Nutritional status is largely not systematically assessed and therefore the patient's nutritional plan is not yet included in the usually medical plan. There is a lack of data on the perioperative nutritional risk and on the prevalence of perioperative malnutrition in thoracic surgery units. The aim of this study was to assess the nutritional status of patients undergoing thoracic surgery using different nutritional tools.

MATERIAL AND METHODS

The study took place in Thoracic Surgery Department in Clinical Pneumology Hospital, Iaşi, Romania. In this prospective study, we enrolled all consecutive patients who underwent thoracic surgery between July-September 2011. The Ethics Committee of the Clinical Hospital of Pneumology Iaşi approved the study (no. 13953/2011), which was conducted in accordance with the Helsinki Declaration and all patients signed informed consent form before being enrolled. The demographic data (gender, age) of patients were assessed. We evaluated weight, height and thus calculated body mass index (BMI). All anthropometric measurements were performed by the same investigator. The weight was recorded *a jeun*, to the nearest 0.1 kg, using calibrated medical portable scales with 4 sensors (model 27236; Liamed, Brasov, Romanian). Height was measured using a stadiometer to the nearest 0.1 m (Practical Metrology, Lancing, UK). The BMI was calculated by the current weight (kg) over height $(H)^2$ (m^2) ratio and classified according to World Health Organization. Subjects were grouped in well-nourished (BMI $\geq$ 18.50 kg/m^2) and malnourished (BMI $<$ 18.50 kg/m^2) patients. Preoperative serum level of TTR was analysed by ELISA. All patients were examined by the Subjective Global Assessment (SGA) and the Nutritional Risk Screening 2002 (NRS 2002). SGA was carried out based on patient's history regarding weight loss, dietary intake, gastrointestinal symptoms, functional capacity, and physical signs of malnutrition (loss of subcutaneous fat or muscle mass, oedema, ascites), according to the method described by Detsky et al. (7). Patients were classified as well-nourished (A), moderately or suspected of being malnourished (B) or severely malnourished (C). The Nutritional Risk Score (NRS-2002) is the recommended screening tool (for nutritional risk) by the European Society of Parenteral and Enteral Nutrition/European Society of Clinical Nutrition and Metabolism (ESPEN) and was performed according to the recommendations set by Kondrup et al. (8). Nutritional risk was evaluated through impaired nutritional status and disease severity. Evaluated nutritional parameters were: BMI, recent weight loss, and food intake during the week before admission. The worst indicator was taken into account. Patients were scored in each of the two components: undernutrition and disease severity. A score between 0 and 3 was given for each of the two components according to the recommendations for each

parameter. When the age of the patient was>70 years, a value of one was added to the total score. Patients with a total score of three or more were considered at nutritional risk. The results of the study were expressed as mean ± standard deviation (x±SD). Unpaired t-tests were used to compare data in different groups of patients. Statistical significance was set at $p \leq 0.05$ for all tests.

RESULTS

Forty three patients who underwent thoracic surgery were included in this study (tab. I). Subjects had a median age of 54.51 ± 16.70 years and 79 % (n=33) were male. The average BMI in the study group was 24.14 ± 5.44 kg/m^2, the women having an average BMI of 25.25 ± 5.32 kg/m^2, and the men with an average BMI of 23.72 ± 5.35 kg/m^2 ($p>0.05$).

Analysing BMI, we noticed that 13.8 % of the subjects had a BMI < 18.5 %, the others being: 44.8 % of normal weight, 24.1 % overweight and 17.2 % obese. The average TTR level in the study group was 20.8 ± 8.9 mg/dl, and there was no statistically significant difference between the TTR of patients with BMI < 18.5 kg/m^2 (TTR = 19.2 ± 8 mg/dl) and those with BMI ≥ 18.5 kg/m^2 (TTR = 21.1 ± 9.2 mg/dl).

TABLE I

Distribution of series of 43 thoracic patients according to pathology

Pathology		**N**	**%**
Neoplastic	NSCLC	17	39,53
	SCLC	1	2,32
	Esophageal squamous cell carcinoma	1	2,32
	Breast Cancer with malignant pleural effusion	1	2,32
	Basal cell cancer of central face with malignant pleural effusion	1	2,32
	Cancer with no clear origin and malignant pleural effusion	2	4,65
Non-neoplastic	Spontaneous pneumothorax	4	9,30
	Pleural empyema	4	9,30
	Sarcoidosis	2	4,65
	Pulmonary tuberculoma	2	4,65
	Tuberculous thoracic parietal abcess	1	2,32
	Pulmonary abcess	1	2,32
	Posttraumatic pneumothorax	1	2,32
	Posttraumatic hemopneumothorax	1	2,32
	Spontaneous chest wall hematoma	1	2,32
	Miastenia gravis with thymus hyperplasia	1	2,32
	Pulmonary hamatochondroma	1	2,32
	Costal osteoid osteoma	1	2,32

NSCLC - non-small cell lung cancer; SCLC - small cell lung cancer

After performing SGA, we concluded that 67.9 % of the patients were well-nourished (score A), 21.4 % were moderately or suspected of being malnourished (score B) and 10.7 % were severely malnourished (score C). When taking into account only patients with a BMI ≥ 18.5 kg/m^2, we still found that 25 % were in the categories with risk, that is: 20.8 % were moderately or suspected of being malnourished (score B) and 4.2 % were severely malnourished (score C). The level of TTR was significant-

ly lower in the group with scores B or C according to SGA, compared to those considered well-nourished (TTR = 15.5 ± 7 mg/dl vs. TTR = 23.1 ± 8.9 mg/dl, p=0.05).

The Nutritional Risk Score (NRS-2002) showed that 42.9 % of the patients included in our study were considered at nutritional risk. Moreover, out the patients with a BMI ≥ 18.5 kg/m^2, 37.5 % were at nutritional risk, based on the NRS-2002. The level of TTR of the patients considered at nutritional risk was lower than the level of TTR of the patients without nutritional risk, but statistical significance was not obtained (TTR = 17.9 ± 6.9 mg/dl vs. TTR = 23 ± 9.8 mg/dl, p>0.05).

DISCUSSION

Protein-energy malnutrition represents acute or chronic protein loss leading to a state of nutritional deficiency that causes a decrease in defense mechanisms against infection, delay healing and alteration of certain important functions (respiratory, cardiac, endocrine). It is associated with a lower response of the organism during a certain condition and may be reversed in an anabolic state by medical intervention. It is common in hospitalized patients, being associated with increased mortality (9).

Disease-related malnutrition varies in different studies and has been reported in 10% to 55% of people in hospital and the community (2). The prevalence of malnutrition in people with cancer, chronic diseases and after major surgery has been reported to be around 10% (2). A large percentage of our study population had or was at risk of malnutrition.

There is no "gold standard" for determining nutritional status. A modification of protein and energy intake leads to changes in the same direction of circulating concentrations of TTR (10). Like albumin, TTR is considered a negative acute phase reactant, as it may decrease in an inflammatory context (11).

Currently TTR is the most commonly used visceral protein to assess nutritional status (12). TTR is a useful marker in monitoring malnourished patients because its serum concentrations are influenced by changes of early nutritional status and respond to nutritional support (13). TTR level measurement is a sensitive and cost-effective method for assessing the severity of disease in critically or chronically ill patients (14).

TTR is a more reliable nutritional marker than albumin due to short half-life (48 hours), a relatively low spread in the body and the rapid synthesis which is influenced by protein intake (15). TTR concentration decreases significantly in just three days after inadequate nutritional intake and increases by 1 mg/day when the nutritional requirements are satisfied (16). Hypoalbuminemia is an independent strong risk factor, depending on the concentration, for morbidity, mortality, acute renal failure and other negative clinical results (17), but due to the widespread in the body, the half-life of 20 days, and sensitivity to the patient's hydration status, albumin is a less sensitive parameter in assessing protein-energy malnutrition. A study on 1760 patients demonstrated that serum albumin and BMI alone underestimated the prevalence of malnutrition at hospital admission (18).

TTR concentration decreases in the presence of inflammation (negative acute phase reactant). Rapid changes in C-reactive protein may lead to overestimation of malnutrition. While these limitations must be taken into account, the study conducted by Devoto et al. (6) concluded that serum TTR can still be reliable in cases associating inflammatory processes.

SGA is a nutritional score created over two decades ago to assess nutritional status

and risk of infectious complications in patients undergoing surgery. This score takes into account changes in body composition and physiological functions. In one study, patients considered in the category of preoperative severe malnutrition showed higher postoperative infectious and non-infectious morbidity (19). In our study, 32 % of subjects had moderate or severe malnutrition, according to SGA.

In Western Europe, 25-30% of patients who are undergoing surgery are considered at increased nutritional risk preoperatively (20). For nutritional screening in hospitalized patients, the European Society for Clinical Nutrition and Metabolism recommends the use of Nutritional Risk Screening score (NRS 2002). In a general population of hospitalized patients, this score is easy to implement, reliable and reproducible for identifying patients at nutritional risk (8). Compared with other screening tools, this system is unique in that it has been developed from the idea that the indication for nutritional support depends on the severity of malnutrition and increased nutritional needs that may result from underlying disease. Therefore, severe malnutrition or severe disease, alone or together, constitutes an indication for the need for nutritional support (8, 21). NRS 2002 is also used increasingly for preoperative classification and risk assessment (22), although it has been designed to predict therapeutic effects, not complications (8).

A study conducted by Kawai et al. aimed at identifying biomarkers that might be useful in predicting postoperative early recurrence of lung cancer. They evaluated the perioperative nutritional status of the patients by measuring the serum level of TTR and analyzed the correlation between this factor and early recurrence, in a sample of forty-four patients with NSCLC. The conclusion of the authors was that low serum TTR level in the perioperative period was associated with a poorer prognosis in NSCLC patients and could serve as a marker for identifying patients at high risk, even at an early clinical stage. (23).

Devoto et al. studied the prevalence of protein-energy malnutrition and TTR serum concentrations in 108 hospitalized patients. The Detailed Nutritional Assessment (DNA) was used as the reference method to determine protein-energy malnutrition. TTR performance was compared with that of 2 other methods, the Subjective Global Assessment (SGA) and the Prognostic Inflammatory and Nutritional Index score (PINI). According to the DNA reference method, 41% of patients were classified with mild malnutrition and 19% with severe malnutrition. TTR showed the best concordance with the standard DNA method (concordance index, 76.8%) and a good sensitivity/specificity profile (83.1%/76.7%) compared with SGA and PINI. They concluded that TTR could represent a feasible and reliable tool in the evaluation of malnutrition, especially in settings where it is difficult to obtain a more detailed and comprehensive nutritional assessment such as the DNA (6).

CONCLUSIONS

Our data support the assessment of nutritional status of in-patients undergoing thoracic surgery. The nutritional state per se is predictive for postoperative outcome and must be seriously assessed in every thoracic patient undergoing surgery. BMI is not a reliable tool on its own in assessing nutritional status of patients undergoing thoracic surgery. Subjective Global Assessment (SGA) and the Nutritional Risk Screening 2002 (NRS 2002) are useful in identifying patients with nutritional risk, so that appropriate nutritional management could be initialized even before surgery.

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RESEARCH STUDIES ON *IN VITRO* AND *EX VIVO* YIELD OF THE MICONAZOLE NITRATE FROM ORAL BIOMUCOADHESIVE TABLETS

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RESEARCH STUDIES ON *IN VITRO* AND *EX VIVO* YIELD OF THE MICONAZOLE NITRATE FROM ORAL BIOMUCOADHESIVE TABLETS (Abstract): Among the various routes of drug administration, the oral mucosa is perhaps the most often preferred by patients and medical staff. However, oral administration of drugs has disadvantages, which may limit or prevent oral administration of some drugs, especially peptides and proteins, little when they are inserted in special administration systems for the colon. The disaggregation of some oral biomucoadhesive tablets and the *in vitro* yield of the miconazole nitrate was evaluated and in parallel with this, the evaluation of the *in vivo* yield of the antifungal from the pharmaceutical form. Thus, for a clear determination of the oral mucobioadhesive tablets' disintegration with miconazole nitrate, it was necessary to implement a method to simulate the conditions of the oral cavity at a flow of solution (artificial saliva) similar to that of the human one. **Materials and methods:** materials: miconazole nitrate. Methods: The determination of disintegration time according to method A (FRX); the disaggregation of oral biomucoadhesive tablets with miconazole nitrate by means of simulation methods of *in vitro* conditions; the quantitative determination of the miconazole nitrate by means of HPLC method, after the *in vitro* dissolution test; the study of miconazole nitrate's yield in dynamic condition from biomucoadhesive tablets in the presence of artificial saliva (AFNOR). **Results:** The yield profile of the miconazole nitrate in the disintegration solutions by means of classical method from FR X, by HPLC dosage was researched. The release of miconazole nitrate from the oral mucobioadhesive tablets was determined, that varies in time, depending on the type and relation of matrix forming polymers; a low yield speed of the miconazole nitrate from the tablets was determined; the yield profile of miconazole nitrate in disintegration solutions by means of the new suggested method was researched. **Conclusions:** The release of miconazole nitrate from the formulated biomucoadhesive tablets is of swelling and erosion. **Keywords:** MICONAZOLE NITRATE, MUCOBIOADHESIVE TABLETS, *IN VITRO* YIELD.

The influence of some formulation factors on the release of miconazole nitrate from biomucoadhesive oral tablets was studied. Biomucoadhesive oral tablets with miconazole nitrate by the method of direct compression as a result of the completion of the mixture of suitable excipients (sodium carboxymethylcellulose and lutrol 6000 excipients used for the bioadhesion, mannitol used as a sweetening agent and aerosil as lubricant) (1).

The bioadhesive systems ensure an in-

timate contact between the dose and the absorbent tissue, which can lead to high local concentrations and thus a strong flow through the tissue. The efficiency of bioadhesive systems for oral administration of the active substance is influenced by the environment and biological properties of the polymer and of the drug (2, 3).

The researches on bioadhesive administration systems continue at a rapid pace, aiming at an efficient treatment of commonly encountered lesions (sores, ulcers, etc.) of periodontitis, gingivitis and caries. Optimizing the systemic treatment of disease by transmucosal drug administration in the oral cavity continues to be investigated using a variety of dosage forms containing new bioadhesive polymers (4).

Determining the disintegration time of tablets during tests was an old requirement in most pharmacopoeias. Currently, there are severe limitations of the disintegration test, this being applied especially to conventional tablets. The dissolution test was included as a consequence also for other solid forms with extended release. Both the disintegration time and the *in vitro* yield (or the dissolution tests) are determined by official methods (5, 6).

The mucobioadhesive tablets as pharmaceutical form and as a release system of the active substance requires the implementation of a new method of disaggregation method that better simulates the conditions of the oral cavity (7). Thus, for a clearer determination of the oral mucobioadhesive tablets' disintegration with miconazole nitrate it was necessary to implement a new method for determining the flow rate of solution (artificial saliva) similar to that of the human one.

MATERIALS AND METHODS

Materials: Miconazole nitrate (Sigma Aldrich, Germany); *Sodium carboxymethyl cellulose (*Lubrizol, U.S.A); *Mannitol (East Chemical, Weifang City, China) Polyethylene glycol, PEG 6000 microcrystalline powder (*Lutrol,*BASF Pharma, Germany)*; *Aerosil* (Degussa, Germany). All the materials used had the purity degree in conformity with the normatives in force. The miconazole nitrate, the antifungal was used as active substance with 99% purity in oral mucobioadhesive tablets (1) (tab. I).

TABLE I

Formulations of mucobioadhesive tablets with miconazole nitrate

Pharmaceutical substances mg/tablet	Formula I	Formula II	Formula III	Formula IV	Formula V
Miconazole nitrate	25	25	25	25	25
Sodium carboxy methylcellulose (CMC-Na)	20	60	100	100	140
Mannitol	90	90	90	50	30
PEG 6000(Lutrol)	80	40	-	40	20
Aerosil	4	4	4	4	4
Total (mg)	219	219	219	219	219

Methods: 1. The assay of disintegration time according to method A (FRX). For the disintegration of mucobioadhesive tablets with miconazole nitrate one has used 6 bioadhesive tablets from every formulation and as a device. DISINTEGRATION TESTER QC-21 (4). 2.*B. The disintegration of mucobioadhesive tablets with miconazole nitrate by means of simulation method of in vivo conditions*. The equip-

ment used in the disintegration of mucobioadhesive tablets and in the desorption of miconazole nitrate –a personal conception using a burette provided with a dropper, which regulates the artificial saliva flow that goes under dynamic condition on the tested tablet (0.5 mL /minute), an adjustment system of the artificial saliva flow, a support vial of the tablet , a vial for collecting the mixed solution (artificial saliva with active substance), muco-bioadhesive tablets with miconazole nitrate (1 tablet from each formula), 6 porcelain capsules, 10 ml graduated vials, thermostate bath GRANTw6 and artificial saliva solution with pH 7.24 AFNOR (0.26 g of disodium phosphate , 6.7 g sodium chloride, 0,33 g of potassium sulfocyanide, 0.2 g of monopotassium dihydrogen phosphate, potassium chloride 1,2g, sodium bicarbonate 1.5 g, distilled water up to 1000g) .

The human saliva is released into the oral cavity with a flow of 1000mL up to 1500mL/day, resp. 0,5-1mL / minute, a flow rate that is regarded by the equipment. A tablet from each formula was placed in the equipment for the desorption of the miconazole nitrate and the equipment was started so that 0,5mL AFNOR artificial solution per minute falls on the tablet. The tablet is kept in this installation until it totally disintegrates. The artificial saliva solution that has disintegrated the table is being collected every 20 minutes, at a quantity of 10 mL.*3.The quantitative assay of miconazole nitrate by means of HPLC method, after performing the in vitro dissolution test.* The dosage method HPLC was confirmed, which constitutes the objective of this paper, of the miconazole nitrate from oral mucobioadhesive tablets with long yield following the specificity, the exactness (accuracy), the retrieval capacity and also the precision (fig.1).

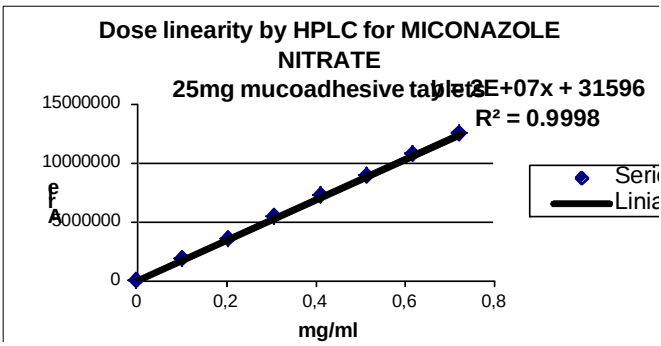

Fig. 1 Calibration curve of the miconazole nitrate by means of HPLC method

The gradual yield of the active substance from mucoadhesive tablets was studied using the current USP dissolution equipment. For the quantitative assay of the antifungal and of the HPLC method's validity one has used an equipment no. 2 (with paddles), dissolution agent respectively phosphate buffer solution to pH = 6.8 (the agent's volume of 500 ml), the rotation speed of 50 revolutions /minute, the temperature of the dissolution agent of 37 ° C $\pm$ 0.5 ° C, a standard solution consisting of 25.0 mg of miconazole nitrate was dissolved in methanol in a 25ml graduated flask. Subsequently, 0.5ml was diluted with 5ml methanol and 5ml phosphate

buffer pH=6.8 in a 10ml graduated flask. 2.5ml of this solution was diluted with phosphate buffer to pH=6.8 in a graduated flask of 10ml (concentration of 0.0125 mg/ml miconazole nitrate). The principle of the method: the dosage of the miconazole nitrate is performed by liquids' chromatography under pressure (HPLC). 4. *The study of miconazole nitrate's yield in dynamic condition from mucobuccal-adhesive tablets in the presence of artificial saliva (AFNOR).* In order to achieve the yield in the miconazole nitrate from oral mucobioadhesive tablets one has used an equipment, which requires the use of as proof of mucobioadhesive tablets with miconazole nitrate dissolved in a solution of artificial saliva AFNOR at pH 7.24 and pure substance, miconazole nitrate, in order to carry out the calibration curves and as an auxiliary system one has used thermostate bath GRANTw6, colorimeter type 252SHERVEDWOOD, spectrophotometer MICROLAB 300- high-performance semiautomatic equipment.

The reagents used for the performance of miconazole nitrate's yield are: reagents for elution from the capsule (0.5ml methanol + 0.5 ml of chloroform), update rate reagent of 10 mL (1,2 dichloroethane), the complexation reaction reagent(solution 0.01M iodine in 1,2-dichloroethane-volume 1mL). Benchmark for spectrophotometer MICROLAB 300: 0.25 mg miconazole nitrate+ 1 mL mixture (chloroform, methanol, 1:1 relation). Over the homogeneous mixture add solution of 1 mL iodine 0.001 M in 1,2 dichloroethane it is brought to a graduaded flask of 10 mL with 1,2-dichloroethane preserving it at 25° C temperature for 50 minutes.

The study of miconazole nitrate's yield under dynamic conditions was approached in order to simulate the almost similar conditions of the oral cavity. The study under dynamic conditions was carried out based on the traditional methodology in the literature, at a value of pH 7.24, taking into account also the average salivary flow rate (0.5ml/min) (1.b).

A tablet from each formula was placed in the equipment for the desorption of the miconazole nitrate and the equipment was started so that 0,5mL AFNOR artificial solution per minute was dropping on the tablet. The tablet is kept in this installation until it totally disintegrates. The artificial saliva solution that has disintegrated the tablet is being collected every 20 minutes. Therefore, every 20 minutes one has collected 10mL from the sample, which was introduced in porcelain capsules, being evaporated on dry, on a water bath.

The residue of the porcelain cap was eluted with 1 mL of solution of methanol with chloroform. Also, in the porcelain capsule one has added 8 mL 1,2 dichloroethane and 1 mL iodine solution 0.01M. From the homogenized sample one has collected 500µL for the spectrophotometric method performed with the device MICROLAB 300 and for the colorimetric method one has collected 1 mL sample.

RESULTS AND DISCUSSION

By determining the disintegration time according to method A from FR X (5) above mentioned, one has been achieved for the first formulation a disintegration time of 25 minutes, for the second one a disintegration time of 45 minute, for the IIIrd formula 60 minutes, formula IV 65 minutes and for the final formulation a 90 minutes time (fig. 2).

The decomposition of mucobioadhesive tablets with miconazole nitrate by means of

the new method leads to the following results: the first formulation disintegrates in 30 minutes, the second in 80 minutes, the third in 340 minutes, the fourth in 360 minutes and the last formula, respectively the fifth formula in 380 minutes (fig. 3).

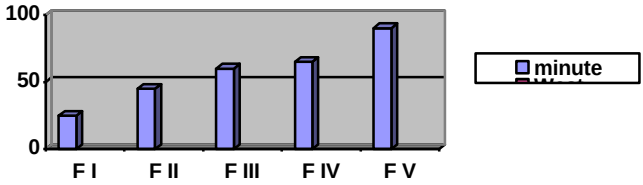

Fig. 2 Decomposition time of oral biomucoadhesive tablets with miconazole nitrate by method A (FR X)

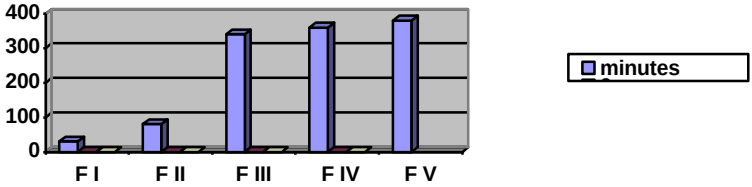

Fig. 3 Decomposition time of oral biomucoadhesive tablets with miconazole nitrate by simulating *in vivo* conditions.

From the above results we can see a prolonged disintegration time when we simulate the conditions at the level of oral mucosa. Thus, the pH, the saliva flow and also the temperature are important factors which influence the disintegration time. Formulae III, IV and V have a much higher disintegration time compared to formulae I and II, a phenomenon explained by: the presence of sodium carboxymethyl cellulose in a higher quantity for the last three formulae(100 mg-F3;100 mg-F4;140 mg-F5) and also the presence of polyethylene glycol in admixture with CMC-Na added in a higher quantity for the last two formulae(100 mg CMC-Na /40 mg PEG /cpr; 140 mg CMC-Na / 20 mg PEG /cpr). Formula III contains CMC-Na but not PEG, the substance added in the highest quantity (140 mg/cpr) in formula V.

The yield profile of the miconazole nitrate in the solutions from disintegration by the classical method from FR X (method A) by HPLC dosage (tab.II, fig. 4) was researched.

It is noted that the release of miconazole nitrate from oral biomuco-adhesive tablets varies in time, depending on the type and ratio of the matrix forming polymers. The antifungal's yield depends on the amount of polymers. The most important factor influencing the rate of release of the miconazole nitrate is the ratio between it and the polymers: CMC-Na and PEG 6000.

An increase in concentration of CMC-Na polymers increases the viscosity of the gel formed with the dissolution agent and also the forming of a gel layer around the

tablet, which reduces the rate of diffusion of the active substance. The release mechanism appears to be a swelling or erosion one of the tablets, which is observed during the yield study and also at the dynamic dissolution test.

TABLE II

***In vitro* yield of the miconazole nitrate from mucobioadhesive tablets (HPLC dosage)**

Time/ Miconazole nitrate %	30’	60’	90’	120’	150’	180’	210’
Formula I	8,32	9,75	10,0	10,52	13,53	14,00	14,61
Formula II	5,80	6,15	8,35	10,30	12,16	12,36	14,48
Formula III	3,88	6,43	7,60	7,80	10,20	10,50	10,70
Formula IV	4,25	6,00	10,00	10,20	10,50	11,50	12,40
Formula V	3,50	6,00	9,70	9,90	10,00	10,40	11,50

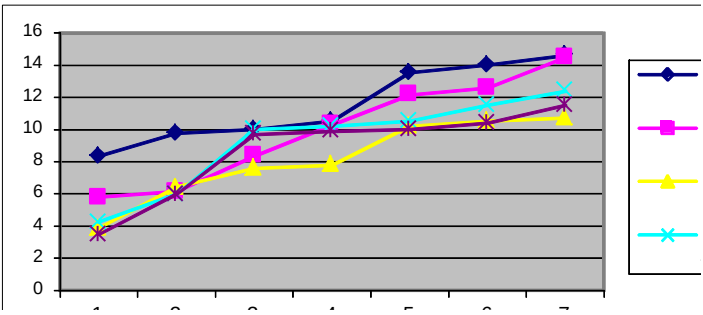

Fig. 4 The yield profiles of miconazole nitrate from I-V formulae after decomposition according to A FRX decomposition

Also, there was a low yield rate of the miconazole nitrate from mucobioadhesive tablets during the research time: the largest yield of 14.51% within 3h and 30'o represents formula I and 14.48% represents formula II followed by formula IV with 12.4%, formula V with 11.5% and formula III with 10.7%.

The yield profile of miconazole nitrate in solutions of disaggregation by the new method, by colorimetric and spectrophotometric dosage was studied. Experimental results are processed in Figure 5. A more effective yield was registered for tablets of formulas I and II, which are noted as having a lower concentration of CMC-Na.

Further one presents the method used in determining the concentration of miconazole nitrate involved in AFNOR saliva solution to pH = 7.24 and D = 0.5 ml/min. The total percent of miconazole nitrate released from the tablet of formula I, in the presence of AFNOR saliva is of 83,49%, in formula II -79 943%, in formula III -45 717%, formula IV - 49.392% and the formula V is of 52.264%.

It is important to keep in mind this fact, so that during drug administration, one maintains the required concentration for the destruction of fungi in a certain time, so that they do not form as in the case of the antibiotic, drug-resistant strains (fig. 5).

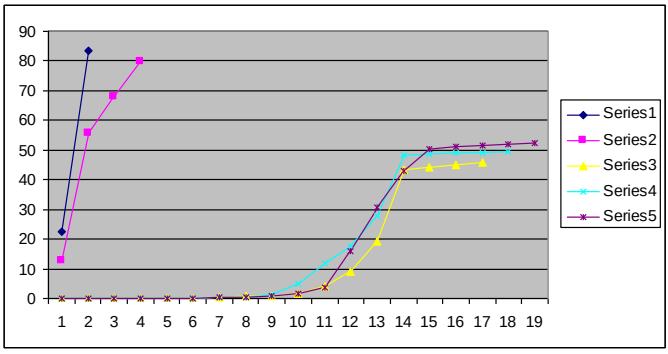

Fig. 5 Yield percents of miconozole nitrate from mucobioadhesive tablets, presented in a graphic

CONCLUSIONS

From the results of this research regarding the tablets' disintegration, it is noted that formula V has the longest disintegration time, that is of 90 minutes, as determined by the method of FRX and of 380 minutes by means of dynamic method, which simulates *in vivo* conditions. Validation study performed on a dosage method adapted for the miconazole nitrate by means of HPLC shows that the method is linear, selective, accurate, precise and robust. The release of *in vitro* miconazole nitrate from oral bio-mucoadhesive tablets varies in time depending on the type, concentration and the ratio of polymers: CMC-Na/PEG.

In the study performed, one has observed both *in vitro* and *ex vivo* yield. These results explain the role of yield conditions (pH, temperature, flow of saliva, etc). Basically, I have noticed a substance release up to 16% in *in vitro* yield and up to 83.49% in *ex vivo* yield. The release of miconazole nitrate from formulated and studied biomucoadhesive tablets, concerning swelling and erosion.

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A COMPLEX SYSTEM FOR TELEMONITORING OF MEDICAL VITAL SIGNS

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A COMPLEX SYSTEM FOR TELEMONITORING OF MEDICAL VITAL SIGNS (Abstract): A complex goal in biomedical information technology today is the design and implementation of telemedicine and e-health solutions, which provide (chronic) patients and elderly people services that can enhance their quality of life. **Material and Methods:** We used wireless sensor network technology, low-power integrated embedded systems and wireless communications, which have enabled us the design of low-cost, miniature, precise and intelligent physiological sensor modules. These modules are capable of measuring, processing, communicating one or more physiological parameters, and can be integrated into a wireless personal area network (WPAN). **Results:** We have designed, deployed, produced and tested both hardware and software medical sensors for WPAN, dedicated to vital signs acquisition, processing and transmitting, as well as the whole telemedical system for these physiologic parameters monitoring in real time. **Conclusions:** This paper is dedicated to the most complex Romanian telemedical pilot project, TELEMON, that has as goals the design and implementation of a system for automatic and complex telemonitoring, everywhere and every time, in (almost) real time, of the vital signs of persons with chronic illnesses, of elderly people, of those having high medical risk and of those living in isolated regions. The final objective of this pilot project is to enable personalized telemedical services, and to act as a basis for a public service for telemedical procedures in Romania and abroad. **Keywords:** TELEMEDICINE, TELEMONITORING, BIOMEDICAL DEVICES, WIRELESS PERSONAL AREA NETWORK, BIOMEDICAL SIGNAL ANALYSIS.

Ageing of the population has resulted in a sustained increase in family and state healthcare expenses – it is now close to 10% of EU gross domestic product (GDP) and is growing every year. Successful ageing means maintain physical, cognitive, and social activities, live an independent life of one's own choice and maintain an appropriate quality of life (QoL). Senior citizens wish to remain living at home for as long as possible, despite the appearance of motor and/or cognitive impairment. Some of these individuals require moral and/or physical support 24 hours a day, a

situation difficult to deal with without sustained family support or institutionalization. Solutions supporting independent living at home and outdoors are one of the main alternatives to institutionalization, implying a significant reduction in healthcare costs. Telemonitoring is one of the alternatives that provide users and their families with confidence and satisfaction, since it allows elderly patients with chronic diseases or very frail to live independently in their own home with direct contact to the professionals, relatives and friends.

Our project, TELEMON, enables designing of a secure multimedia transmission system (medical records, digital images, video, and text) in order to enhance the telemedical consultancy services. The main objective of this project is to enable personalized teleservices delivery and patient safety enhancement based on an earlier diagnosis with medical telemetry using biosignals, images, text transmissions, and also applying the suitable treatment according to the remote medical experts' recommendations (1, 2). Our project allows persons with different (chronic) diseases and to elderly/lonely people to be monitored from medical and safety points of view. In this way the medical risks and accidents are diminished. The TELEMON system acts as a pilot project destined to the implementation of a public e-health service, "everywhere and every time", in real time, for people being in different hospitals, at home, at work, during the holidays, on the street, etc.

MATERIALS AND METHODS

The main objective of this project is the achievement of an integrated system, mainly composed by the following components in a certain area: a personal network of wireless medical sensors on the ill person (fig. 1), a personal server on the same patient (a smartphone), and a personal computer (PC). After local biosignal processing, according to the specific monitored feature, the salient data are transmitted via one of internet, GSM/GPRS or a telephonic line to the database server of the Regional Telemonitoring Centre. The personal network of sensors includes at least one medical device for vital signs acquisition, or a fall detection module, all these components having radio microtransmitters, which allows an autonomic movement of the subject.

The project also approaches the situation of *the mobile patient*. Data processing is done by a Personal Digital Assistant or a smartphone with GPS localization, and data transmission is done by the GSM module of the PDA.

Concerning the application programs, they act and correlate on two levels: a local data processing, near the patient, as well as another processing on database central server. So, the general software architecture is a client-server one, and the project develops a so-called *SOA – Service Oriented Architecture* – which is a standards-based approach to manage services made available by different software packages for reuse and reconfiguration (3).

The results of data processing are in principal and if necessary different locally generated alarms, transmitted to the central server, to the family or specialist doctor, to the ambulance or to a hospital. Other results of locally or on server data processing are different medical statistics, necessary for the evaluation of health status of the subject, for the therapeutic plan and for the healthcare entities. The TELEMON system is built around a database server that re-

ceives data from local subsystems, also from mobile subsystems, and stores them. Also, the database server can be connected to another database server, for example a hospital server. The subsystems are connected to the database server through Internet (if it is available) or a GSM connection.

The following medical devices were chosen to allow a good monitoring of the vital parameters:

a) A *3-leads ECG module* to record and transmit data through a radio transceiver interface. This sub-system allows detection of various abnormalities of electric heart activity, focusing only on those which can be life threatening and thus a medical emergency, such as: (i) rhythm modifications: severe bradychardia (<45/min) or tachycardia (> 140/min or even asystole – the heart rate equals 0 for at least 3 sec.); (ii) recently installed AV blocks; (iii) signs of myocardial ischemia: new, significant pathological Q wave, elevation of the ST segment > 200 μV or depression of the ST segment < –150 μV, negative T wave; (iv) enlargement of the QRS complex > 0,12 sec; (v) prolonged QT interval > 0,45 sec. We have adapted algorithms for the extraction of the ECG features based on digital filters (4), especially algorithms for the QRS complex and ST segment detection. The module is useful for patients with heart complications or at risk for myocardial/vascular problems, who represent more than 80% of the elderly population.

b) The *arterial pressure module*, with serial interface. This module identifies significant variation of blood pressure such as hypotension or hypertension and is very important for elderly persons, who are prone to this kind of oscillations. Postural hypotension is one of the most frequent situations a senior person must deal with; its complications are severe, impairing the quality of life and becoming life threatening (e.g.: falls, syncope, and stroke).

As practical solution, a commercially available A&D UA-767PC BPM was used. The blood pressure monitor (BPM) takes simultaneous blood pressure and pulse rate measurements. It includes a bi-directional serial port connection communication at 9600 kbps. A eZ430-RF2500 module communicates with the BPM on this serial link to start the reading process and receives the patient's blood pressure and heart rate readings. Once the readings are received, the eZ430-RF2500 communicates with the network and transmits them to the Personal Server (PDA). PDA computes blood pressure and defines the status of the patient by using the following blood pressure values (by default), that can be adjusted and personalized by specialized physicians (cardiologists): Hypotension: systolic < 90 mmHg or diastolic < 60 mmHg; Normal: systolic 90–119 mmHg and diastolic 60–79 mmHg; Pre-hypertension: systolic 120–139 mmHg or 80–89 mmHg; Stage 1 Hypertension: systolic 140–159 mmHg or diastolic 90 – 99 mmHg; Stage 2 Hypertension: systolic ≥ 160 mmHg or diastolic ≥ 100 mmHg.

c) The *oxygen saturation module* (SpO_2). A large number of elderly patients have respiratory insufficiency due to chronic pulmonary diseases. The evolution of their respiratory capacity is strongly influenced by weather, exposure to allergens, humidity and compliance to treatment, therefore detection of oxygen saturation is a very useful thing. A decrease of arterial blood oxygen < 90% triggers the alarm system.

The *pulsoximeter sensor* used is Micro Power Oximeter board from Smiths Medi-

cal (5). The same sensor can be used for heart rate and SpO_2 detection. The probe is placed on a peripheral point of the body such as a fingertip, ear lobe or the nose. The pulsoximeter communicates with the eZ430-RF2500 through asynchronous serial channel at CMOS low level voltages. Data provided includes $\%SpO_2$, pulse rate, signal strength, and plethysmogram.

d) The use of wireless *breathing module* is suitable for continuous long-time monitoring of human respiration for a number of medical conditions requiring analysis of respiratory rhythm, sleep-related breathing disorder and ischemic heart disease or can be useful during recovery from an acute event or surgical procedure. Our module uses one of the most usual methods to sense breathing, i.e. using a nasal thermistor (6). The sensor is designed using MSP430F2274 microcontroller with an on-chip 10 bit A/D converter for data acquisition (10 Hz sampling frequency) and CC2500 2.4 GHz wireless transceiver. The thermistor detects changes of breath temperature between ambient temperature (inhalation) and lung temperature (exhalation). The used thermistor is a 0603 SMD type and has as main characteristics: R_{nom} = 10 kΩ at 25°C, B = 3380, 1% tolerance. The Personal server on patient computes the following parameters: Breathing amplitude - calculated for every breathing cycle as a difference between minimum (Inhalation) and maximum thermistor voltage (Exhalation); Breathing interval – measured between two signal minima; Breathing frequency, calculated as a number of breaths per minute. Normal breathing frequency is 12-20 cycles/minute. We consider two types of respiration: normal respiration, when every breath lasts more than 0.5 seconds; apnea, when the breathing is missing for more than 10 seconds.

e) The *body temperature module* gives important information about occurrence of fever, especially for persons with mild cognitive impairment who cannot sense temperature modifications (> 38 °C or < 35 °C). We used the TMP275 temperature sensor (Texas Instruments). The TMP275 is directly connected to the eZ430-RF2500 module. The accuracy for the 35-45°C interval is below 0.2 °C. The Personal server samples the signal from the temperature sensor once per second and computes the status of the patient for the following temperature values: low temperature – <35°C; high temperature – >38°C; normal temperature – between the above values.

f) The *fall detection module* should be recommended to all senior persons who live alone. Elderly people are exposed to falls due to several causes: (i) postural hypotension (induced by inadequate hydration, cervical spondilosys with vertebrobasilar circulatory problems or even inappropriate medication for hypertension); (ii) inappropriate house conditions such as poor lighting conditions, narrow halls or staircases, slippery surfaces which predispose losing balance and fall; (iii) sensory disturbances (visual, postural) that induce imbalance and fall; (iv) inappropriate shoeing and/or clothing. Early detection of an elderly who had a fall is of extreme importance for recovering personal health and mental state after such a traumatizing event. Our fall detection module is based on accelerometer technique, that can recognize four kinds of static postures: standing, bending, sitting, and laying. Motions between these static postures are considered as dynamic transitions. If the transition before a lying posture is not intentional, a fall event is detected.

Our method uses the ADXL330 3-axes accelerometer and eZ430-RF2500 Wireless Modules. The ADXL330 is a small, thin, low power, complete, three axial accelerometer with signal conditioned voltage outputs, all on a single monolithic IC. We determine if the subject has fallen if the condition $a_A = \sqrt{a_{A_x}^2 + a_{A_y}^2 + a_{A_z}^2} > 0.4g$ is valid.

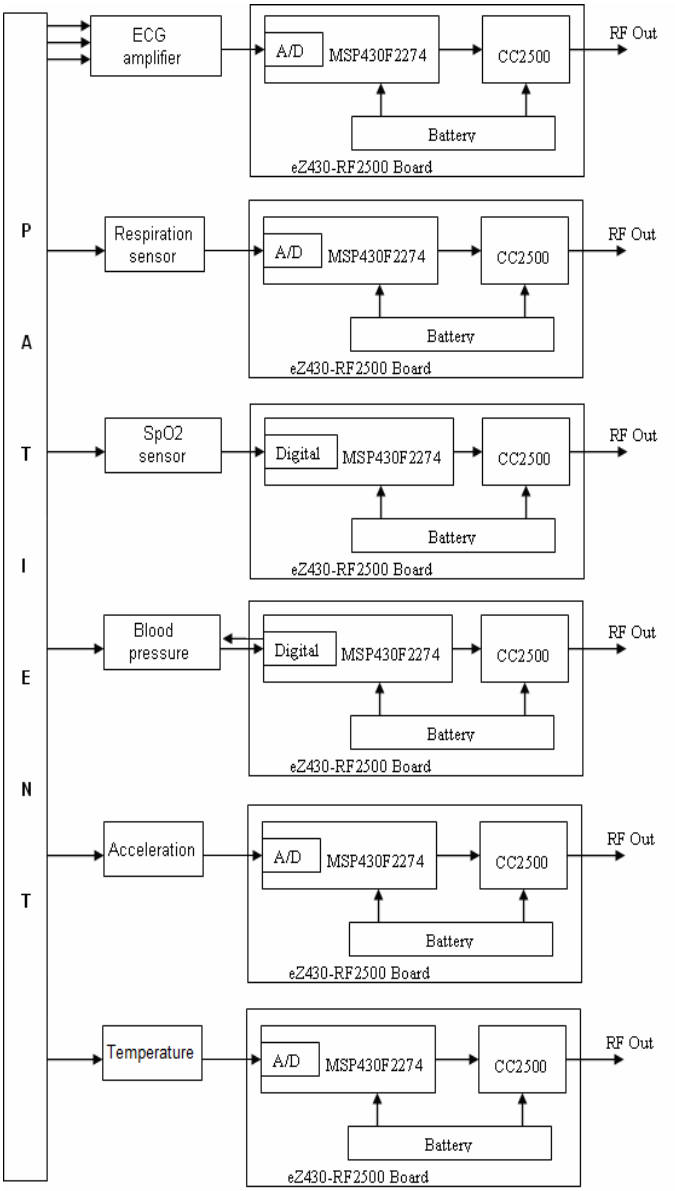

Fig. 1. The local subsystem for home monitoring of the patient

The modules (a, d, e, f) were made by our research team, while modules (b) and (c) were chosen from the market and were integrated in TELEMON system (www.bioinginerie.ro/telemon) (Fig. 1). These modules transmit data to a PDA through radio transceivers (eZ430RF2500 boards), can operate in the 2.4 GHz band, and have 5m /10m range indoors /outdoors. The eZ430-RF2500 is a complete MSP430 wireless development tool (7) providing all the hardware and software for the MSP430F2274 microcontroller and CC2500 2.4 GHz wireless transceiver (8). Operating on the 2.4 GHz unlicensed industrial, scientific and medical (ISM) bands, the CC2500 provides extensive hardware support for packet handling, data buffering, burst transmissions, authentication, clear channel assessment and link quality. The radio transceiver is also interfaced to the MSP430 microcontroller using the serial peripheral interface. The USB interface enables eZ430-RF2500 to remotely send and receive data through USB connection using the MSP430 Application UART.

RESULTS

The personal server was implemented by means of a PDA (Toshiba TG01C) (Fig. 2). This personal medical monitor is responsible for a number of tasks, providing a transparent interface to the wireless medical sensors, an interface to the patient, and an interface to the central server. Its USB interface is realized by using a serial-to-USB transceiver (FT232BL) from FTDI (9) and enables eZ430-RF2500 radio module to remotely send and receive data through USB connection using the MSP430 Application UART. All data bytes transmitted are handled by the FT232BL chip.

The software on the Personal Server receives real-time patient data from the sensors and processes them to detect anomalies. The software working on the Personal Server (Fig. 3) was written by using C# from Visual Studio.NET, version 8. The software displays temporal waveforms, computes and displays the vital parameters and the status of each sensor (the battery voltage and distance from the Personal Server). The distance is represented in percent, based on RSSI (received signal strength indication, measured on the radio power present in a received radio signal).

If the patient has a medical record that has been previously entered, information from the medical record (limits above the alarm become active) is used in the alert detection algorithm. So, when an anomaly is detected in the measured patient vital signs, the Personal server application generates an alert in the user interface and transmits the information to the TELEMON Server. For instance, the following physiological conditions cause important alerts:

low SpO2, if SpO2 < 90%; bradycardia, if HR < 40 bpm (HR = heart rate); tachycardia, if HR > 150 bpm; HR change, if ΔHR / 5 min > 20%; HR stability, if max HR variability from past 4 readings > 10% ; BP change, if systolic or diastolic change is > ±10% .

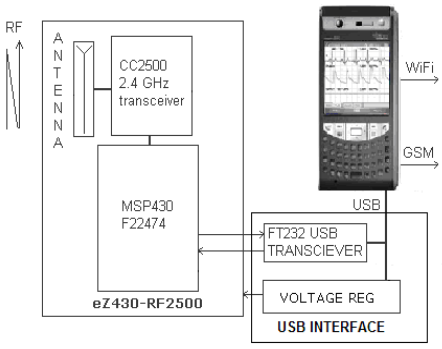

Fig. 2. The Personal server (block diagram)

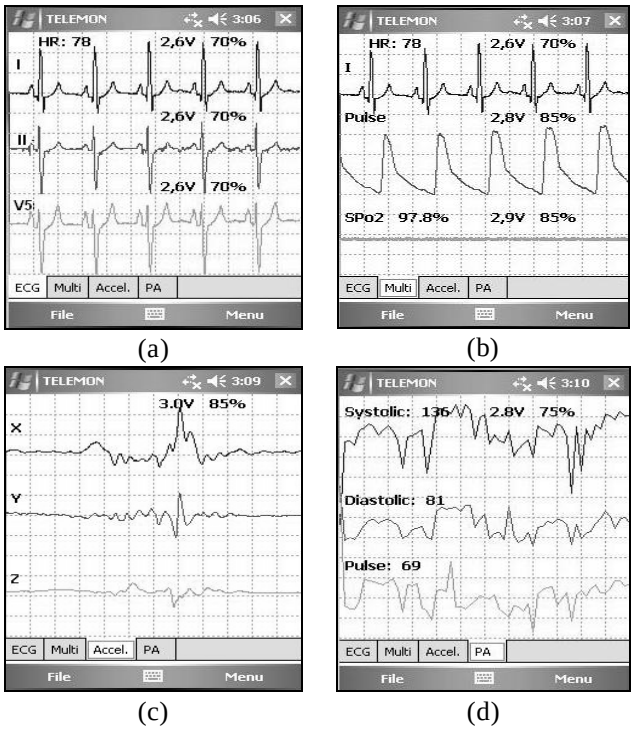

Fig. 3. The Personal server interface: (a) 3 ECG traces, (b) one ECG trace, pulse waveform and SpO2, (c) 3 accelerometer traces, (d) systolic and diastolic pressure from the blood pressure monitor

DISCUSSION

In this paper it is presented a project that acts as base for developing a secure multimedia, scalable system, designed for medical consultation and telemonitoring services. The main goal is to build a complete pilot system that will connect several local telecenters into a regional telemedicine network. This network enables the implementation of teleconsultation, telemonitoring, homecare, urgency medicine, etc. for a broader range of patients and medical professionals, including elderly people and those people living in rural or isolated regions (10–14).

The Regional Telecenter in Iasi / Romania, situated in the Faculty of Medical Bioengineering, allows local connection of hospitals, diagnostic and treatment centers, as well as a local network of family doctors, patients, paramedics and even educational entities. As communications infrastructure, we have developed a combined fix-mobile-internet (broadband) links. The proposed system is also used as a warning tool for monitoring during normal activity or physical exercise. Such a regional telecenter acts as a support for the developing of a regional medical database, that should serve for a complex range of teleservices such as teleradiology, telepathology, teleconsulting, telediagnosis, and telemonitoring. It should also be a center for continuous training and e-learning tasks, both for

medical personal and for patients.

Also, the telemonitoring of elderly people who want to preserve as long as possible their independence but are of medical risk (cardiovascular, respiratory, proneness to falls) represents one of the most important solutions to an aging Europe. Besides the medical and technical objectives, TELEMON project also proposes important economic objectives: (i) the decrease of budgetary and personal expenses dealing with the unjustified transport of patients to the hospital; (ii) „zero costs" for the hospitalization in the case of patients who may be treated at home.

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NEW HYDRAZONES WITH PYRAZOLONE STRUCTURE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION

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NEW HYDRAZONES WITH PYRAZOLONE STRUCTURE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION(Abstract): **Aim:** To design new hydrazones with pyrazolone structure with improved biological properties. **Material and methods**: New hydrazones of antipyrine have been prepared by reaction of 4-aminophenazone (4-aminoantipyrine) with chloracetylchoride and hydrazine hydrate and then condensation with various aromatic aldehydes. **Results:** The synthesized compounds were screened for their antibacterial activity against Gram positive (*Staphylococcus aureus* ATCC 25923, *Sarcinalutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*) and Gram negative bacterial strains (*Escherichia coli* ATCC 25922) and pathogenic yeasts (*Candida albicans* ATCC 10231, *Candida sake*, *Candidaglabrata*). Some of them were found to have good antibacterial and antifungal activity. The antioxidant activity of these compounds was also evaluated using the total antioxidant capacity test. **Conclusions:** The chemical modulations performed on antipyrine structure have a good influence on the biological activity of the synthesized compounds. **Keywords:** ANTIPYRINE, HYDRAZONES, ANTIBACTERIAL, ANTIFUNGAL, ANTIOXIDANT.

Non-steroidal antiinflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically active compounds used in the management of acute and chronic inflammation, pain and fever. Their mechanism of action is related to their ability to inhibit both COX-1 and COX-2 isoforms of the cyclooxygenase enzyme (COX) (1). COX-1 is a constitutive isoform and it is responsible for synthesis of prostaglandins with cytoprotective effects in gastrointestinal tract and control of renal function. The inductible COX-2 isoform is selectively activated by pro-inflammatory stimuli and facilitates the release of prostaglandins involved in the inflammatory process (2). Because most of NSAIDs are nonselective COX inhibitors their long-term clinical use is associated with significant side effects, such as gastrointestinal damage, bleeding and nephrotoxicity. The pyrazolone derivatives are important drugs among NSAIDs. Starting with 1884, when antipyrine was marked as drug used in management of pain,

inflammation and fever, great attention was focused on pyrazolone derivatives as potent anti-inflammatory, analgesic and antipyretic agents. As a result, a large number of pyrazolone derivatives have been developed and some of them marked as important drugs (3). For example, Celecoxib is a selective COX-2 inhibitor with anti-inflammatory, analgesic properties and few gastrointestinal side effects. Up to now, broad properties of antipyrine derivatives have been investigated as antitumor, antimicrobial, antiviral, analgesic and anti-inflammatory agents (4-8).

This study presents the synthesis, physico-chemical and spectral characterization and also evaluation of the antioxidant and antimicrobial activity of new antipyrine derivatives.

MATERIAL AND METHODS

4-amino-antipyrine, chloroacetyl chloride, organic solvents (*p.a.* quality), standard reagents for antioxidant and antimicrobial tests were purchased from Sigma Aldrich Company. All solvents and reagents were used without further purification.

Chemistry.*Synthesis of antipyrine derivatives.*4-aminoantipyrine (1,5-dimethyl-2-phenyl-4-amino-1,2-dihydro-3H-pyrazol-3-one) (1) was reacted with chloroacetyl chloride in dry acetone and in the presence of anhydrous potassium carbonate (fig. 1). The resulting N-chloroacetyl-aminoantipyrine (2) was then reacted with hydrazine hydrate in anhydrous ethanol and the hydrazine derivative (3) was then reacted with various aromatic aldehydes when new hydrazone derivatives (4-9) were obtained.

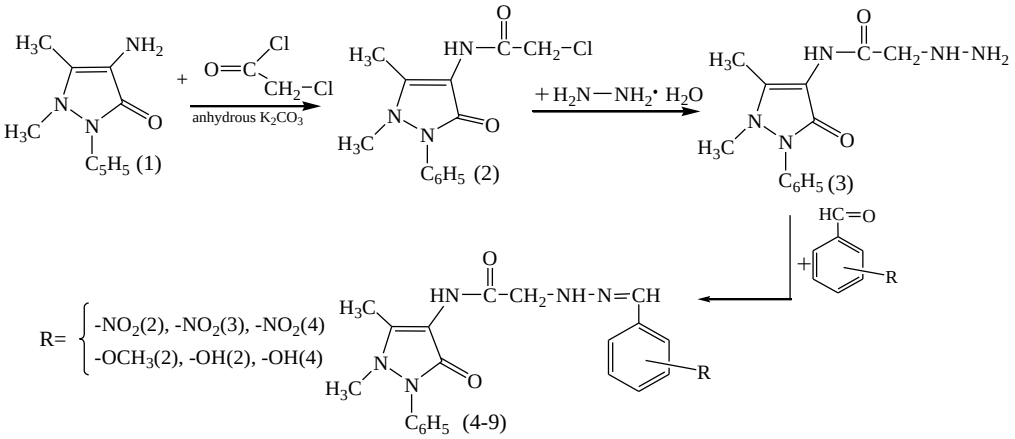

Fig. 1. Synthesis of new hydrazones (4-9) with pyrazolone structure.

Synthesis of 2-chloro-N-(1,5-dimethyl-3-oxo-2-phenyl-1,2-dihydro-3H-pyrazolyl) aceta-mide(1). 4-aminophenazone (10 mmol, 2.03 g) was dissolved in 20 ml chloroform and then chloracetic chloride (10 mmol, 0.8 ml) and anhydrous potassium carbonate (10 mmol) were added. The reaction mixturewas heated under reflux for 8 h and then filtered off and evaporated. The reaction progress was monitored by Thin Layer Chromatography (TLC) (ethyl acetate: acethone: hexane 5:2:1; Rf 0.62). The crude product was treated with crushed ice and the solid was filtered off, washed with sodium bicarbonate (10 ml) and water (10 ml) and then recrystallized from ethanol.

White-yellow powder, yield 87%, m.p. 189-190°C. IR (cm^{-1}): 1690 (CO-NH), 654 (C-Cl).

Synthesis of N-(1,5-dimethyl-3-oxo-2-phenyl-1,2-dihydro-3H-pyrazolyl)-2-hydrazinyl-acetamide (2).Hydrazine hydrate (5 mmol) and magnesium sulphate (0.5 g) were added in 25 ml absolute ethanol at 0°C and then chloracetyl derivative of aminoantipyrine (1 mmol, 0.279 g) (2) in 10 ml ethanol was added drop wise for 15 min. The reaction mixture was stirred at room temperature for 8 h, left overnight and then filtered off in order to remove the excess of magnesium sulphate. The solution was evaporated and the product was washed three times with chloroform. The progress of reaction was monitored by TLC (dichlormethane : methanol 1:1; Rf 0.25). White powder, yield 58 %. IR (cm^{-1}): 1654 (CO-NH), 3433, 3327 (NH_2).

General procedure for synthesis of 2-(2-benzylidenhydrazinyl)-N-(phenazon-4-yl) acetamide(4-9).To a solution of hydrazine derivative of aminoantipyrine (1 mmol, 0.28 g) (2) in ethanol (10 ml), aromatic aldehydes (1 mmol) and glacial acetic acid (2-3 drops; pH 4-5) were added. The reaction mixture was heated under reflux for 5 h. The obtained solidwas filtered off and recrystallized from ethanol.

Characterization by FTIR Spectroscopy. Fourier transform infrared (FTIR) spectra were recorded on a FT-IR Bomem MB-104 spectrometer (Canada). FTIR spectra were acquired after 32 scans between 4000 and 600 cm^{-1}, with spectral resolution of 4 cm^{-1}. Spectra processing was carried out with a Grams/32 program (Galactic Industry Corporation).

Antioxidant assays. *Determination of total antioxidant capacity*.

The antioxidant activity of tested compounds was evaluated using phosphomolybdenum method according to the procedure of (9) with minor modifications. Briefly, 300 µL sample solution of various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml) in DMSO was mixed with the reagent solution (2 ml 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated at 95°C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank (DMSO mixed with the reagent solution). Ascorbic acid (AA) was used as standard antioxidant in the same concentration of the samples.

Antimicrobial assessment. The antimicrobial activity was studied using Gram positive (*Staphylococcus aureus* ATCC 25923, *Sarcinalutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*,), and Gram negative bacterial strains (*Escherichia coli* ATCC 25922) and pathogenic yeasts (*Candida albicans* ATCC 10231, *Candida sake*, *Candidaglabrata*). Antimicrobial activity was initially evaluated by agar disc diffusion method according to described protocols (CLSI, 2012). Sterile stainless steel cylinders (50 mm internal diameter; 100 mm height) were applied on the agar surface in Petri plates. 200 µL of the tested compounds (4-9) (10 mg/ml) were added to each cylinder. Commercially available discs containing ampicillin (25 mg/disc), chloramphenicol (30 mg/disc) and nystatin (100 mg/disc) were used. The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts). After incubation the diameters of inhibition zones were measured.

RESULTS AND DISCUSSION

Chemistry. The physical measurements

of the compounds are presented in tab. I and the characteristic IR spectroscopic bands are given in tab. II. In the IR spectra of the hydrazone derivatives of aminoantipyrine (compounds 4-9) the absorption bands characteristic for all functional groups were observed. The absorption band for the C=O group was observed in the range of 1603-1653 cm^{-1}, for the C=N group in the range of 1578-1597 cm^{-1} and for C-N group the characteristic band appears in the range of 1107-1140 cm^{-1}. The bands of benzilydene ring substituents appeared in the range of 1516 -1522 cm^{-1} (asymmetric vibration of NO_2 group), 1342-1356 cm^{-1} (symmetric vibration of NO_2 group), 3070 cm^{-1} (OCH_3), 1200-1230 cm^{-1} (OH). The presence of these bands confirmed the chemical structure of the synthesized compounds.

TABLE I

Physical characteristics of pyrazolone derivatives (4-9)

Sample	R	Yield (%)	Weight	Formula	m.p. °C
4	-NO_2(2)	83.85	408.35	$C_{20}H_{20}O_4N_6$	200 - 202
5	-NO_2(3)	83.25	408.35	$C_{20}H_{20}O_4N_6$	183 - 185
6	-NO_2(4)	88.32	408.35	$C_{20}H_{20}O_4N_6$	258 - 260
7	-OCH_3(2)	78.25	393.37	$C_{21}H_{23}O_3N_5$	137 -139
8	-OH(2)	98.70	379.35	$C_{20}H_{21}O_3N_5$	210 - 213
9	-OH(4)	64.52	379.35	$C_{20}H_{21}O_3N_5$	174 - 176

TABLE II

Characteristic spectral bands of pyrazolone derivatives (4-9)

Sample	R	IR characteristic band (cm^{-1})			
		C= 0	**C=N**	**C –N**	**C-R/ R**
4	-NO_2(2)	1647	1591	1128	NO_2: 1520 (asymm), 1356 (symm)
5	-NO_2(3)	1645	1593	1136	NO_2: 1522 (asymm), 1346 (symm)
6	-NO_2(4)	1639	1597	1107	NO_2: 1516 (asymm), 1342 (symm)
7	-OCH_3(2)	1647	1591	1136	O-C (CH_3): 3070; C-H (CH_3): 2934, 2837
8	-OH(2)	1653	1591	1138	C-O: 1200
9	-OH(4)	1603	1578	1140	C-O: 1238

Antioxidant assays*. Determination of total antioxidant capacity*. The assay for total antioxidant capacity was based on the reduction of Mo^{+6} to Mo^{+5} by the tested compounds followed by formation of a green phosphate/ Mo^{+5} complex at acidic pH. The results of the synthesized compounds are presented in tab. III. At concentrations of 500 μg/ml, 250 μg/ml, 125 μg/ml and 62.5 μg/ml all tested compounds were more active than ascorbic acid (AA) at the same concentrations. The most active compounds were derivatives (7, 9), which were obtained through condensation of hydrazine derivative of antipyrine with 2-methoxybenzaldehide and 4-hydroxybenzaldehyde, respectively.

TABLE III

Absorbance of the tested compounds at various concentrations compared with ascorbic acid (AA)

Sample	Absorbance of sample at various concentrations				
	500 µg/ml	**250 µg /ml**	**125 µg /ml**	**62.5 µg /ml**	**31.25 µg /ml**
4	2.5863±0.046	1.1768±0.0144	0.5870±0.0477	0.2782±0.0072	0.1183±0.0114
5	2.0863±0.2194	0.8372±0.0949	0.5317±0.0353	0.1828±0.0130	0.0713±0.0020
6	2.5933±0.1558	1.1817±0.0921	0.5695±0.0560	0.2832±0.008	0.1974±0.0215
7	2.8822±0.4053	1.3421±0.1361	0.6569±0.0902	0.3272±0.0365	0.1738±0.0241
8	2.6239±0.2091	1.2056±0.0199	0.5675±0.0075	0.2701±0.020	0.1100±0.0110
9	2.8210±0.1309	1.2194±0.031	0.6283±0.0312	0.3084±0.0473	0.1700±0.034
AA	1.8088±0.405	0.8740±0.1804	0.2863±0.0282	0.2387±0.0306	0.2708±0.0317

*Antimicrobial assessment.*The inhibition zone diameters (mm) of the tested compounds on bacterial strains and yeasts are presented in figs. 2 and 3. According to the results, the most active compounds were (5), (R = -NO_2(3)) and (8) (R = -OH(2)). Compound 5 was active both on bacterial strains and yeasts, while compound 8 was active especially on bacterial strains. The antimicrobial activity of these compounds was comparable with that of ampicillin and chloramphenicol (bacterial strains) and nystatin (yeasts).

The inhibition zone diameters for compound 5 on yeasts were 22 mm(*Candida albicans* ATCC 10231), 25 mm (*Candida sake*) and 20 mm (*Candidaglabrata*). As to the antibacterial activity of compound 5, it was noticed that it was active especially on *Pseudomonas aeruginosa* and *Sarcinalutea* strains. Compound 8 also had an important antibacterial activity on Gram positive and Gram negative bacteria.

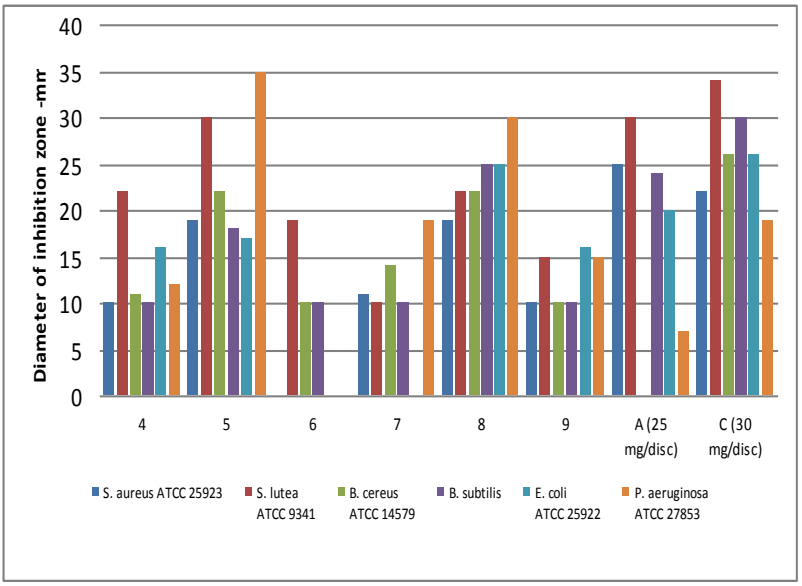

Fig. 2. Antibacterial activity of compounds(4-9)(A-Ampicillin, C-Chloramphenicol)

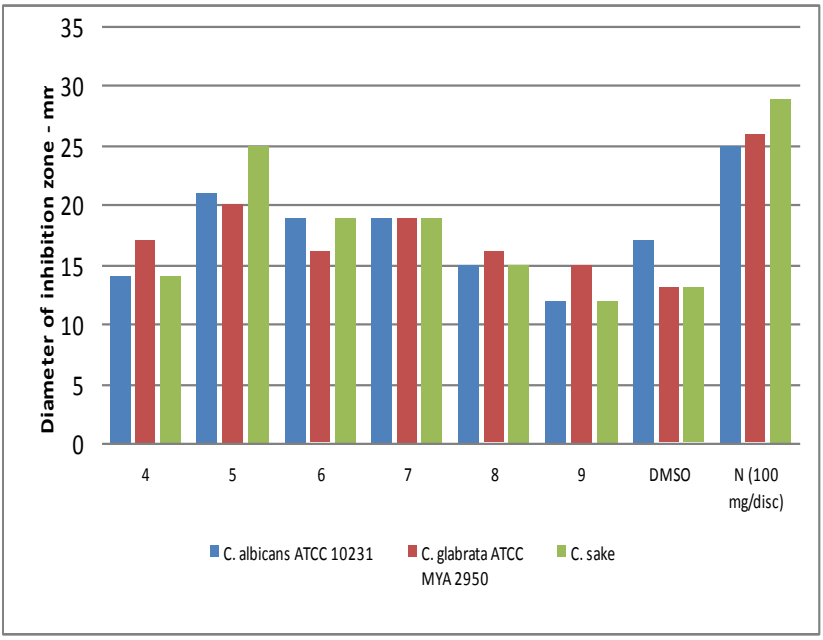

Fig. 3. Antifungal activity of compounds (4-9) (N-Nystatin)

CONCLUSIONS

Some new hydrazones with pyrazolone structure have been synthesized and optimal conditions of reaction were established for obtaining compounds with high yields and advanced purity. The synthesized compounds have been characterized by their physical constants (melting point, yield, molecular formula, molecular weight, solubility in different organic solvents) and their chemical structure was confirmed by FT-IR spectroscopy. Their antibacterial and antioxidant activities have been also evaluated. Some compounds possessed important antimicrobial and antioxidant activity.

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NEWS

HERBAL MEDICINES WITH ANTI-PARKINSONIAN ACTIVITIES

Parkinson's disease is a chronic neurological disorder. In recent years, increasing interest has been devoted to the treatment or prevention of Parkinson's disease by herbal medicines. Li et al. summarized and analyzed the anti-Parkinsonian activities of several herbal medicines and herbal formulations. All the herbal medicines and herbal formulations were tested on Parkinson's disease models both *in vitro* and *in vivo*. A total of 38 herbal medicines and 11 herbal formulations were analyzed. The relevant compounds, herbal extracts and formulations were reported to be effective on Parkinson's disease models by modulating multiple key events or signaling pathways implicated in the pathogenesis of the disease. The plant species of these herbal medicines belonged to 24 genera (*Acanthopanax*, *Alpinia*, *Astragalus*, *Camellia*, *Cassia*, *Chrysanthemum*) and 18 families. These herbal medicines can be an alternative and valuable source for anti-Parkinsonian drug discovery. According to this study, the plant species in these genera and families may be the most promising candidates for further investigation and deserve further consideration in clinical trials (Li XZ, Zhang SN, Liu SM et al. Recent advances in herbal medicines treating Parkinson's disease. *Fitoterapia* 2013; 84: 273-285).

Ana Maria Zbancioc

NEW HYDRAZINES WITH SULPHONAMIDIC STRUCTURE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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NEW HYDRAZINES WITH SULPHONAMIDIC STRUCTURE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY (Abstract): Infections caused by bacterial species are common in immunocompromised patients and carry significant treatment costs and mortality. The emerging resistance of microorganisms to some synthetic antimicrobial agents makes it necessary to continue the research for new antimicrobial drugs. **Aim:** To design new sulphonamide compounds with potential antibacterial and antioxidant activity. **Material and methods**: New N-hydrazino acetyl-sulphonamides were prepared by condensation of some sulphonamides with chloroacetyl chloride and amination of intermediate compounds with hydrazine hydrate. **Results:** The synthesized compounds were screened for their antibacterial activity against Gram positive (*Klebsiellapneumoniae*, *Proteus vulgaris*, *Citrobacter freundii*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*) and Gram negative bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*). Some of them were found to have good antibacterial activity. The antioxidant activity of these compounds was also tested using different methods: total antioxidant capacity, reducing power, and DPPH radical scavenging activity. **Conclusions:** Chemical modulations performed on sulphonamide structure have a good influence on the biological activity of the synthesized compounds, especially on their antioxidant effects. **Keywords:** SULPHONAMIDES, HYDRAZINES, ANTIBACTERIAL ACTIVITY, ANTIOXIDANT ACTIVITY.

Sulphonamides and their different derivatives are extensively used in therapy due to their antibacterial activity. They interfere with the use of *p*-aminobenzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid, which is an essential growth factor, vital to the bacterial metabolism (1). The chemistry of sulphonamides has been recently recognized in the preparation of various valuable biologically active compounds used especially as antibacterial agents (2). On the other hand the production of free radicals is relatively increased during infections. Starting from these observations and continuing our research on bioactive molecules, we synthesized some novel hydrazine acetyl-sulphonamides in view of studying their biological activities.

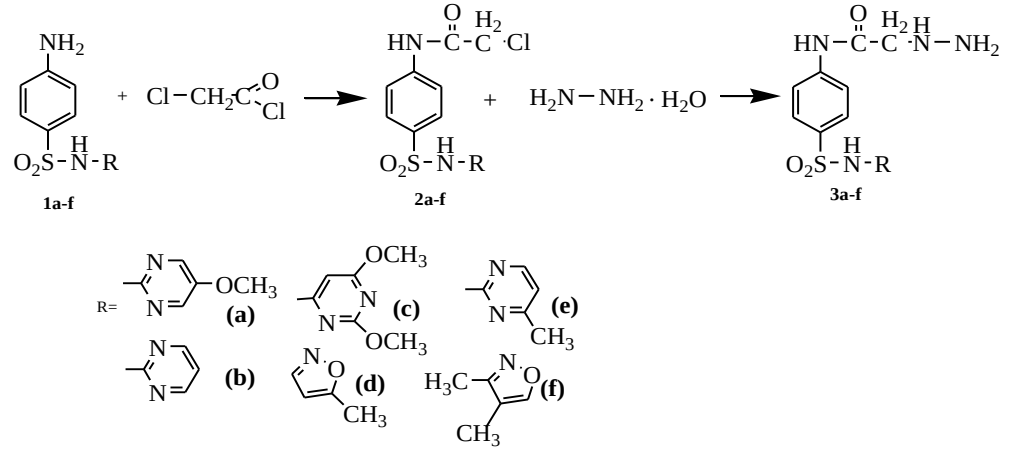
Given their antibacterial and antioxidant potential, these compounds could be used in protecting biological systems from oxidative damage and bacterial infections.

MATERIAL AND METHODS

Sulphonamides, chloroacetyl chloride, organic solvents (*p. a*quality), standard reagents for antioxidant and antimicrobial tests were purchased from Sigma Aldrich Company. All solvents and reagents were used without further purification.

Synthesis of sulphonamide derivatives. Six sulphonamides (sulfamethoxydiazine, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfisoxazole and sulfamethoxazole) were subjected to reaction with chloroacetyl chloride in dry acetone in the presence of anhydrous K_2CO_3 (Scheme 1).

The resulting N-chloroacetyl-sulphonamides have then reacted with hydrazine hydrate in anhydrous ethanol. The reactions were carried out by adapting similar methods used for derivatization of some compounds with primary aromatic amine groups (3, 4, 5, 6).



Scheme 1. Synthesis of new hydrazine with sulphonamide structure

***General procedure for synthesis of N-hydrazinoacetyl-sulphonamides* (3a-f):**

A mixture of each of the compounds **2a-f** (0.04 mole) and hydrazine hydrate (99%, 0.12 mole) in ethanol (150 ml) was refluxed for 10 hours. From the resulting mixture excess ethanol was removed under reduced pressure. The resulting solid residue was purified by recrystallization using the proper solvent and separated by filtration.

Characterization by FTIR Spectroscopy. Fourier transform infrared (FTIR) spectrum was recorded on a Bomem MB-104 FT-IR spectrometer (Canada). All spectra were obtained in the 4000 - 600 cm^{-1}range at a resolution of 4 cm^{-1} over 32 scans. Spectroscopy data were processed with a Grams/32 software (Galactic Industry Corporation).

Antioxidant assays

Determination of total antioxidant capacity. The antioxidant activity of tested compounds was evaluated by the phosphomolybdenum method according to the standard procedure (7) with minor modifications. Briefly 50 µl sample solution (5 mg/ml) in DMSO was mixed with the rea-

gent solution (2 ml, 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank (DMSO mixed with reagent solution). Ascorbic acid (AA) in concentration matching that of the samples was used as standard antioxidant.

*Reducing power.*The reducing power of the compounds was quantified by the method described in the literature (8) with minor modifications. Briefly, 1 ml of reaction mixture, containing 5 mg of samples in DMSO, was mixed with 0.2 M sodium phosphate buffer (1 ml, pH=6.6). The reaction was initiated by addition of potassium ferricyanide (1% w/v, 1 ml). After 20 min of incubation at 50°C, the reaction was terminated by trichloroacetic acid solution (10% w/v, 1 ml). The mixture solution was centrifuged at 4500 rpm for 15 min. 1 ml of supernatant was diluted with 1 ml of deionised water, and 0.2 ml ferric chloride (0.1% w/v) solution was added. After 5 min of incubation, absorbance was measured at 700 nm against a blank (the mixture of DMSO with the reagents). Higher absorbance indicates higher reducing power. Effective concentration (EC50: the concentration of a sample in which the absorbance is 0.5 and 50% of substrate is reduced) was calculated.

DPPH radical scavenging activity. The radical scavenging activity of tested compounds against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured as described by (9) with a few modifications. 50 µl of each sample solution (20 mg/ml in DMSO) was added to 2950 µl of 0.1 nM DPPH methanol solutions. The mixtures were left at room temperature and in the dark for 30 min, and then absorbance was measured at 517 nm. Ascorbic acid (AA) in concentration matching that of the samples was used as standard.

DPPH methanol solution was used as control sample. The radical scavenging activity was calculated using the following formula: % inhibition = [(control absorbance-sample absorbance) /control absorbance] x 100.

Antimicrobial assessment

The determination of minimum inhibitory concentrations (MICs) of samples was performed by agar dilution method. Nine reference bacterial strains (*K. pneumoniae* CIP 53153, *P. vulgaris* CIP 104989, *C. freundii* CIP 5732, *E. cloacae* CIP 103475, *S. aureus* ATCC 6583, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922, *E. faecalis* ATCC 25912, *P. aeruginosa* CIP 82118) were included in this study which was performed according to EUCAST standard. The stock solutions were prepared by dissolving 200 mg of each compound in 19.5 ml of DMSO. All stock solutions were stored at 4 °C and used within 24 h. Then 20 ml (19 ml of molten agar to 1 ml of sample solution) were used in 9 cm Petri dishes for agar dilution MICs. The MIC value was defined as the lowest concentration exhibiting no visible bacterial growth at 15°C for 44 to 48 hours and/or 68 to 72 hours.

RESULTS AND DISCUSSION

Chemistry

The hydrazines with sulfonamide structure are crystalline light yellow to light brown powders very soluble in DMF and DMSO. The characteristic IR spectral bands of sulphonamid-hydrazines are shown in table 1. The presence of these bands confirms the chemical structure of

the compounds.

Antioxidant asays

Determination of Total Antioxidant Capacity. The total antioxidant capacity assay is based on the reduction of Mo^{+6} to Mo^{+5} by the tested compounds followed by formation of a green phosphate/ Mo^{+5} complex at acidic pH. The results of the synthesized compounds in comparison with their parent sulphonamides are presented in fig. 1, 2. It was noticed that the most active compound as compared to the parent sulphonamide was 3d while as compared to ascorbic acid 3e.

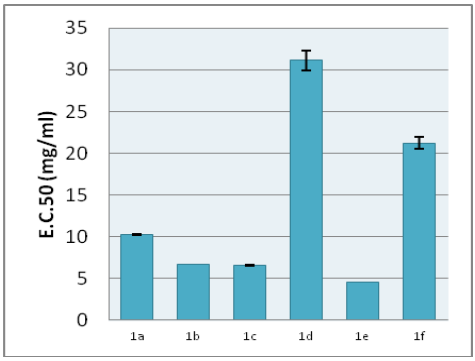


Fig. 1. Total antioxidant capacity of (1a-f)

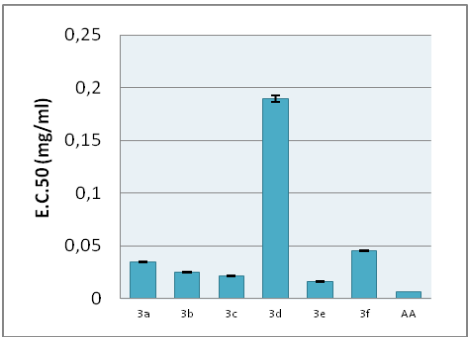


Fig. 2. Total antioxidant capacity of (3a-f)

Reducing power. The synthesized compounds (3a-f) caused the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form (Fe^{2+}), the colour of the test solution changing from yellow to green depending on the reducing power of each sample. The derivatization of parent sulphonamides with acetyl-hydrazine chain led to increased antioxidant capacity, all tested compounds being more active than parent sulphonamides (fig. 3, 4).

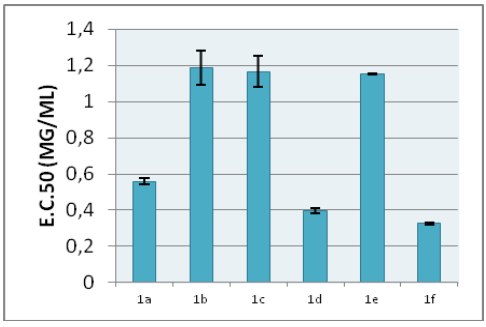


Fig. 3. Reducing power of (1a-f)

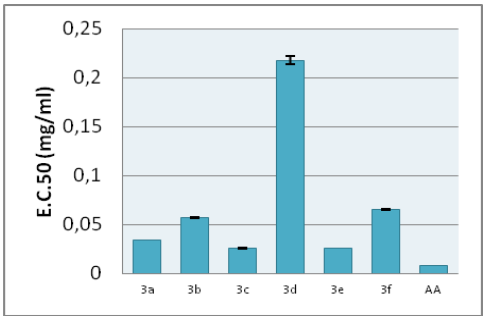


Fig. 4.Reducing power of (3a-f)

*DPPH Radical Scavenging Activity.*The results are presented in figs. 5 and 6. In all six sulphonamides the percentage of inhibition of free radical DPPH is very low (I%=0-36.53), while all six hydrazines have an inhibition percentage (I%=51.25%-97.45%) comparable with that of ascorbic acid (I%=97.59).

*Antimicrobial assessment.*CMI values of the tested compounds are presented in fig. 7, 8. According to the obtained results only N^4 hydrazin acetylamino-sulpha dime-thoxine has a better antimicrobial activity as compared to its parent sulphadimethoxine.

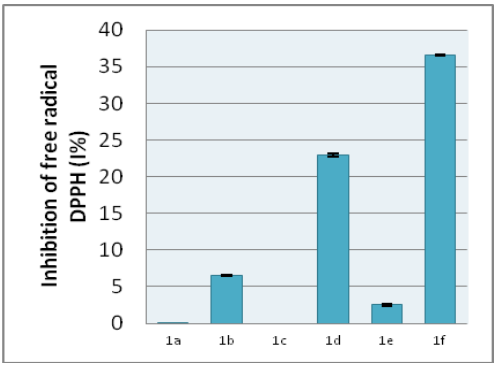


Fig. 5. Radical scavenging activity of (1a-f)

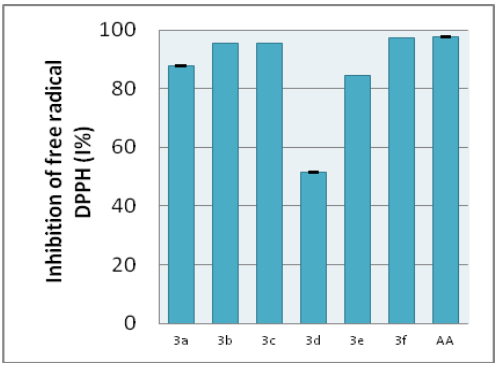


Fig. 6. Radical scavenging activity of (3a-f)

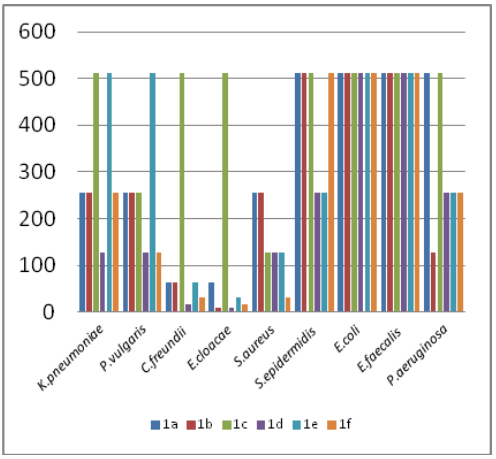


Fig. 7. MIC values of (1a-f).

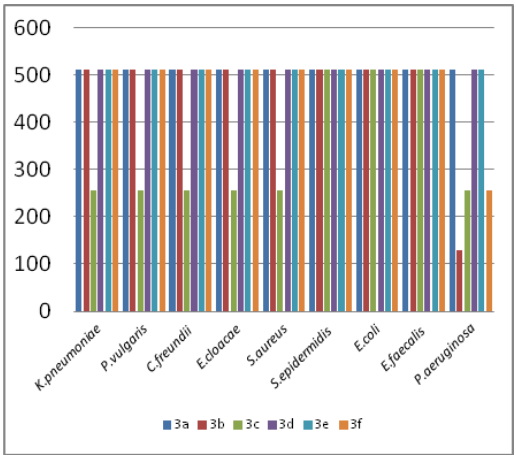


Fig. 8.MIC values of (3a-f).

TABLE I

FTIR characterization and physical measurements of N- hydrazinoacetyl-sulphonamides (3a-f)

Comp.	Infrared (cm-1)			Physical characterization		
no	δ_{HN-NH2}	δ_{CH2-NH}	δ_{HN-CO}	Molecular formula	m.p (°C)	Yield (%)
3a	3351	2853	1686	$C_{13}H_{16}O_4N_6S$	181	85,12
3b	3353	2850	1674	$C_{12}H_{14}O_3N_6S$	165	65,09
3c	3350	2850	1684	$C_{14}H_{18}O_5N_6S$	102-105	84,75
3d	3320	2886	1703	$C_{12}H_{15}O_4N_5S$	220-222	75,90
3e	3307	2843	1670	$C_{13}H_{16}O_3N_6S$	155-160	75,41
3f	3350	2875	1688	$C_{13}H_{17}O_4N_5S$	135-140	63,10

CONCLUSIONS

Some new hydrazines with sulphonamide structure have been synthesized and optimal conditions of reaction were established for obtaining compounds with high yields and advanced purity. The synthesized compounds have been characterized by their physical constants (melting point, yield, molecular formula, molecular weight, solubility in different organic solvents) and their chemical structure was confirmed by FT-IR spectroscopy. Their antibacterial and antioxidant activities have been evaluated. All compounds have marked antioxidant activity compared to the parent sulphonamides and, for some of them; the antioxidant activity is comparable to that of ascorbic acid at the same concentration.

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CLINICAL AND BIOLOGICAL FEATURES OF ADULT TOXOCARIASIS

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CLINICAL AND BIOLOGICAL FEATURES OF ADULT TOXOCARIASIS (Abstract): **Aim:** To determine the main clinical signs and symptoms of adult toxocariasis and the relationship between anti-*Toxocara canis* antibody levels, symptoms, and the other biological parameters. **Material and methods**: Prospective study conducted in the interval 2008 – 2011 in a series of 457 adult patients from Moldova, Romania. The ELISA method was used to detect the presence of IgG antibodies against *T. canis*. Fasting blood samples were collected from all patients who then underwent the following tests by standard methods: hemoglobin, hematocrit, eosinophils, lymphocytes, white blood cell count, SGOT, SGPT. **Results**: An IgG titer of 1/100 was most commonly found in case of allergies, physical asthenia, sensation of constriction in the anterior cervical area, right hypochondriac pain, paresthesies, nervousness (anxiety) and lipotimic states; a 1/50 titer was most often associated with hive reactions, dysphonia, lower limb edema, and pale/yellow axy skin. In patients with laterocervical adenopathy the most frequent determinations were 1/100 (34.0%), followed by 1/50 (22.6%). **Conclusions**: The statistically significant clinical signs and symptoms may represent a starting point in making a diagnosis of toxocariasis. Of the non-specific laboratory findings we mention: hyperleukocytosis , hypereosinophilia and low hematocit, elevated transaminases level having a high sensitivity and specificity. The diagnosis of certainty is made by determining IgG for *T. canis* or the presence of larvae in the biopsy tissues. **Keywords**: *T.CANIS*, TOXOCARIASIS, CLINICAL DIAGNOSIS, BIOLOGICAL MARKERS

Human toxocariasis is a cosmopolitan helminthic zoonosis due to human parasitization by larvae belonging to species of the Toxocara genus. The most common source of human visceral larva migrans is *Toxocara canis*. "Larva migrans" syndrome was first described by Beaver and colleagues (1952) (1).

In 1954 Beaver mentions the dog as the main source of infection for parasitic diseases. Toxocariasis is a zooanthroponosis with different clinical manifestations depending on the location of larva migrans , degree of activity and number of larvae. The parasitic disease caused by S2 larva is characterized by delayed growth, general, digestive, nervous, ocular and respiratory disturbances and many other pathologic actions (2,8,10,11, 15). Hypereosinophilia, hyperleukocytosis, sometimes ESR and gammaglobulin elevation are biological changes commonly found in toxocariasis, most studies being conducted in children (1.9). For the serological diagnosis of *Toxocara canis* infection the

ELISA method is used.

The aim of this study was to determine the main clinical signs and symptoms of adult toxocariasis and the relationship between anti-*Toxocara canis* antibody levels, symptoms, and the other biological parameters.

MATERIAL AND METHODS

This prospective study was conducted in the interval 2008 - 2011 on a series of 457 adult patients. Patient selection and analysis of medical records were in agreement with the health legislation in force. The study database, retrospective analytical study, was created by using history and physical examination data and interpretation of laboratory tests results.

Positive *T.canis* contact was assessed by determining IgG antibody titer by ELISA method. In all patients the following laboratory test were done by standard methods in fasting blood samples: hemoglobin, hematocrit, eosinophils, lymphocytes, white blood cell count, SGOT, SGPT.

Data from patients' medical observation sheets were entered into a Microsoft Excel spreadsheet, then statistically processed using SPSS 16.0. The numerical variables on subgroups defined by qualitative variables were compared by using ANOVA test. The homogeneity of variances was checked by using Levene test. In cases where this condition was not met, to evaluate the same phenomenon we used the nonparametric Kruskal-Wallis alternative for rank comparison. We sought evidence of biological clinical features suggestive of a diagnosis of Toxocariasis and their correlation with IgG titer for *T. canis.*

RESULTS AND DISCUSSION

All types of clinical manifestations were statistically significantly associated with positive Toxocara determination , except for stress/panic, sweating, palpitations, headache, dizziness and weight loss. *T. canis* IgG titer of 1/100 was most commonly found in allergies, physical asthenia, feeling of constriction in the anterior cervical region, right upper quadrant discomfort, paresthesias, nervousness, and faintness; a titer of 1/50 was often associated with urticarial reactions, dysphonia, lower leg edema, pale / yellow- waxy skin.

In the patients with laterocervical adenhopathy the most common determinations were 1/100 (34.0%), followed by 1/50 (22.6%), significantly statistically different from the values of the same determinations in patients without this diagnosis (21.4% and 19.7%, respectively) (fig. 1).

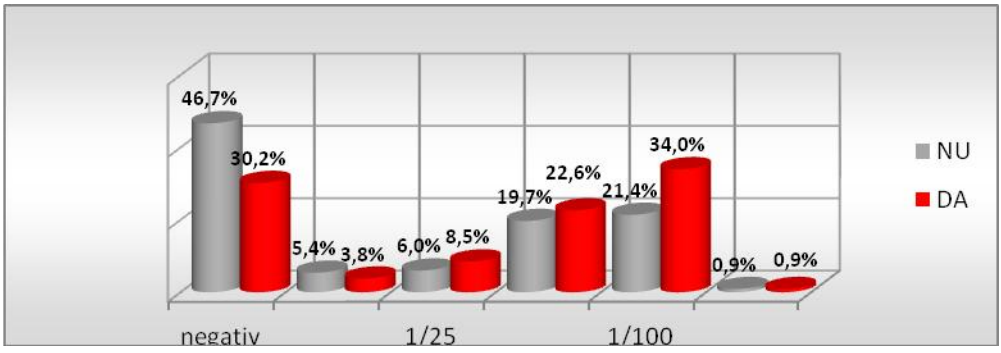

Fig. 1. *T.canis* IgG titer in the patients with laterocervical adenopathy

The sensitivity and specificity for the diagnosis of toxocariasis of the performed biochemical tests was calculated in view of identifying the tests that could lead us to this diagnosis. We determined the ROC curve and interpreted the results. For being useful in making a diagnosis of toxocariasis, the area under the corresponding curve should be greater than 0.5, ie this curve to be placed above the reference line (gray-shaded in our graph). There are tests that can actually calculate with some significance the difference from the 0.5 value of this surface (fig. 2).

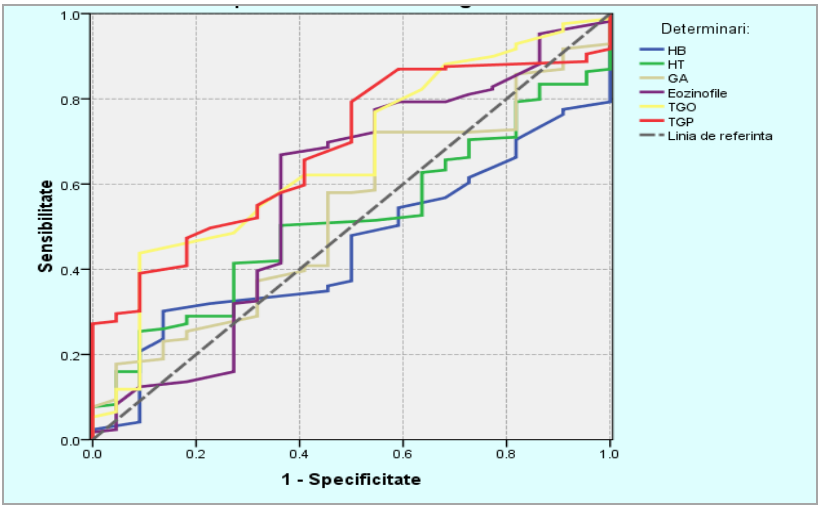

Fig. 2. ROC curve for the identification of the diagnosis of Toxocariasis

Then we calculated the area under the curve and tested the difference between this and the 0.5 threshold value. By analyzing the sensitivity and specificity for the diagnosis of toxocariasis of the performed blood tests and ROC curve calculations, we found out that the only important determinations were SGOT and SGPT, covering an area under the curve of 0.661 and 0.681, respectively, significantly greater than the 0.5 limit surface (tab. I).

Likelihood ratio tests were used to analyze the effect of each independent variable on the system; basically, we checked if each of the performed blood tests had a role in making the diagnosis.

TABLE I
Sensitivity and specificity of biological parameters

Determination	Surface	Standard error	Signif. asimpt. p	Asymptotic confidence interval 95%	
				Lower limit	Upper limit
HB	**.452**	**.054**	**.463**	**.347**	**.557**
HT	**.513**	**.055**	**.841**	**.406**	**.620**
GA	**.534**	**.062**	**.601**	**.414**	**.655**
Eosinophils	**.581**	**.072**	**.216**	**.440**	**.722**
SGOT	**.661**	**.059**	**.014**	**.545**	**.778**
SGPT	**.681**	**.052**	**.006**	**.580**	**.783**

Tests with a significance level less than 0.05 have influence on the model – this being the case of SGPT test - which has also been identified as important for determining the presence of Toxocara diagnosis by ROC curve method. Other determinations seem to have no influence on the model.

CONCLUSIONS

The clinical signs and symptoms found statistically significant can be a starting point for making a diagnosis of toxocariasis. Nonspecific laboratory findings are hyperleukocytosis, hyper-eosinophilia, and low hematocrit level, and elevated transaminase levels have high sensitivity and specificity. Accurate diagnosis is established by determining *T. canis* specific IgG and detection of *Toxocara* larvae in biopsy tissues.

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INTERACTIONS BETWEEN THE OXIDATIVE AND NITROSATIVE STRESS IN NOCICEPTIVE PROCESSING IN RAT

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THE INTERACTION BETWEEN OXIDATIVE AND NITROSATIVE STRESS IN NOCICEPTIVE PROCESSING IN RAT (Abstract): During the last years, special emphasis was directed towards the relation between the molecular and cellular alterations produced by free oxygen radicals and the normal and pathologic implications of nitric oxide. Moreover, lately it is believed that a true radical cascade might exist between the reactive species of oxygen and nitrogen during stress of various causes. In this way, their succession would produce a temporal prolongation of the complex cellular alterations produced by stressing aggressions. In the present paper, we were interested in presented some selective aspects regarding the interactions between oxidative and nitrosative stress in the modulation of the nociceptive behavior in rats. We report here a significant correlation between the results of the hot-plate latency time and the values of some oxidative and nitrosative stress markers. **Key words:** OXIDATIVE STRESS, NITROSATIVE STRESS, NOCICEPTION

Researches carried on in the last two decades with more and more advanced technical means, starting from the level of the entire organism continuing to a cellular and molecular level, have established that during the exaggerated oxido-reductive energogenic reactions there are made up variable quantities of free radicals of oxygen, generating multiple biochemical alterations, generically called oxidative stress (1).

In normal conditions of rest, the molecular oxygen, as a strongly oxidant element which is indispensable to the cellular energetic metabolism, suffers processes or bivalent reduction by the capture of two electrons with the participations of the mitochondrial respiratory chain, represented by the system cytochromes - cytochrome oxidase. Only small quantities (of 2-3%) of the molecular O_2 reaching the cellular level "escapes" from the bivalent mitochondrial oxidative-reductive reactions and take part to the process of univalent partial reduction by a single electron for making up the superoxide anion ($O_2\bullet$), as a precursor of the radical reactions producing hydrogen peroxide (H_2O_2 – oxygenated water), hydroxyl radicals ($HO\bullet$) and singlet oxygen ($1O_2$).

The intensity of the univalent reduction making new reactive species of O_2 depends both on the activation of pro-oxidant enzymes of the type of NADPH-oxidase, xanthine oxidase and myeloperoxidase, and on the participation of the means of endogenous anti-radical counteraction and defense, represented by the antioxidant enzymes – superoxide dismutase (SOD), catalase and glutathione-peroxidase (GPX) (2, 3).

Being much more reactive than the molecular O_2 reduced on bivalent way, the radical species of the oxygen determine the holding or the dislocation of some molecular components of lipids, proteins and cellular nucleoproteins (DNA, RNA), producing multiple tissue injuries, mainly cardiovascular and cerebral ones (4-6).

Disequilibria created by the oxidative stress by the predominance of the radical species of O_2 on the activity of antioxidant enzymes contribute on functional plan both to the modulation of some physiological reactions and to the genesis and evolution of different pathologic conditions.

One of these is represented by pain. Pain as complex psycho-sensorial cerebral-spinal phenomenon, having as a basis the integration and modulation of nociceptive information existent both at the level of the posterior cusp of the spinal marrow and at the level of the spinal-thalamic-cortical supraiacent structures, is one of the most common cause of human suffering generating stress. Concomitantly with the specific sensorial, perceptual, affective and behavioral manifestations, pain releases non-specific neuro-endocrino-metabolic disequilibria, similar to the other types of stressing aggressions inducing the oxidative and nitrosative stress (7).

Generally, the variable reactivity to pain and stress is based both on the multiple individual features of the cerebral chemical mediators, of the type of opioid neuropetides (endorphins, enkephalins, dinorphins), and on the genic substratum at the level of the cortical-subcortical polyneural matrix, recently described under the denomination of „Body-self neuro-matrix" (8).

Depending on the genetic substratum and on the previous experience, the central cortical-subcortical structures evaluate and release individual reactions of different intensities and durations, depending on the constitutional type, on the features of stressing aggression and on the capacity of adaptation of the subject.

Furthermore, recently, more and more studies remind of the implications of the oxidative and nitrosative stress in the nociceptive processes. As a matter of fact, in the last years, a special attention is given to the relations between the cellular and molecular alterations produced by the free radicals of O_2 and the normal or pathological implications of the nitric oxide (NO). This results from the oxidation of the terminal azoth of the amino-acid L-arginin on the shunted course of the cycle of urea, under the influence of one of the three isoenzymes of nitroxide sintetase (constitutive NOS calcium dependent), which is present in almost all the tissues and organs (9).

On a functional plan, it was proved that NO, as the free radicals of O_2, possesses dual biological properties, depending on concentration and on the individual reactivity. In small physiological concentrations, NO contributes to fix the local vasodilatation, the cardiovascular and cerebral circulation, to platelet aggregation and to antiproliferative and protective action against bacterial and viral infections. The multiple biological properties of NO are achieved both by the mechanism of activating the

guanilatcyclase forming the cyclical GMP, as an intracellular main secondary messenger, and by nitrosation of proteins forming oxidant nitrosotiols of some amino-acids (cysteine, methionine, glutathione, tyrosine) (10).

In this context, in the present study we have intended to describe the possible relations that establish between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes. These aspects shall be proved by the use of a model of induced pain and by the administration of angiotensin II, of which was initially proved to detain a hyperalgesic role, as well as the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and the determination of correlations that occur between these markers and the indices of the painful perception.

MATERIAL AND METHODS

Experiments were carried on lots of male *Wistar* rats, with an average weight of 230-250 g, kept in normal laboratory conditions (constant temperature of 21˚C ± 2˚C, food and water *ad libitum*, alternating lighting regime – 12 hours light – 12 ore dark). The experimental techniques that were used have been in compliance with the international regulations regarding the work on laboratory animals.

Techniques of Neurosurgery

For the purpose of the stereotaxic intervention, *Wistar* rats were anesthetized, by intraperitoneal injection (i.p.) with a solution of Nembutal (45 mg/ kg b.w.) (Sigma). The heads of animals were fixed in the stereotaxic device so that the plan that passes through the auditory channels and the back of incisors to form an angle of 11°. Angiotensin II has been administered (3 consecutive days, 0.1µg/kg b.w.) by a plastic cannula (Portex, with the diameter of 0.44), stereotaxically implanted in the left ventricle, with the following coordinates: 0.5 mm posterior to the bregma; 1.3 mm laterally to the median line; 4.3 mm ventrally of the surface of the cortex.

The false-operated animals have been injected with physiological serum, the behavioral testing starting 7 days after the treatment.

Hot-plate test

The investigation of painful sensitivity was achieved by means of the hot-plate test. For this test, rats were placed in a closed cylindrical space, provided with an electrically hot plate and maintained constant at the temperature of 55ºC with the aid of an ultrathermostat. The hot plate produces two behaviors and their times of reaction can be measured: paw licking and the bounce.

Stress of contention

Stress of contention consists in the immobilization of the animal for 30 minutes. In this manner, it was considered that the stress of contention is one of the most repeatable experimental protocols, with the possibility of minimizing the implied variables and which in the same time presents enough similarities with the other types of nociceptive stress.

Determination of the markers of the oxidative and nitrosative stress was carried on as described above (6, 7). As the **statistical analysis of data is concerned**, it was used ANOVA one-way (the values of $p \leq 0.05$ have been considered significant) for the results of the behavioral tests and the Pearson correlations in order to establish the relations between the markers of the oxidative/nitrosative stress and the

behavioral indices.

RESULTS

As mentioned above, the administration of angiotensin II has determined a significant decrease (F (1.8)=13, p=0.006) of the time of latency for the hot-plate test, related to the witness lot (fig. 1), fact that suggests the nociceptive effects of angiotensin II.

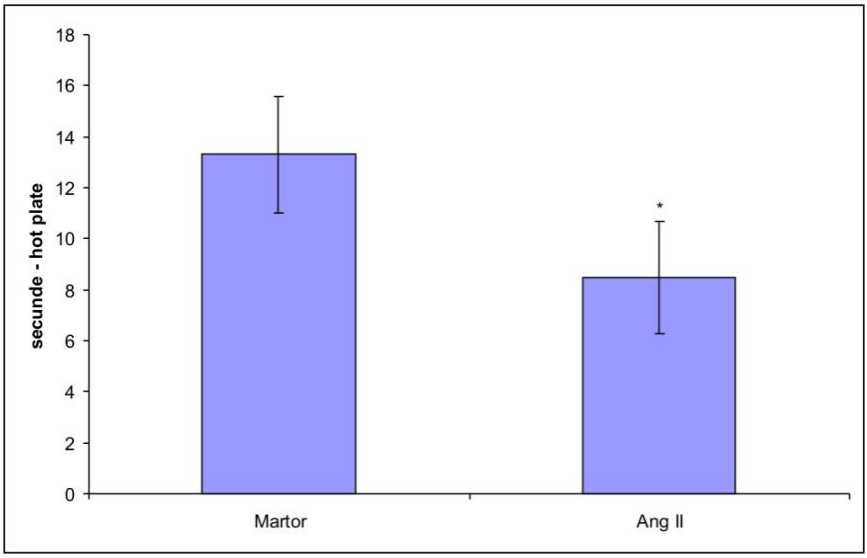

Fig. 1. The effects of administrating Ang. II on the time of latency of hot-plate.
The results are expressed by average ± E.S.M. (n=5).
*p=0.006 compared to the control.

Furthermore, we have noticed a significant correlation between these effects and the levels of some different markers of the oxidative stress that were previously determined (11). In this way, as the relation between the time of latency of hot-plate vs. the specific activity of superoxide dismutase (SOD) are concerned, the Pearson correlations have shown an r = - 0.798 and a p of 0.006, for n= 10 (fig. 2). In the same manner, in the case of the second determined antioxidant enzyme have been noticed significant correlations with the time of latency in the hot-plate (n=10, r = - 0.541, p=0.045) (fig. 3). Moreover, we have obtained significant correlations between the level of lipidic peroxidation (shown by the concentrations of MDA) vs. the time of latency (n=10, r=0.857, p=0.002) (fig. 4).

As the level of markers of the nitrosative stress is concerned, it was opted for the determination of the nitrite anion (NO_2^-), considering that this is the main mediator in the reaction of nitrosylation of the tyrosine amino-acids in the structure of different proteins, this reaction representing the foundation of the phenomenon of nitrosative stress. In this case was also noticed an important correlation with the behavioral processes, considering the decreased level of NO_2^- after those 30 minutes of stress of contention (F(1,8)=60, p<0.0001), compared to the witnesses (fig. 5).

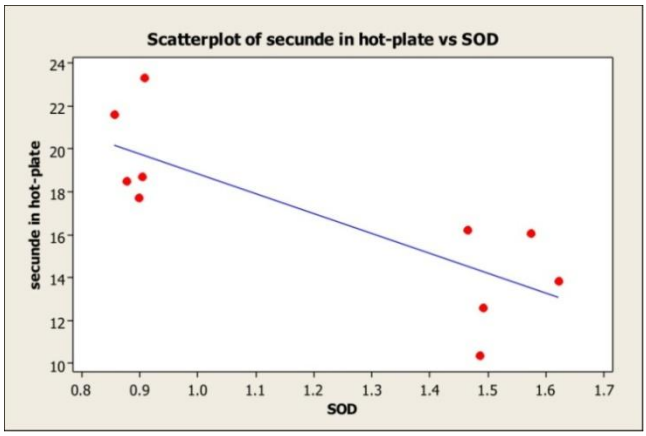

Fig. 2. The degree of correlation between the time of latency in the hot-plate and SOD.

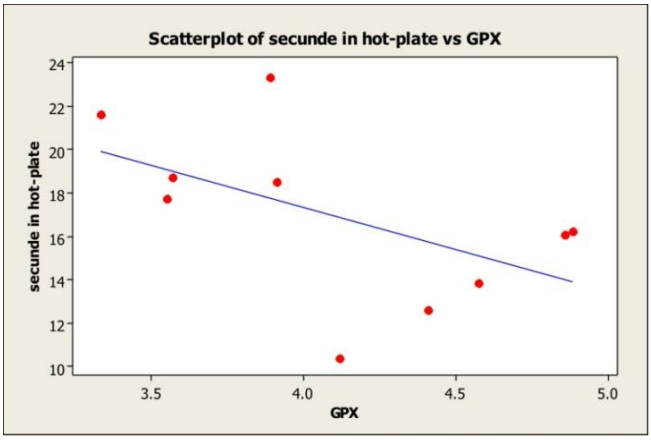

Fig. 3. The degree of correlation between the time of latency in the hot-plate and GPX.

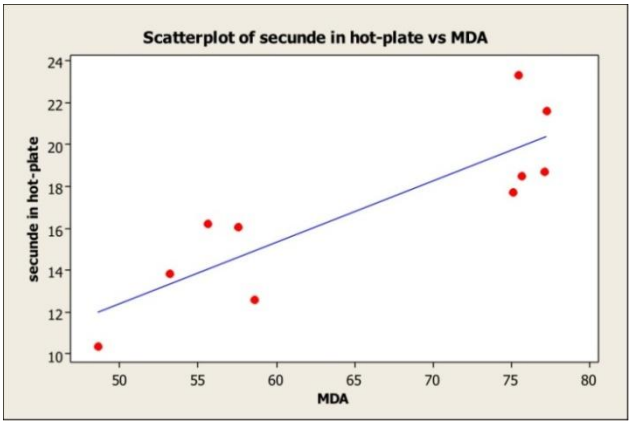

Fig. 4. The degree of correlation between the time of latency in the hot-plate and MDA.

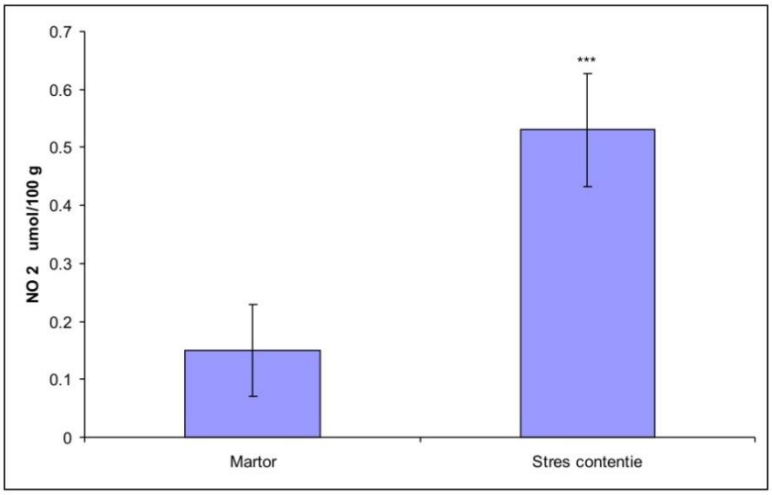

Fig. 5. The effects of the contention stress on the levels of NO_2^-. Results are expressed by average ± E.S.M. (n=5). *** $p < 0.0001$ compared to the control.

DISCUSSION

In the present study we have confirmed the existence of some relations between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes, by the use of a model of pain induced by the administration of angiotensin II, as well as by the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and proving the correlations that occur between these markers and the indices of the painful perception.

In this manner, previous studies have proved the involvement of angiotensin II in the modulation of the nociceptive perception. Therefore, Kaneko and collaborators have proved that the intracerebroventricular administration of angiotensin II has led to the diminishment of the analgesia induced by the initial administration of morphine (12). Also, it was proved that the administration of some inhibitors of the conversion enzyme, as well as of some antagonists specific for angiotensin II lead to the diminishment of nociceptive responses in specific tests such as the hot-plate test (13). Similarly, in the case of the present paper we have proved that the administration of angiotensin II determines a significant decrease of the time of latency for the hot-plate test, compared to the witness lot, fact that suggests its nociceptive effects.

Moreover, in the present article we have reported a direct correlation between the above-mentioned effects in the behavioral tests of pain (hot-plate) and the markers of the oxidative stress, represented by two enzymes with antioxidant effect (SOD and GPX), as well as of a marker of the processes of lipidic peroxidation (MDA).

Also, there was presented a possible relation between an important marker of the stress (NO_2^-) and the stress of contention (it was considered that the stress of contention is one of the most repeatable experimental protocols, with the possibility of minimizing the implied variables and which in the same time presents enough similarities with the other types of nociceptive stress). Further-

more, nitrite anions are the main mediators in the reaction of nytrosilation of the tyrosine amino-acids in the structure of different proteins. This reaction represents the basics of the phenomenon of nitrosative stress and as a result, the variations of concentration of these radicals are direct witnesses of the nitrosative stress. Therefore, from a statistical point of view, there was a significant increase of the quantity of nitrite anions, fact that is an incontestable proof in the favor of the occurrence of the nitrosative stress at the level of the nervous system of animals subjected to the stress of contention.

Therefore, the nociceptive stress produces modifications at the level of the central nervous system, of oxidative and nitrosative type, modifications that can variably affect the capacity of transmission and perception of nociceptive signals by the animal of experience. Moreover, these data seem to suggest the fact that the intraneural and/or extraneural effects of modification of proteins involved in the phenomena of transduction of the painful signal are truly due to the effects of the presence of mediators of the nitrosative stress, which are in increased quantities.

As a matter of fact, it is considered that a real radical cascade would produce between the reactive species of the oxygen, azoth and newly of the carbon during the stress having different causes (14, 15). Their succession would realize amplification and implicitly the prolongation in time of the complex cellular disorders produced by the stressing aggressions, such as those related to nociception. Also, on a pharmacotherapeutic plan there occur differences between the three categories of radical species involved in stress: while the oxidative component of the stress benefits of antioxidant therapy based on the high consumption of vitamins C, A and E, beta-carotene, flavonoids, selenium, polyphenols, anthocians and procyanidins, fighting the stress caused by pain situates to the fore the adequate analgesic, anxiolitic and hormonal medication with glucocorticoids or non-steroidal inhibitors of the cyclo-oxigenases of the type of indomethacin, diclophenac, ketoprophen or celebrex in the case of the inflammatory pain (16-18). Moreover, new therapeutic opportunities open the clinical-experimental researches referring to the neurosedative and anti-stressing properties of melatonin or inhibitors of receptors of the hypothalamic CRF (Corticotropin Releasing Factor) and the rennin-angiotensin cerebral system (19, 20), discussed in the present paper.

Although these studies show the involvement of the free radicals in different nociceptive mechanisms, much more detailed studies are necessary for the independent replication of these results and the use of the proper antioxidants as a treatment, having in regard the complexity of the biochemical systems and numerous interactions between the antioxidant and the prooxidant molecules from the biological processes.

CONCLUSIONS

In this study we have confirmed the existence of some relations between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes, by the use of a model of pain induced by the administration of angiotensin II, as well as by the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and proving the correlations that occur between these markers and the indices of the painful per-

ception. These aspects could have a special importance in the therapy of different mechanisms of nociception.

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CONTRIBUȚII LA STUDIUL METODOLOGIEI DE EVALUARE FUNCȚIONALĂ A ARTICULAȚIEI TEMPORO-MANDIBULARE ÎN SINDROMUL ALGO-DISFUNCȚIONAL

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CONTRIBUTIONS TO THE METHODOLOGY OF STUDY IN THE FUNCTIONAL ASSESSMENT OF TEMPOROMANDIBULAR JOINT DYSFUNCTIONAL SYNDROME (Abstract): **Aim:** Dysfunctional syndrome of the temporomandibular joint through the complexity and variability of the symptoms may result in significant structural and functional changes leading from discomfort to disability and a negative impact on quality of life. **Materials and methods:** A prospective study was conducted between October 2009 - December 2010, in 52 subjects diagnosed with rheumatoid arthritis and complaining of symptoms in the temporomandibular joint. The functional examination of the joint and muscle has included the assessment of the active range of motion, passive range of motion, resistance range of motions of the temporomandibular joint, dermatomes and the pain in the region of masseter, temporalis, median and lateral pterigoids, digastric, sternocleidomastoid and longus colli muscles. The ultrasound examination has included clinical aspects of alignment, shape, dynamics of the mandibular condyle, of the temporal fossa, aspects regarding the temporal and masseter muscles at rest and at contraction phases. **Results**: The clinical examination highlighted the presence of pain at temporomandibular jointlevel for all tested pacients. The majority complained the pain from moderate to severe level. For the majorityof cases(59,60%) the pain stars spontaneously, during the day time (80,80%) and the night time(50,60%). Over 50% from the research cases complained cracks at the temporomandibular joint level released by a movement. At the oral and dental examination of the research group 55,8% from the tested pacients presented dental cavities toothless partially or totally duet o additionally overworking temporomandibular joint. The limitation of the range of motion is not the most important detected dysfunction, affecting approximately 30% of the cases that have been studied. Pain and spasm in a very large proportion are present in the masseter and temporal muscles regions. Atrophy is present in a lesser extent. The dysfunctional index shows an involvement of the temporomandibular joint, for over 45% of patients tested. The ultrasound examination revealed degenerative inflammatory lesions at a rate of 38.46%, inflammatory lesions at a rate of 28.85% and mixed lesions at a rate of 9.62%.**Conclusions**: The research regarding the clinical paraclinical and functional aspects of temporomandibular joint, permited to highlight the fact that rheumatic inflamatory and degenerative, pathology, characterises it self through a high index of morbidity and disability that lowers the life quality. Establishing a treatment plan involves customizing all clinical, functional and laboratory data.
Key words: TEMPOROMANDIBULAR JOINT, DYSFUNCTIONAL SYNDROME, FUNCTIONAL ASSESSMENT

Mişcarea reprezintă principala funcție pe care o realizează omul şi datorită căreia acesta beneficiază de independență şi se integrează în mediul înconjurător. Articulația temporo-mandibulară (ATM) este o articulație complexă, specială sub raport morfologic şi funcțional (1). Problematica controversată generată de sindromul algodisfuncțional al ATM reține interesul practicienilor şi cercetătorilor din mai multe domenii.

Terapia prin mişcare, utilizând procedeele non-invazive poate ameliora simptomatologia din sindromul algodisfuncțional al articulației temporo-mandibulare modificând viziunea pacienților în legătură cu afecțiunea de care suferă.

OBIECTIVE

Cercetarea noastră are ca obiectiv evidențierea incidenței sindromului algodisfuncțional în rândul populației cu boli reumatismale prin evaluări obiective clinice, funcționale şi paraclinice.

Scopul principal este de a sensibiliza personalul medical şi a conştientiza pacienții de resursele şi importanta tratamentului kinetic în combaterea durerii şi menținerea unei funcționalități favorabile la nivel cranio-facial.

MATERIAL ŞI METODE

Studiul prospectiv realizat în perioada octombrie 2009 - decembrie 2010 a inclus un lot de 52 de pacienți şu afectări reumatice inflamatorii şi degenerative

Informațiile au fost obținute pe baza unui chestionar cu referire la durere, zgomote articulare temporo-mandibulare, dificultăți şi rigiditate la mişcarea mandibulei, obiceiuri vicioase, antecedente şi tratamente efectuate.

De asemenea, bolnavii au fost evaluați pe baza unei fişe ce acuprins: datele anamnestice generale; evaluarea clinică realizată in colaborare cu medicul curant; examinarea funcțională articulară (testarea mişcărilor active de deschidere şi închidere a gurii, lateralitate, protuzie, retruzie menționându-se amplitudinea de mişcare, existența deviației, restricției sau durerii in realizarea lor, mişcările pasive (*end-feel*-ul), mişcările cu rezistență, mişcările de joc articular la nivel temporo-mandibular pentru parametrii menționați anterior); examinarea musculaturii mobilizatoare a mandibulei şi a celei cervicale realizată prin palpare digitală, pentru determinarea contracturii musculare şi a durerii la nivelul muşchilor maseter, temporal, pterigoid intern şi extern (fig. 1), digastric, sterno-cleido-mastoidian şi lungul gâtului (2); examinarea paraclinică ecografică a urmărit aspecte legate de alinia-

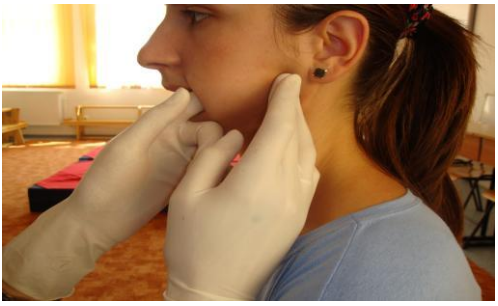

Fig. 1. Testarea muşchiului pterigoid extern

ment, formă, dinamică a condilului mandibulei, aspecte ale formei fosei temporale, ale spațiului intrarticular în repaus şi dinamică (fig. 2) (1,2), aspecte ale meniscului legate de starea de repaus şi contracție ale muşchilor temporal şi maseter; examinarea s-a realizat cu o sondă de 3,5MHz, cu pacientul în decubit dorsal cu transductorul plasat paralele cu lungul ax al mandibulei.

Pe baza parametrilor obținuți la testarea clinică generală, testarea articulară şi testa-

rea musculară am evaluat disfuncţia la nivelul ATM prin intermediul unui indice cu scorul de afectare maximă 30.

Fig.2. Ecografie ATM calcificare meniscală

REZULTATE ŞI DISCUŢII

Distribuţia pe sex a pacienţilor incluşi în studiu a evidenţiat existenţa unor date similare cu cele publicate de alţi autori (5, 6).

Deşi a predominat afectarea sexului feminin, s-a remarcat totuşi o incidenţă moderat crescută la pacienţii de sex masculin cu boală artrozică în comparaţie cu cei cu afectare reumatoidă (fig.3).

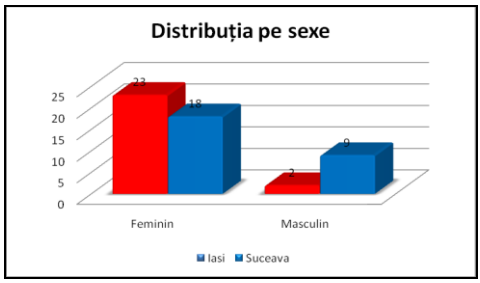

Fig. 3. Distribuţia cazurilor pe sex

În ceea ce priveşte durata de evoluţie a bolii reumatismale de bază, s-a constat prezenţa atât a bolnavilor cu debut recent cât şi a celor cu un istoric de peste 21 ani. Afectarea articulaţiei temporo-mandibulare a dovedit a avea o durată mai scurtă, aspect vizualizat şi de media de vârstă a bolii de bază comparativ cu cea a afectării temporo-mandibulare (tab. I).

În urma analizei corelaţiei dintre vârsta pacienţilor şi durata afecţiunii lor de bază s-a constatat o relaţie cu intensitatea medie, cu semnificaţie statistică, coeficienţii Pearson şi Spearman prezentând valoare de 0,605.

TABELUL I

Durata de evoluţie a bolii de bază şi temporo-mandibulare

Durata Afectării	Nr.	Media de vârstă	Min	Max	Deviaţia Standard	Semnificaţia Mediei
Vârstă(ani)	52	56,36ani	27	79	12,831	1,546
Boala bază	52	9,74 ani	0,5	21	5,464	1,051
ATM	52	3,58 ani	2	13	2,474	0,476

În ce priveşte relaţia dintre vârsta pacienţilor şi durata afectării secundare s-a evidenţiat că intensitatea acestei legături scade din punct de vedere statistic, cei 2 indicatori de corelaţie calculaţi au de această dată valori mult mai mici ceea ce indică o semnificaţie slabă (0,306 respectiv 0,486).

Explicaţia este legată de faptul că afectarea temporo-mandibulară poate fi sau nu prezentă în cadrul bolii de bază şi poate apare în orice perioadă evolutivă a acesteia.

Examenul clinic a evidenţiat existenţa durerii la nivel temporo-mandibular la toţi pacienţii testaţi. Majoritatea au prezentat o intensitate moderată sau severă, fiecare

categorie având valori de 42,30% (tab. II).

TABELUL II
Intensitatea durerii – Scala analogă vizuală-VAS

Intensitate (VAS)	Nr	%(x)
Fără durere (0-1)	0	0
Durere uşoară (2-3)	6	11,53
Durere moderată (4-5)	22	42,30
Durere severă (6-7-8)	22	42,30
Durere insuportabilă (9-10)	2	3.87

(x) Pentru valori mai mici de 10 proporţiile sunt orientative

La pacienţii luaţi în studiu durerea a fost simţită în diferite circumstanţe: la palparea conductului auditiv extern sau a pretragusului, în timpul masticaţiei, la mobilizarea mandibulei sau la palparea muşchilor masticatori.

În majoritatea cazurilor (59,60%) durerea apare spontan, diurn (80,80%) şi nocturn(50,60%).

La pacienţii cu artroză durerea a fost semnalată mai mult provocată şi diurn, iar la cei cu manifestări de tip inflamator aceasta a fost de obicei spontană şi cu intensificare nocturnă. Este important de evaluat şi diagnosticul bolii de bază deoarece determină caracterul durerii şi prognosticul afecţiunii.

Peste 50% din cazurile noastre au prezentat zgomote la nivelul articulaţiei temporo-mandibulare declanşate de angajarea unei mişcări; fiind considerate semne majore ale disfuncţiei temporo-mandibulare posibil a exprima o situaţie anormală indusă de leziunile articulare. Cu cât stadiul bolii de bază este mai avansat cu atât prezenţa zgomotelor intrarticulare este mai frecventă.

La evaluarea orală şi dentară a lotului de studiu s-a dovedit a fi manifestări importante ce pot determina în timp, disfuncţionalităţi la nivelul articulaţiei temporo-mandibulare.

În cazul nostru 55,8% din pacienţii evaluaţi au prezentat carii dentare şi 38,5% edentaţii parţiale sau totale, afecţiuni care solicită suplimentar articulaţia temporo-mandibulară.

Cercetările noastre au constatat că amplitudinea de mişcare la nivel temporo-mandibular era deteriorată în proporţie variabilă în funcţie de tipul mişcării: 15,38% pentru deschiderea gurii, 29,9% de lateralitate, 21,15% de protuzie, 1,92% de retruzie. Limitarea mişcării a înregistrat valori totale de sub 30%.

Testarea musculară s-a realizat pentru muşchii maseter, temporal, pterigoid intern, pterigoid extern, digastric, sternocleidomastoidian sau lungul gâtului şi a urmărit existenţa durerii, spasmului şi atrofiei. Durerea într-o proporţie foarte mare este prezentă la nivelul muşchilor maseter (84,61%) şi temporal (65,38%), (principalii muşchi masticatori), iar într-o proporţie moderată la ceilalţi muşchi testaţi.

Spasmul este însoţit sau generat de durere a fost prezent într-o proporţie de 51,91% la nivelul maseterului, 26,92% a temporalului, 13,46% a pterigoidului intern şi extern şi 17,30% la nivelul sterno-cleido-mastoidianului.

Cu ajutorul valorilor acordate fiecărei disfuncţionalităţi s-a estimat evoluţia indicelui ATM astfel a rezultat o medie de 13,56 ceea ce denotă o afectare temporo-mandibulară de peste 45% la bolnavii aflaţi in studiu.

Examinarea ecografică a permis depistarea leziunilor la nivelul articulaţiei temporo-mandibulare contribuind astfel la confirmarea diagnosticului clinic (fig. 4).

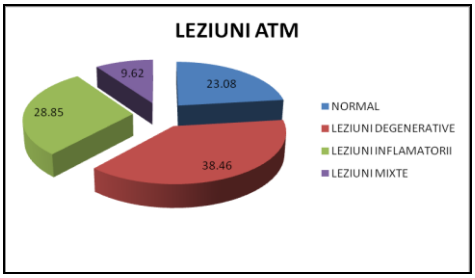

Fig. 4. Ponderea leziunilor ATM depistate ecografic

La evaluarea paraclinică ecografică s-au depistat leziuni degenerative în proporţie de 38,46% (demineralizări ale condilului şi ale fosei, osteofite, eroziuni marginale, diminuarea spaţiului articular); leziuni de tip inflamator în proporţie de 28,85% (contur dinţat şters al condilului mandibular şi fosei temporale, osteoporoză, geode, lichid sinovial, calcificări meniscale, mobilitate limitată a condilului) şi leziuni mixte (9,62%).

În cazul a 23,08% pacienţi au lipsit leziuni ecografice evidenţiate la nivelul articulaţiei temporo-mandibulare.

Investigaţia ecografică a muşchilor maseter şi temporal în repaus şi în contracţie determinarea grosimii muşchiului în cele două faze (tab. III).

TABELUL III

Evaluarea ecografică la nivel muscular

Niveluri	Muşchi							
	Temporal				Maseter			
	Repaus		Contracţie		Repaus		Contracţie	
	D	S	D	S	D	S	D	S
Media	6,53	6,50	8,03	8,02	8,57	8,61	10,44	10,50
Max	8,5	7,9	10	9,3	10,2	11	13	13
Min	4,9	5,4	6	6,3	6	6,4	7,3	8
DS	0,724	0,584	0,810	0,641	0,783	0,787	0,940	0,793
Correl	0,719		0,721		0,909		0,859	

În urma analizei statistice a corelaţiei dintre valorile musculare din parte dreaptă şi stângă se poate concluziona că există o legătură de intensitatea mare din punct de vedere statistic, coeficientul obţinut pentru muşchiul temporal-în repaus este de 0,719; coeficientul obţinut pentru muşchiul temporal-în contracţie este 0,721; coeficientul obţinut pentru muşchiul maseter-în repaus 0,909; coeficientul obţinut pentru muşchiul master în contracţie 0,859 (fig. 5).

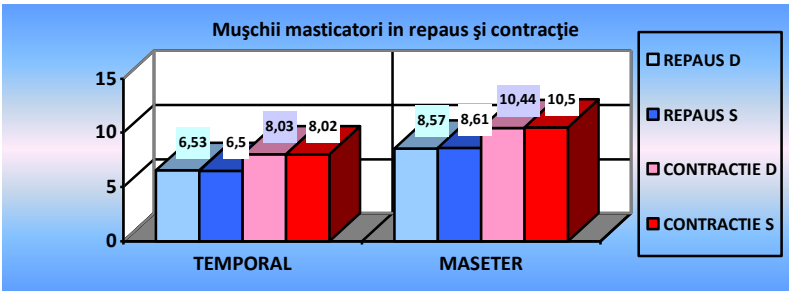

Fig. 5. Dinamica mediei în repaus şi contracţie pentru muşchii maseter şi temporal

DISCUŢII

Articulaţia temporo-mandibulară are structură particulară şi funcţii generate de sistemul cranio-facial a cărui subsegment este activitatea sa, în condiţii de articulaţie cu o parte fixă şi una mobilă, de joc funcţional concomitent bilateral şi încărcare, crează o biomecanică specială; articulaţia va înregistra mecanismele patologice ale bolilor reumatismale sistemice şi totodată va avea o patologie condiţionată de cauze locale (7).

ATM este frecvent implicată în determinarea durerii oro-faciale recunoscută pentru indicatorul de morbiditate şi dizabilitate (2). În cadrul patologiei sale, în ultima perioadă s-a particularizat sindromul algo-disfunctional la originea căruia s-au grupat trei elemente: ATM, musculatura şi structurile adiacente (6).

În practica medicală sindromul algic la nivelul ATM este recunoscut pentru impactul clinic şi epidemiologic crescut, pentru caracterul dizabilitant şi necesitatea unei abordări multidisciplinare ceea ce presupune stabilirea unui algoritm diagnostic şi terapeutic.

Studiul de faţa sensibilizează asupra acestui aspect analizând un lot de bolnavi cu boală reumatismală inflamatorie şi degenerativă şi afectare de tip sindrom algo-disfuncţional. Pentru a evalua din punct de vedre clinic şi funcţional ATM s-au utilizat o serie de parametri fizici, articulari şi musculari care au evidenţiat participarea în special a componentei musculare în generarea manifestărilor clinice şi disfuncţionale ale sindromului.

Utilizarea ecografiei în diagnosticul suferinţei (standardizată pe cuantificarea elementelor morfologice şi de dinamică a condilului) subliniază, o dată în plus, aportul diagnostic al metodei în suferinţele aparatului locomotor.

Evidenţierea indicelui de disfuncţie ATM prin măsurarea unor parametri fizici, articulari, musculari şi ecografici cu valoare medie de 13,56 exprimând o deterioare mai mare de 45% a structurilor zonale este în acord cu datele *The European Leaque Against Rheumatism* (EULAR) şi *American College of Rheumatology* (ACR) îl promovează pentru bolile aparatului locomotor, de determinare obiectivă a deficitelor.

CONCLUZII

Cercetările noastre multidisciplinare privind aspectele clinice, paraclinice şi funcţionale a articulaţiei temporo-mandibulare au permis a se evidenţia că patologia reumatică inflamatorie şi degenerativă se caracterizează prin indici de morbiditate şi dizabilitate crescuţi, ceea ce degradează calitatea vieţii.

Afectarea articulaţiei temporo-mandibulare la pacienţii cu reumatism inflamator şi degenerativ amplifică suferinţa clinică şi dizabilitatea bolnavului.

Examenul funcţional a evidenţiat limitarea amplitudinii pe anumite direcţii de mişcare şi afectarea grupurilor musculare adiacente articulaţiei temporo-mandibulare.

Investigarea paraclinică a oferit argumente care demonstrează deteriorarea morfologică şi funcţională a ATM, examenul ecografic dinamic având o putere de diagnostic mare oferind condiţii optime de evaluare a cost - eficienţei unei terapeutici optime, efectuate concomitent cu investigaţiile funcţionale şi paraclinice.

Programul terapeutic personalizat presupune analizarea datelor referitoare la aspectele clinice, funcţionale şi paraclinice precum şi sinteza tuturor indicilor clinico-biologici, funcţionali, socio-economici şi psiho-comportamentali ai pacientului.

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NOUTĂŢI

p53 MUTANT - ŢINTA TRATAMENTULUI ANTITUMORAL

Un nou studiu descrie un compus care distruge selectiv celulele canceroase prin restaurarea structurii şi funcţiei uneia dintre proteinele care suferă cel mai frecvent mutaţii în cancerul uman, supresorul tumoral p53. Gena care codifică p53 a suferit mutaţii în mai mult de jumătate din cazurile de cancer uman, şi pierderea funcţiei p53 a fost legată de multe aspecte, inclusiv de agresivitatea cancerului, metastazarea şi răspunsul slab la chimioterapie şi radiaţii. Restaurarea funcţiei p53 mutant cu un medicament reprezintă o strategie terapeutică atractivă în cancer, cu toate acestea, s-a dovedit a fi dificil să se găsească compuşi care restabilesc funcţia pierdută a unui supresor tumoral defectuos. Dr. Alexei Vazquez, a dezvoltat o metodă computerizată de screening pentru a identifica compuşi care au ca ţintă celulele tumorale cu mutaţii p53, dar nu şi celule p53 normale. Metoda de screening a implicat celulele canceroase, cu genotipuri diverse şi a identificat doi compuşi din familia tiosemicarbazonă care au ucis celulele canceroase care conţin p53 mutant prin restaurarea structurii şi funcţiei unei mutante p53-R175H. Aceste cercetări susţin tendinţa dezvoltării unor terapii antitumorale pe baza cunoaşterii genotipului tumorii pacientului. Xin Yu, Alexei Vazquez, Arnold J. Levine, Darren R. Carpizo. Allele Specific p53 Mutant Reactivation. *Cancer Cell*, 25 May 2012 DOI: 10.1016/j.ccr.2012.03.042.

Cătălina Luncă

CARACTERISTICI CLINICO-EPIDEMIOLOGICE ALE PACIENȚILOR CU CANCER GASTRIC ASISTAȚI ÎN SPITALUL MUNICIPAL DE URGENȚĂ MOINEŞTI ÎN PERIOADA 2000-2010

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CLINICAL EPIDEMIOLOGICAL CHARACTERISTICS OF PATIENTS WITH GASTRIC CANCER IN EMERGENCY MUNICIPAL HOSPITAL MOINESTI (Abstract): Cancer is now a major public health problem by the size of the human, medical, economical and social implications as well as in industrialized and also in developing countries. Gastric cancer is a neoplasia with a starting point in gastric mucosa, representing one of the most common malignant visceral locations, being on the second most common after lung neoplasm. **The aim** is to highlight the incidence of gastric cancer in Emergency Municipal Hospital in Moinesti in relation with certain factors (age, sex, area of origin, personal habits, personal and family pathological history) for a period of eleven years. **Material and methods:** The study has been conducted on a period of eleven years (2000-2010) and it included 77 patients with gastric cancer. The following parameters were evaluated: age, sex, area of origin, personal habits, personal and family pathological history. **Results:** Distribution by years of study revealed that most of the cases were in 2009 (19 patients). The incidence of gastric cancer was over two times higher at men than at women, the ratio men/women being 2,34 to 1. The most common cases (n=40) were reported in people over 61 years (the mean age was 60,32±11,87) and from rural areas (ratio rural/urban areas=1,5:1). Only 15 patients (19,5%) had in the personal antecedents stomach or duodenal ulcer and half of the cases were smokers (49,35%). **Conclusion:** The profile of patient with gastric cancer was contoured: male, over 60 years, from rural areas, smoker and with a personal history of stomach or duodenal ulcer. **Key words:** HEALTH PROBLEM, GASTRIC CANCER, INCIDENCE.

Cancerul gastric (CG) este o neoplazie cu punct de plecare în mucoasa gastrică, reprezentând una dintre cele mai frecvente localizări viscerale maligne, situându-se pe locul al doilea ca frecvență, după neoplasmul bronhopulmonar (1, 2).

Cu largă răspândire la nivel mondial, CG are o variabilitate importantă de la o țară la alta, atât ca morbiditate cât şi ca letalitate. În Europa, rata de incidență la bărbați este mai mare în est (29,6/100.000) şi în sud (18/100.00), față de femei în nord (5,9/100.000) şi în vest (6,6/100.000) (3-5). Grupul de vârstă cel mai afectat de CG este între 50-70 ani, cu o ascensiune în jur de 60 şi foarte rar sub 30 ani (6), iar frecvența

bolii la populația masculină este dublă în raport cu cea feminină (7).

Lucrarea își propune realizarea unui studiu epidemiologic, retrospectiv, descriptiv pentru evaluarea incidenței cazurilor de CG asistați în Spitalul Municipal de Urgență Moinești, pe o perioadă de unsprezece ani, în raport cu anumiți factori (vârstă, sex, mediu de proveniență, obiceiuri personale, antecedente patologice personale și familiale).

MATERIAL ŞI METODE

Studiul realizat în perioada 2000-2010 a inclus 77 de pacienți cu CG asistați în Spitalul Municipal de Urgență Moinești.

Lotul studiat a cuprins pacienții nou diagnosticați cu CG și internați în Spitalul Municipal de Urgență Moinești. Diagnosticul a fost susținut pe baza anamnezei, examenului clinic, investigațiilor de laborator și imagistice, aspectului morfologic intraoperator și examenului anatomopatologic postoperator.

Din lotul studiat au fost excluși pacienții diagnosticați și tratați în alte spitale și cei care, deși au prezentat la internare semne și simptome ce orientau diagnosticul către CG, acesta nu a putut fi confirmat prin endoscopie cu biopsie și examen anatomo-patologic.

Datele au fost obținute atât prin studiul documentelor medicale din Spitalul Municipal de Urgență Moinești cât și prin ancheta clinico-epidemiologică.

Informațiile acumulate au fost evaluate prin metode epidemiologice și statistice (metoda de analiză dispersională ANOVA și testul „t" privind egalitatea a două medii aritmetice – metoda Kaplan Meier).

REZULTATE

1. *Distribuția anuală* a pacienților cu CG asistați în Spitalul Municipal de Urgență Moinești, a evidențiat o distribuție relativ uniformă pe durata a 11 ani, cu două ascensiuni, în anul 2005 (11 cazuri) și în 2009 (19 cazuri) (fig.1).

2. *Evaluările pe sex* au stabilit că, în întreaga perioadă, din cele 77 cazuri de CG au fost înregistrate 70% la barbati, cu un raport M/F de 2,34/1 (tab.I, fig.2).

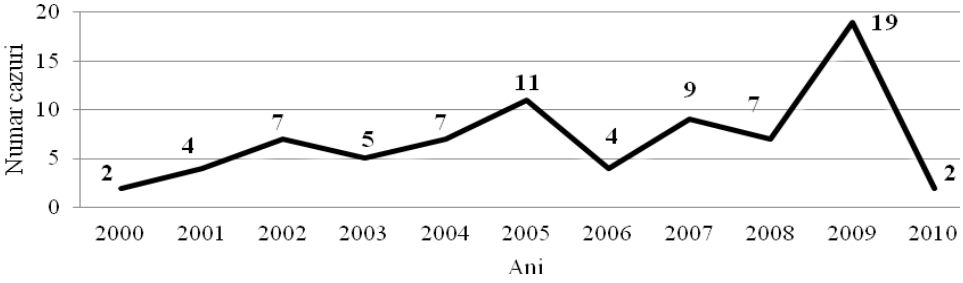

Fig. 1. Distribuția anuală a bolnavilor cu cancer gastric incluși în studiu, în perioada 2000-2010

TABELUL I

Distribuția pe sex a pacienților incluși în studiu

Sex	Nr.	%	Raport M/F
Masculin	54	70	2,34/1
Feminin	23	30	
TOTAL 77 100%			

Fig. 2. Distribuția pe sex a cazurilor de cancer gastric incluse in studiu

3. *Vârsta medie* a pacienților din lotul de studiu a fost de 60,32±11,87, variind de la 25 la 85 de ani. Extremele grupurilor de vârstă au fost cuprinse între 25 şi 85 de ani, iar grupul de vârstă 61-70 de ani a inclus cei mai multi pacienți:23 (30%) (tab. II, fig. 3).

4. *Distribuția pe medii de proveniență* a evidențiat valori de prevalență mai mari pentru pacienții proveniți din mediul rural (58,44%) (fig. 4), cu predominanța bărbaților (59,25%) (fig. 5).

TABELUL II

Distribuția pe grupuri de vârstă

Grupul de vârsta (ani)	Pacienți		Barbați		Femei (x)	
	Nr	%	Nr	%	Nr	%
<20	0	0,0	0	0,0	0	0,0
21-30	2	2,6	2	3,7	0	0,0
31-40	1	1,3	0	0,0	1	4,4
41-50	14	18,2	9	16,7	5	21,8
51-60	20	26,0	12	22,3	8	34,6
61-70	23	30,0	20	37,0	3	13,0
71-80	15	19,3	10	18,5	5	21,8
81-90	2	2,6	1	1,85	1	4,4

(x) Pentru valorile mai mici de 10, proporțiile sunt orientative.

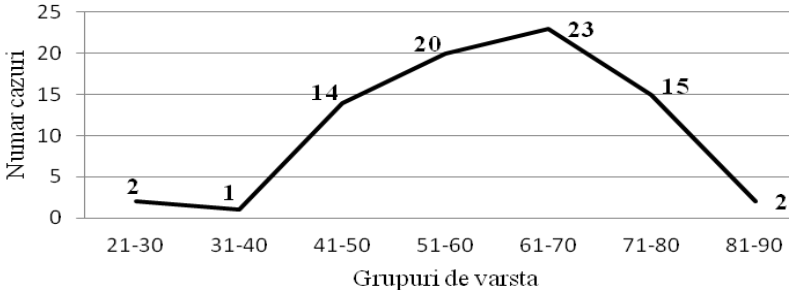

Fig. 3. Distribuția pe grupuri de vârstă a pacienților cu cancer gastric incluşi în studiu

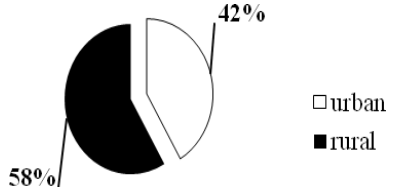

Fig. 4. Distribuția pe medii de proveniență a pacienților cu cancer gastric asistați în Spitalul Municipal de Urgență Moineşti

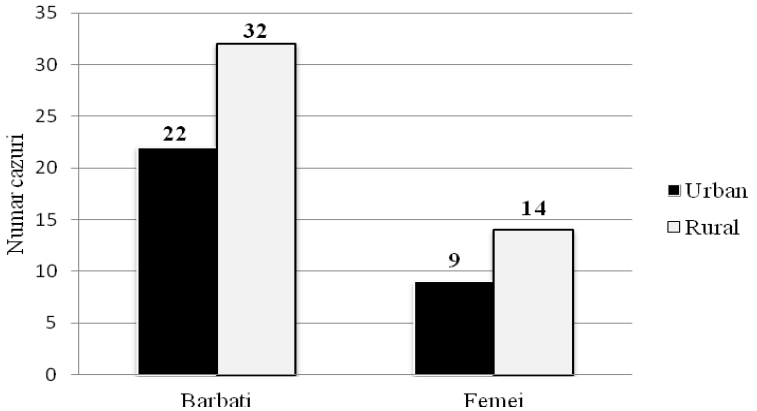

Fig. 5. Distribuția pe sex şi medii de proveniență a lotului de 77 pacienți cu cancer gastric

5. *Antecedentele heredocolaterale*. La bolnavii cu cancer gastric incluşi în studiu s-au evidențiat următoarele grupuri de afecțiuni:

- tulburări metabolice la 19 (24,67%), din care: obezitate 12 (15,58%) şi diabet zaharat 7 (9,09%)
- boli cardiovasculare la 46 (59,74%), din care cu: HTA 31 (40,25%) şi IC 15 pacienți (19,48%)
- boli digestive la 21 (27,27%), din care: ulcer gastric/duodenal 9 (11,68%) şi cancer digestiv: 12 (15,59%) (fig. 6).

6. *Antecedentele patologice personale* ale pacienților. Cercetările au constatat că doar 15 (19,5%) au prezentat ulcer gastric sau duodenal, pentru celelalte comorbidități s-au înregistrat valori între 2 şi 46 cazuri (HTA: 46; ICC: 17; adenom de prostată: 13; litiază biliară: 4; astm bronsic: 2) (fig. 7).

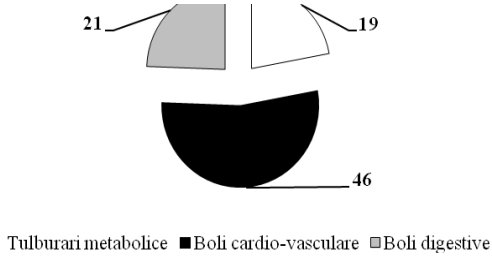

Fig. 6. Distribuția pacienților în relație cu antecedentele heredocolaterale

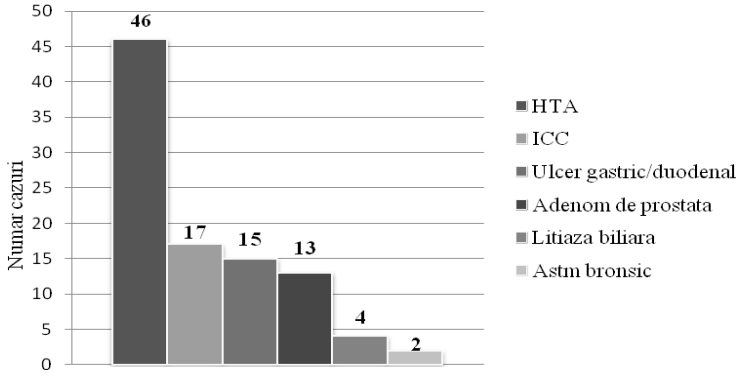

Fig. 7. Distribuția cazurilor în relație cu unele antecedente personale patologice

DISCUȚII

Analizând incidența cazurilor noi de cancer gastric în Spitalul Municipal de Urgență Moineşti în perioada 2000 – 2010 s-a remarcat anul 2005 cu 11 (14,28%) şi 2009 cu 19 (24,67%) cazuri, iar incidența cea mai scăzută a fost în anii 2000 şi 2010, cu 2 cazuri/an (2,6%). Media pe cei 11 ani de studiu a fost de 7 cazuri noi/an.

Rezultate similare cu noi au obținut şi alți autori. Astfel, Gherman et al. (8) într-un studiu pe 158 bolnavi tratați în clinica Chirurgie II din Cluj-Napoca, în perioada 1992-2002, au găsit o distributie anuală relativ omogenă a cazurilor, între 6 şi 16, cu o medie de 10 pacienți pe an.

Incidența CG în studiul nostru evaluată în relație cu sexul a fost de peste 2 ori mai mare la bărbati decât la femei: 54 (70%) cazuri pentru masculin şi respectiv 23 (30%) feminin. Raportul bărbați/ femei a fost de 2,34/1.

Valoarea raportului determinată pentru lotul studiat este în concordanță cu rezultatele cercetărilor altor autori (9,10,11) care indică o afectare mai frecventă a bărbaților decât a femeilor, atât pentru CG în general, cât si pentru cel cu alte localizari, cu un raport de aproximativ 2/1.

Evaluarea pe grupuri de vârstă a aratat că aproape 78% dintre pacienții studiați au avut vârsta peste 51 de ani. Incidența CG a înregistrat o crestere de la 2,6% la sub 30 de ani, la 26% la grupul de vârstă 51-60 ani, după care creşte la 51,9% la pacienții în vârstă de peste 61 de ani. Şi alti autori (12) menționează că, sub vârsta de 30 ani, incidența CG are valori scăzute pentru ca după să crească rapid şi continuu, cea mai mare frecvență fiind semnalată la pacienții vârstnici.

Şi în studiul nostru, cele mai frecvente cazuri (52%) s-au înregistrat la persoane în vârstă de peste 61 ani, iar vârsta medie a pacienților a fost de 60,32±11,87. Mitrut şi Genunche (13), la cei 415 pacienți diagnosticați cu CG, au evaluat vârsta medie la 48±7 ani.

Incidența cazurilor noi de CG asistate în Spitalul Municipal de Urgență Moineşti în relație cu mediul de proveniență a fost exprimată printr-un raport dintre rural şi urban de 1,5/1, rezultând că numărul de pacienți cu această afecțiune provin din mediul rural mai mult decât cei din urban (59,7% / 40,2%).

Popescu şi Pascu (14) au obținut rezultate diferite într-un studiu efectuat pe 257 de cazuri cu CG, semnalând diferențe minime între statusul socio-economic şi mediul de proveniență urban–rural.

În studiul noastru, la 12 (15,59%) bolnavi s-a observat prezența în antecedentele eredocolaterale a cancerului cu diverse localizări, ceea ce pune în discuție rolul amprentei genetice în determinarea neoplaziilor.

Analiza distribuției cazurilor noi de CG în relație cu antecedentele personale patologice a arătat că 15 (19,5%) pacienți au avut ulcer gastric/duodenal.

Studiul privind incidența CG pentru acelaşi lot evaluat în relație cu condițiile de viață a evidențiat că 52% au consumat diferite toxice, îndeosebi tutun (49,35%) şi alcool (35,06%).

CONCLUZII

Deşi valorile incidenței CG au scăzut simțitor în unele tări, ca de exemplu SUA (7,5/100000), acestea continuă să rămână ridicate în altele, cum este cazul Japoniei (95,5/100000) şi al României (21,15/100000), reprezentând o parte semnificativă din valorile mortalității prin cancer.

În studiul efectuat de noi am constatat

preponderența CG la sexul masculin față de feminin (raport: 2,34/1).

Cele mai multe cazuri au fost înregistrate la persoanele de peste 61 ani cu 40 (52,4%) cazuri, față de numai 2 (2,6%) la grupurile de vârstă sub 30 ani.

Etiologia bolii considerată plurifactorială, în 12 (15,6%) cazuri boala canceroasă a fost prezentă în familie, sugerându-se implicarea şi a factorului genetic.

Într-un număr de 15 (19,5%) cazuri din lotul studiat în antecedentele patologice s-a semnalat ulcerul gastric/duodenal.

Frecvența ridicată a consumatorilor de tutun şi/sau alcool, a sugerat posibilitatea implicării în etiologia CG.

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CALITATEA VIEȚII LA PACIENȚII CENTRULUI REGIONAL HIV/SIDA DIN IAŞI

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THE QUALITY OF LIFE IF HIV/AIDS PATIENTS IN THE REGIONAL CENTER FROM IAŞI (Abstract): Quality of life in relation to health is a consequence of disease and treatment on the patient's perception of his ability to have a full and useful life. Due to its nature, the quality of life is a multidimensional and subjective construct, based on the patient's experience. We operationalized the quality of life using the following parameters: CD4 count and HIV viral levels in the patient's blood, the number of days of hospitalization, number of antiretroviral plans, types of side effects and their frequency, psychological issues (anxiety, depression, and neurotic tendencies). Methods: We centralized data from a total of 600 patients from the Regional Center in Iaşi in 2010-2011. Side effects accused by the patients emerged from discussions with the infectious disease doctor and psychologist, and psychological aspects were measured by specific instruments (PA Inventory, Beck Depression Scale). **Results**: Most patients enrolled in the study were male (59%) with mean age of 21.1 years. 34% of them came from rural areas. Average schooling level was 6 primary classes, with extremes between the two classes of primary and higher education. Only 14% of patients had a job, while the rest did not have a stable job or were not employed. 38% came from broken homes or foster care. 85% of patients were in therapy at the time of data centralization. 55% were in the second, third or fourth regimen. 25% were at their first scheme, while 10% in the seventh - eighth scheme. The mean CD4 count ranged between 13 and 269/mmc, while the average viral load varied between 1730 and 3.180.000/mmc. The average number of days of hospitalization was 4. Among the antiretroviral side effects patients complained nausea and vomiting in 85% of cases, lipodystrophy symptoms in 25% of cases, diarrhea 15%. With regard to psychological aspects, 65% of patients showed an above average level of anxiety, 40% showed depressive symptoms, while 10% had specific obsessions-compulsions, and 10% neurotic and hysterical tendencies. **Conclusions**: By measuring the quality of life through the above parameters, we obtained a clear and comprehensive picture of the situation facing the HIV patient. We believe that close cooperation between the team of doctors and psychologists assisting the infectious patient, on the one hand, and specialists in other medical fields that interact with the patient, on the other hand, can enhance the quality of life of the HIV patient and maintain it at a comfortable level.
Key words: QUALITY OF LIFE, HIV/AIDS, SIDE EFFECTS, ARV THERAPY.

Calitatea vieții, în raportul sănătate/boală

Calitatea vieții se referă în esență la "caracterul mai mult sau mai puțin "bun" sau de "satisfăcător" al vieții oamenilor. Este un concept complex, cu multiple laturi

şi posibilități de abordare. Deşi este folosit frecvent în viața de zi cu zi, nu întruneşte o definiție unanimă a specialiştilor.

Calitatea vieții din punct de vedere obiectiv se referă "standardul individual de viață reprezentat de condițiile verificabile caracteristice unei unități culturale" (Evans 1994). Din punct de vedere subiectiv, aceasta e definită ca "gradul în care viața individuală este percepută ca îndeplinind unele standarde interne, explicite sau implicite" (Evans 1994). Însă distincția calitate subiectivă/calitate obiectivă ridică numeroase controverse.

O stare bună de sănătate este un element esențial al bunăstării umane reprezentând o valoare în sine. La nivel individual, o stare bună de sănătate reprezintă o componentă importantă a capitalului uman, permițând oamenilor să îşi desfăşoare activitățile, să îşi îndeplinească țelurile, să aibă o viață completă şi să fie membri activi ai societății. La nivel societal, o stare de sănătate ridicată este un element-cheie al capitalului uman al fiecărei țări, contribuind la competitivitatea ei față de alte țări.

În cercetările de calitatea vieții putem considera ca sănătatea este abordată din două perspective distincte. O primă abordare constă în considerarea sănătății ca o dimensiune, ca un domeniu al calității vieții. Dimensiunea sănătății, ca şi celelalte sfere ale vieții umane, sunt descrise prin indicatori subiectivi şi obiectivi, accentul fiind pus pe modul în care oamenii îşi evaluează propria stare de sănătate, accesul la servicii de sănătate, precum şi evaluarea acestora. Indicatorii utilizați pentru a descrie sfera sănătății pot fi atât subiectivi şi cât obiectivi, situați la niveluri diferite de analiză. Evaluarea propriei stări de sănătate, constrângerile percepute impuse de starea de sănătate, satisfacția față de propria sănătate, percepția accesului la servicii de îngrijire a sănătății, speranța de viață la naştere, mortalitatea infantilă, ratele de mortalitate sau morbiditate sau cheltuielile cu serviciile de îngrijire a sănătății ca procent din PIB reprezintă câțiva dintre indicatori folosiți în cercetarea calității vieții. Sursele de date pot fi multiple: cercetări focalizate pe calitatea vieții, cercetări cu teme diverse, care cuprind şi indicatori de calitatea vieții, cercetări specializate pe domeniul sănătății, sisteme de indicatori de calitatea vieții şi surse de date statistice la nivel național sau internațional. Caracteristic acestei abordări este faptul că evidențiază modul în care oamenii îşi evaluează şi resimt condițiile de viață, resursele şi oportunitățile pe care le au în sfera sănătății. Identificarea inegalităților în ceea ce priveşte starea de sănătate sau de acces la serviciile de îngrijire a sănătății constituie informații esențiale în procesul politicilor sociale.

Cea de-a doua abordare este aceea a calității vieții din perspectiva sănătății, reprezentând o bogată direcție de studiu în cercetările de calitatea vieții. Studiile de calitatea vieții în domeniul sănătății resimt pe modul în care pacienții cu diferite probleme de sănătate îşi experimentează calitatea vieții, pe percepțiile oamenilor care descriu diferite stări de sănătate sau pe timpul de supraviețuire al beneficiarilor de tratamente medicale. În această abordare au fost dezvoltați un număr impresionat de indicatori şi indici de calitatea vieții.

În articolul de față vom privi calitatea vieții prin prisma celei de-a doua abordări.

Calitatea vieții în raport cu sănătatea este consecința bolii şi tratamentului asupra percepției pacientului privind capacitatea sa de a avea o viață deplină şi utilă. Prin natura ei, calitatea vieții este un construct multidimensional şi subiectiv, bazându-se pe experiența pacientului.

Infecția HIV şi boala SIDA – aspecte medicale, sociale şi psihologice

Infecția cu virusul imunodeficienței umane (HIV) în stadiul ei cel mai avansat, sindromul de imunodeficiență dobândită (SIDA), se caracterizează clinic printr-un deficit imunitar complex predominant celular, care predispune la diferite suprainfecții: bacteriene, virale, fungice, cu protozoare şi o evoluție invariabil letală în luni sau ani. Virusul HIV determină infecția CD4, prăbuşirea sistemului de apărare imunitar, determină infecția sistemului nervos central, infecții oportuniste şi apariția de tumori.

Agentul cauzal al infecției HIV este reprezentat de retrovirus, care afectează doar celulele cu receptori CD4-LT4, din țesuturi şi neuroni, şi se multiplică în prezența anticorpilor.

Jonathan Mann, fost Director al Programului Global HIV/SIDA al OMS, făcea referire, în 1987, la trei epidemii mondiale majore, legate de virusul HIV.

Prima epidemie se referă la infecția cu HIV, care intră în comunități silențios şi neobservat, întrucât persoana infectată poate să nu fie conştientă de acest lucru pe toata perioada incubației şi chiar a infecției asimptomatice.

A doua este epidemia SIDA, care apare atunci când infecția cu HIV determină apariția unor boli grave – în organism pătrunde un agent patogen căruia sistemul imunitar nu îi poate face față.

A treia componentă este cea a răspunsurilor sociale, culturale, economice şi politice, date primelor două tipuri de epidemii. „Cercetările sociologice şi epidemiologice focalizate asupra fenomenului SIDA pun accentul pe dimensiunile şi implicațiile socioculturale, demografice, economice şi chiar politice ale acestui fenomen complex, care debutează ca problemă medicală, devenind în timp o adevărată şi tulburătoare problemă socială." (Buzducea, 1997) Este legată de discriminare, stigmatizare, blamare, respingere colectivă, ceea ce împiedică o luptă eficientă împotriva primelor două tipuri de epidemii.

Odată ce infecția a fost detectată, pacientul este sfatuit şi informat despre importanța aderenței la tratament. Medicația previne răspândirea HIV în sânge, contribuie la susținerea sistemului imunitar, şi prelungeşte astfel considerabil viața pacientului.

În prezent există mai mult de 20 de medicamente antiretrovirale aprobate în SUA şi Europa (inclusiv farmaceutice combinate) şi multe altele în curs de aprobare. La fel ca majoritatea medicamentelor, antiretroviralele pot provoca reacții adverse. Aceste efecte nedorite sunt adesea uşoare, dar uneori ele sunt mai grave şi pot avea un impact major asupra sănătății sau calității vieții. De asemenea, în cazuri rare, efectele secundare pot pune viața pacientului în pericol (tab. 1)

TABELUL I

Principalele efecte secundare ale terapiei antiretrovirale

Simptom	Medicament folosit
Diaree	În special inhibitori de protează
Greață şi vomă	Aproape toate medicamentele antiretrovirale
Iritații	Nevirapine, abacavir
Lipodistrofie	Combinații de medicamente din clasa NRTI şi inhibitori de protează
Anomalii lipidice care duc la afecțiuni cardiace	Combinații de medicamente din clasa NRTI şi inhibitori de protează

Caracteristica principală a pacienților care trăiesc cu HIV în România este că aceştie sunt parte dintr-o cohortă ce reprezintă aproape 90% din copiii născuți în perioada 1988-1989. Aceşti pacienți au fost diagnosticați cu infecție HIV la vârsta de 3-6 ani şi au trăit cu HIV întreaga lor copilărie şi adolescență, fiind supravegheați şi monitorizați de către familii şi personalul medical din clinicile de boli infecțioase. Prin urmare, aceştia au crescut odată cu dezvoltarea liniilor terapeutice şi cu trecerea de la mono-şi bi-terapie la terapia HAART. Mulți dintre ei au la activ peste 10 regimuri experimentale, inclusiv utilizarea de inhibitori de protează de ultima generatie şi coformulări ale inhibitorilor nucleozidici. O altă caracteristică specifică pentru aceşti pacienți este coinfecția cu hepatita B şi C în peste 12% din cazuri. Componenta delta a virusului hepatitei este de asemenea prezentă, 1% din toți adolescenții infectați HIV/SIDA suferă de o coinfecție cu hepatită B + D + C.

Conştientizarea infectării cu un virus precum HIV determină la aumite structuri de personalitate o accentuare a stresului şi a neliniştii sub forma manifestărilor anxioase, depresive, însoşite de comportamente obsesiv-compulsive specifice percepției pacientului despre boală (spălarea excesivă, neatingerea obiectelor din jur, neatingerea persoanelor din jur, anumite ritualuri de curățenie a casei sau a obiectelor personale). De asemenea, în cazul persoanelor cu tendințe demonstrative sau hipertimice, pot apărea manifestări nevrotice, de la banalele „crize de nervi", cu o frecvență medie sau ridicată, la aspecte isterice şi crize de isterie.

Am operaționalizat calitatea vieții pacienților infectați HIV în următorii parametri: valoarea CD4 şi nivelul viremiei HIV în sânge; numărul de zile de spitalizare; numărul de scheme terapeutice; tipul de efecte secundare acuzate şi frecvența acestora; aspecte psihologice (anxietate, depresie, tendințe nevrotice).

MATERIAL ŞI METODĂ

Am centralizat datele provenind de la un număr de 600 de pacienți aflați în evidența Centrului Regional Iaşi, în perioada 2010-2011.

Efectele secundare acuzate de pacienți au reieşit din discuțiile cu medicul infecționist şi cu psihologul, iar aspectele psihologice au fost măsurate prin instrumente specifice (Inventarul P.A., Scala de depresie Beck).

REZULTATE

Majoritatea pacienților înrolați în studiu au fost de sex masculin (59%), cu vârsta medie de 21,1 ani.

34% dintre aceştia proveneau din mediul rural. Gradul de şcolarizare mediu a fost de 6 clase, cu extreme între 2 clase primare şi studii superioare.

Doar 14% dintre pacienți aveau un loc de muncă, în timp ce restul nu aveau o meserie stabilă şi nu erau angajați; 38% proveneau din familii destrămate sau din asistență maternală.

85% dintre pacienți se aflau în terapie în momentul centralizării datelor. 55% se aflau la a doua, a treia sau a patra schemă terapeutică. 25% se aflau la prima schemă, în timp ce 10% la a şaptea – a opta schemă (fig. 1).

Valoarea medie a CD4 a variat între 13 şi 269 /mm^3, în timp ce încărcătura virală medie a variat între 1730 şi 3.180.000/mm^3.

Numărul mediu de zile de spitalizare a fost de 4,3, cu extreme între 1 şi 16.

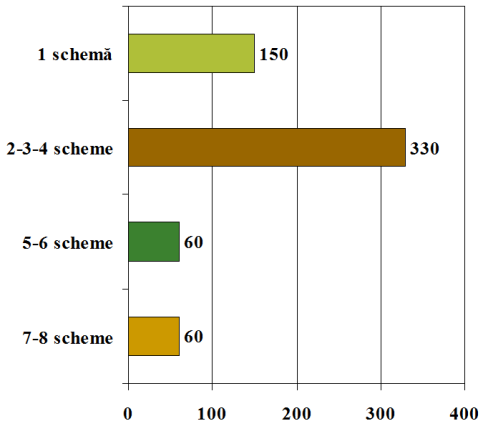

Fig. 1. Numărul de scheme antiretrovirale pentru pacienții monitorizați

Dintre efectele secundare ale antiretroviralelor, pacienții au acuzat greață şi vomă în 85% din cazuri, manifestări lipodistrofice în 25% din cazuri, diaree 15%.

În ceea ce priveşte aspectele psihologice, 65% dintre pacienți au manifestat un nivel pestre medie al anxietății, 40% au evidențiat manifestări depresive, în timp ce 10% au prezentat obsesii-compulsii specifice, şi 10% tendințe nevrotice şi isterice.

CONCLUZII

Operaționalizând calitatea vieții după parametrii prezentați anterior, am obținut o imagine clară şi multilaterală a situației cu care se confruntă pacientul HIV. Considerăm că o colaborare strânsă între echipa de medici infecționişti şi psihologi care asistă pacientul, pe de o parte, şi specialiştii din alte domenii medicale care interacționează cu pacientul, pe de altă parte, poate contribui la creşterea calității vieții pacientului HIV şi la menținerea acesteia la un nivel confortabil.

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CERCETAREA EXPERIMENTALĂ A INFLUENŢEI FACTORILOR DE STRES PE UN MODEL ANIMAL DE SINDROM HELLP

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EXPERIMENTAL RESEARCH REGARDING THE INFLUENCE OF THE STRESS FACTORS IN AN ANIMAL MODEL OF HELLP SYNDROME (Abstract): **Aim**: to study the effects of licht/dark induced-stress in an animal model of HELLP syndrome. **Material and methods**: The experiment was carried out on white female Wistar rats treated intraperitoneally, for 4 weeks, as follows: Group 1: saline solution 0.5ml/100g weight; Group 2: saline solution + dark induced-stress, continuously, 28 days; Group 3: saline solution + light induced-stress, continuously, 28 days; Group 4: endotelin-1; Group 5: endotelin-1 + dark induced-stress, continuously, 28 days; Group 6: endotelin-1 + light induced-stress, continuously, 28 days. Endotelin-1 0.125nmol/kbw was initially administered, followed by 0.25nmol/kbw 30 min later, 2 times/week. **Results**: At the end of the experiment blood pressure was measured and blood samples were taken to assess: leukocyte formula, phagocytic capacity of peripheral neutrophils and serum complement activity, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactic dehydrogenase (LDH) activity, total bilirubin and lipid profile. **Conclusions**:In these experimental conditions we obtained an animal model of partial HELLP syndrome (elevated liver enzyme levels and a low platelet count, values emphasized by light induced-stress, without hemolysis). In chronically stressed animals we found the highest levels of blood pressure, a decrease of plasmatic cortisol level and of phagocytic capacity of peripheral neutrophils and an increase of total cholesterol. **Key words**: EXPERIMENTAL HELLP SYNDROME, BLOOD PRESSURE, STRESS

HELLP este termenul medical pentru una dintre cele mai severe complicaţii ale pre-eclampsiei, în care se combină o afectare a funcţiei hepatice cu tulburări de coagulare a sângelui. **H** reprezintă iniţiala cuvântului hemoliză (fenomen ce se produce în cursul sindromului); **EL** provine de la cuvântul „elevated", din limba engleză, ce exprimă creşterea nivelului enzimelor hepatice, în condiţiile afectării funcţiei şi/sau structurii hepatice; **LP** reprezintă abrevierea de la „low level of platelets", ce exprimă nivelul scăzut al trombocitelor sangvine şi, implicit, alterări ale procesului normal de coagulare (1,2,3).

Sindromul HELLP este caracterizat prin următoarele aspecte: hemoliză, creşterea enzimelor hepatice şi scăderea numărului trombocitelor. De asemenea, pot să apară: cefalee, tulburări de vedere şi edem pulmonar acut (4,5). Sindromul HELLP poate să apară în orice stadiu de evoluţie a sarcinii,

mai ales în trimestrul al treilea de sarcină (6).

Scopul acestei cercetări a constat în evaluarea influenţei factorilor de stres pe un model animal de sindrom HELLP indus experimental.

MATERIAL ŞI METODĂ

Au fost utilizate 6 loturi de câte 6 femele de şobolan Wistar (180-200g), proveniţi de la Institutul de Sănătate Publică din Iaşi, trataţi conform schemei: **L1** = SF = Ser fiziologic (0,5ml/100g corp/zi); **L2** = SF+î = Ser fiziologic (0,5ml/100g corp/zi) + stres indus prin menţinerea la întuneric continuu, timp de 28 de zile; **L3** = SF+l = Ser fiziologic (0,5ml/100g corp/zi) + stres indus prin menţinerea la lumină continuă, timp de 28 de zile; **L4** = ET-1 = Endotelină-1 (0,125nmol/kg corp, intraperitoneal (i.p.), iar, după 30 de minute, 0,25nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni); **L5** = ET-1+î = Endotelină-1 (0,125nmol/kg corp, i.p., iar, după 30 de minute, 0,25nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni) + stres indus prin menţinerea la întuneric continuu, timp de 28 de zile; **L6** = ET-1+l = Endotelină-1 (0,125nmol/kg corp, i.p., iar, după 30 de minute, 0,25nmol/kg corp, i.p., 2 administrări pe săptămână, 4 săptămâni) + stres indus prin menţinerea la lumină continuă, timp de 28 de zile.

Substanţele au fost administrate conform schemei de mai sus, începând cu ora 8 dimineaţa, timp de 28 zile.

După 28 zile de tratament s-a recoltat sânge din plexul retroorbitar (respectând normele instituţionale şi internaţionale de etică ale experimentului pe animale de laborator) şi au fost determinaţi următorii parametri: tensiunea arterială (utilizând sfigmomanometrul HAMEG), nivelul cortizolului sanguin, formula leucocitară, capacitatea de fagocitoză a PMN din sângele periferic (Testul Nitro Blue Tetrazolium), activitatea complementului seric, valorile transaminazei glutamice oxaloacetice (TGO sau aminotransferaza aspartat, ASAT), transaminazei glutamice piruvice (TGP sau aminotransferaza alanina, ALAT), lactat dehidrogenazei (LDH), bilirubinei serice totale, colesterolului total, LDL-colesterolului şi HDL-colesterolului, trigliceridelor şi glicemiei, precum şi nivelurile globulelor roşii, hemoglobinei, hematocritului, globulelor albe şi trombocitelor. Aceşti parametri au fost determinaţi cu un analizor pentru teste biochimice VITROS 750 XRC, folosind reactivi Johnson& Johnson, respectiv un analizor de hematologie HEMA-VET. Rezultatele au fost apoi prelucrate statistic folosind testul „t-Student”.

REZULTATE ŞI DISCUŢII

Administrarea repetată de endotelină-1 determină creşterea valorilor tensiunii arteriale la femelele de şobolan Wistar. Aceste valori sunt semnificativ statistic crescute în condiţiile expunerii la stres comparativ cu animalele care nu au fost menţinute în aceste condiţii experimentale. Cele mai ridicate valori ale tensiunii arteriale au fost înregistrate la şobolanii Wistar cărora li s-a administrat endotelină-1 şi au fost expuşi la lumină continuă (fig. 1).

În condiţiile experimentale ale stresului cronic realizat, s-a constatat o scădere a valorilor cortizolului plasmatic (fig. 2), comparativ cu lotul martor, acest efect fiind semnificativ statistic mai puternic la animalele cu sindrom HELLP indus experimental şi menţinute în condiţii de lumină continuă.

Nivelurile cortizolului seric cresc în timpul unei sarcini normale la niveluri care sunt de 3 ori mai mari. De asemenea, nu-

meroase date din literatură evidenţiază faptul că, într-o sarcină normală, concomitent cu creşterea cortizolului sanguin se realizează, pe de o parte, o creştere a nivelului ACTH şi a nivelului angiotensinei II, iar, pe de altă parte, o scădere a dehidroepiandrosteronului, a hormonului foliculostimulator şi a hormonului luteinizant (7).

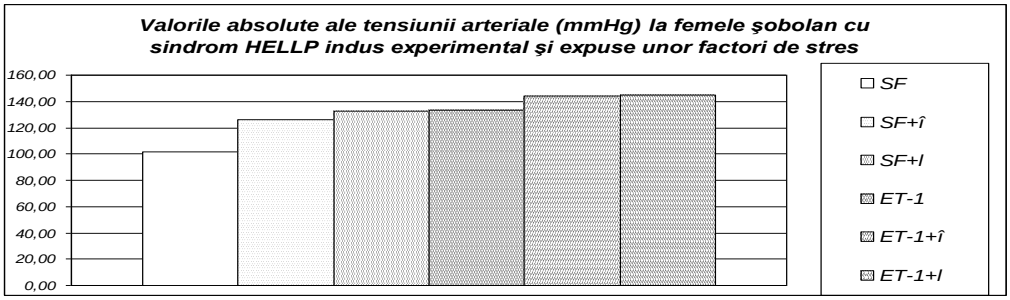

Fig. 1. Valorile absolute ale tensiunii arteriale după 28 de zile

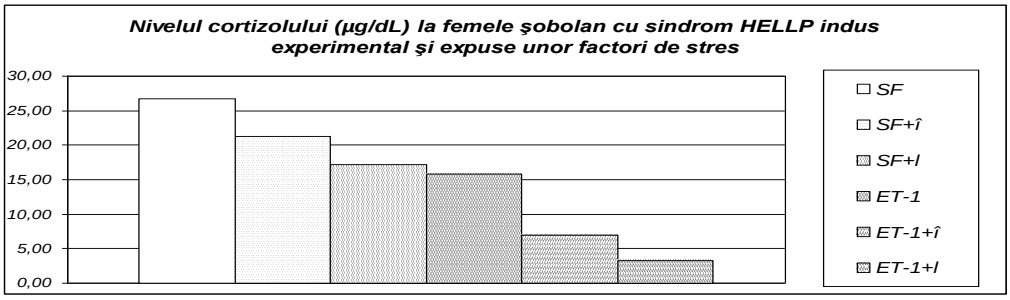

Fig. 2. Nivelurile cortizolului sanguin după 28 de zile

În condiţiile experimentale realizate, nu s-au constatat modificări semnificative statistic ale numărului eritrocitelor, valorilor hemoglobinei sau hematocritului , la nici unul din loturile testate, comparativ cu lotul martor. Nu s-au constatat modificări semnificative statistic ale numărului total de leucocite ale formulei leucocitare, şi nici a activităţii complementului seric.

Pe de altă parte, s-a evidenţiat o scădere semnificativă statistic a capacităţii de fagocitoză a PMN din sângele periferic (testul NBT) la femelele de şobolan cu sindrom HELLP indus experimental şi expuse stresului (fig. 3). Nivelul cel mai scăzut al acestui parametru a fost înregistrat la animalele din lotul expus la lumină continuă.

S-a constatat, însă, o trombocitopenie marcată (fig. 4) faţă de lotul martor, cel mai puternic efect înregistrându-se la femelele de şobolan cu sindrom HELLP indus experimental şi menţinute la lumină continuă, timp de 28 de zile.

În cursul experimentelor, s-a constatat creşterea transaminazelor la toate loturile analizate, comparativ cu lotul martor. Creşterea transaminazelor este efectul alterării permeabilităţii membranei celulare din cauza furtunii steroidiene induse de stres (8). Pe de altă parte, nu s-a constatat hemoliză, ajungând, astfel, la concluzia realizării unui sindrom HELLP parţial. Modelul

experimental realizat pe femele de şobolan reprezintă o alternativă experimentală pentru studiul sindromului HELLP, care a fost realizat pentru prima dată pe femele iepure de către Halim A şi colaboratorii, în 1993 (9). De asemenea, nu s-au înregistrat modificări semnificative statistic ale bilirubinei serice totale comparativ cu lotul martor.

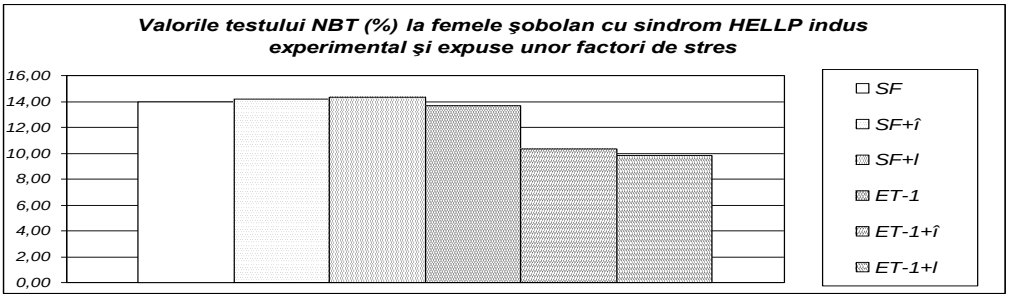

Fig. 3. Valorile testului NBT după 28 de zile

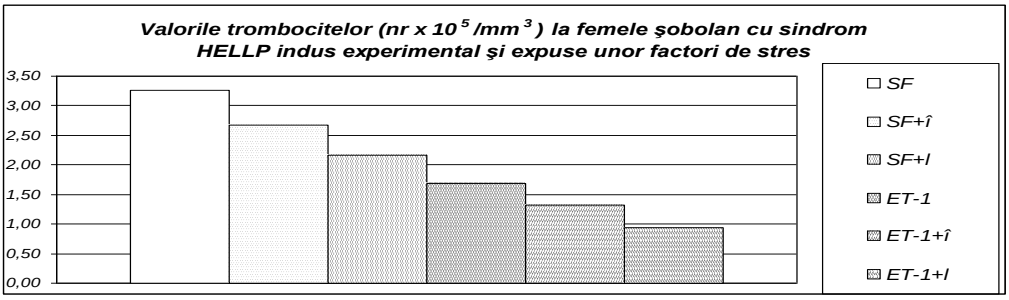

Fig. 4. Valorile trombocitelor după 28 de zile

Datele din literatură sugerează că există o influenţă reciprocă între glucocorticosteroizi şi steroizii sexuali în reglarea funcţiilor imune dependente de timus. De fapt, impactul fondului de hormoni sexuali în răspunsul imun şi activitatea axei hipotalamo-hipofizo-corticosuprarenaliene sunt perfect ilustrate prin prezenţa dimorfismului sexual în cadrul axei şi al răspunsului imun (10). O parte dintre modificările patologice prezente la gravidele diagnosticate cu HTA pot fi explicate prin corelaţiile existente între reacţia organismului la acţiunea unui factor stresor şi „furtuna endocrină şi imunologică" indusă de sarcină. Deşi este o stare fiziologică, sarcina influenţează profund statusul imun matern (11). Imunodepresia corelată cu vârsta gestaţională şi cu factorii de risc materni pentru apariţia HTA poate explica incidenţa mai mare a patologiei hipertensive la primipare.

CONCLUZII

În acest experiment a fost obţinut un sindrom HELLP parţial (creşterea nivelurilor transaminazelor şi trombocitopenie, fără hemoliză), prin administrarea endotelinei-1 conform schemei prezentate, pe fondul unei creşteri semnificative a valorilor tensiunii arteriale la femele şobolan Wistar. Aceste valori sunt semnificativ statistic crescute în condiţiile expunerii la stres, comparativ cu

animalele care nu au fost menţinute în aceste condiţii experimentale. Cele mai ridicate valori ale tensiunii arteriale au fost înregistrate la şobolanii Wistar cărora li s-a administrat endotelină-1 şi au fost expuşi la lumină continuă.

În condiţiile experimentale ale stresului cronic realizat, s-a constatat o scădere a valorilor cortizolului plasmatic şi a capacităţii de fagocitoză a PMN din sângele periferic, comparativ cu lotul martor, acest efect fiind semnificativ statistic mai puternic la animalele cu sindrom HELLP indus experimental şi menţinute în condiţii de lumină continuă.

S-a constatat o trombocitopenie marcată faţă de lotul martor, cel mai puternic efect înregistrându-se la femelele de şobolan cu sindrom HELLP indus experimental şi menţinute la lumină continuă timp de 28 de zile.

În cursul experimentelor, s-a constatat creşterea transaminazelor la toate loturile analizate, comparativ cu lotul martor.

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EVALUAREA SATISFACȚIEI CONSUMATORILOR DE SERVICII DE SĂNATATE IN SISTEMUL PUBLIC DE ASISTENTĂ MEDICALĂ ÎN MUNICIPIUL IAŞI

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ASSESSMENT OF CONSUMER HEALTH SERVICES IN THE IASI TOWN PUBLIC HEALTH CARE SYSTEM (Abstract). **Aim**: The study was designed to assess patient satisfaction regarding the quality of medical care delivered in 2010, the financial accessibility of the used services, the time span accessibility to the services used, doctor-patient relationship, and quality of health services. **Materials and method**: This study was carried out on a series of 150 randomly selected patients, age-range18 to 70 years. These patients were asked to fill out a 44-item questionnaire including demographic data (age, education, environment - rural or urban area, difficult financial situation) and questions on the quality of the delivered medical care and the performance of the qualified medical staff. **Results and discussion**: The global assessment of the quality of health services shows that it is mainly satisfactory (42.00%) and good (31.33%). The primary health services (family medicine) are mostly regarded as very good (38.00%), good (28.67%) and satisfactory (24.67%). As to the specialized outpatient services, most investigated subjects found them satisfactory (32.67%), good (27.33%), and poor and very poor (8.00%). A percentage of 35.33 of the respondents think that the services provided in hospitals are satisfactory, 18,00% good and 9.33% very good. Drug administration was considered satisfactory by 37.33%, while 22.00%and 12.6% believe it to be good and very good, respectively. **Conclusions**: The regular application of questionnaires to measure patient satisfaction and the setting up of a system for measuring the quality of medical care will improve quality of the health services. **Key words:** PATIENT SATISFACTION, DOCTOR-PATIENT RELATIONSHIP, HEALTH SERVICES QUALITY

România are o lungă tradiție în organizarea sistemului de sănătate. În perioada interbelică a existat un sistem de asigurări de boală bazat pe modelul Bismark. Angajații din întreprinderile industriale, comercianții, funcționarii şi familiile acestora, precum şi liberii profesionişti erau beneficiau de asigurări de boală; totuşi, asiguraţii nu reprezentau la vremea respectivă decât 5% din populație. În anul 1949, a fost aprobată Legea Organizării Sistemului de Sănătate de stat şi a urmat o tranziție progresivă către un sistem de sănătate de tip Semaşko. Acesta se baza pe o largă acoperire a populației cu servicii de sănătate. Principalele caracteristici ale sistemului sanitar din România în timpul acestor următoare patru decenii au fost: planificarea şi finanțarea

centralizată de către stat, administrarea rigidă şi monopolul statului asupra serviciilor de sănătate. De asemenea, de remarcat este şi faptul că profesioniştii din sistemul de sănătate erau salariaţi ai statului. Deşi din 1949 au avut loc multe schimbări, noutăţi semnificative au apărut abia în 1978, când a fost promulgată o lege a sănătăţii. Sistemul de sănătate Semaşko din România anterioară lui 1989 a fost un sistem tipic pentru multe dintre ţările din estul şi centrul Europei. Absenţa concurenţei şi a iniţiativei individuale, calitatea precară a serviciilor de sănătate, subfinanţarea cronică, ineficienţa economică, normele rigide şi echipamentele medicale neadecvate au creat în perioada 1989-1997 presiuni pentru schimbarea sistemului. Între 1990 şi 1995, Guvernul şi Ministerul Sănătăţii au adoptat o serie de decrete şi ordine care, în timp, au adud mai multe schimbări. Astfel, după 1995 a fost posibilă implementarea unui sistem de sănătate standardizat şi reglementat bazat pe modelul imaginat de cancelarul german Otto von Bismark (în 1883). Totuşi, moştenirea lăsată de cei patruzeci de ani de comunism şi funcţionarea actuală a sistemului de sănătate românesc reflectă încă, în bună măsură, problemele cronice ale acestuia, cum ar fi: proporţia relativ scăzută din PIB alocată sănătăţii; alocarea centralizată a resurselor; lipsa reacţiei la nevoile locale; calitatea slabă a serviciilor la primul nivel, trimiterile neadecvate şi accentul pus pe serviciile spitaliceşti unde aparatura medicală şi asigurarea cu medicamente sunt deficitare; şi să nu uităm capacitatea managerială redusă a top-managementului sistemului de sănătate şi insuficienţa numerică a personalului care să aibă competenţe în politici publice de sănătate (3).

Începând cu 1995, legi importante privind sistemul de sănătate din România au schimbat practic întreaga structură de organizare şi au stabilit cadrul pentru tranziţia către un sistem bazat pe asigurări sociale de sănătate, mai descentralizat şi pluralist, cu relaţii contractuale între casele de asigurări de sănătate şi furnizorii de servicii de sănătate (2). Astăzi, în România asigurările sociale de sănătate sunt obligatorii şi funcţionează descentralizat pe baza principiului solidarităţii şi subsidiarităţii în colectarea şi utilizarea fondurilor, precum şi a dreptului alegerii libere de către asiguraţi a medicului, a unităţii sanitare şi a casei de asigurări de sănătate (1).

MATERIAL ŞI METODĂ

În studiu au fost incluşi 150 pacienţi. Lotul selectat aleator a cuprins persoane cu vârste între 18 şi 70 de ani. Aceştia au răspuns unui chestionar cu 44 de itemi. Chestionarul include date demografice (vârstă, studii, religie, mediu social rural/ urban, situaţie financiară) şi întrebări (atât închise, cât şi deschise)– privind calitatea actului medical de care au beneficiat, prestanţa personalului medical calificat şi diferenţa pe care acesta o percepe între unităţile medicale publice şi cele private.

Structura pe grupe de vârstă a lotului studiat arată că procentul cel mai mare (48,50%) l-a reprezentat populaţia adultă cu vârste între 30-49 ani, urmat de cel al populaţiei tinere cu vârste între 18-29 ani (20,60%); la polul opus se situează pacienţii cu vârste de 60 ani şi peste (5,80%). Majoritatea pacienţilor luaţi în studiu au fost de sex feminin (62,00%) şi proveneau din mediul urban (56,90%).

Referitor la instrucţia şcolară, ponderea cea mai mare este deţinută de persoanele cu studii medii (27,20%) şi postliceale (13,9%), apoi persoanele cu studii superioare (11,40% învăţământ superior de

scurtă durată, 11,80% învăţământ superior de lungă durată, 16,10% învăţământ postuniversitar), urmat de persoanele cu studii primare (14,7%). Din totalul persoanelor investigate 32,40% erau pensionari.

REZULTATE

Rezultatele studiului reflectă satisfacţia pacienţilor care au beneficiat de servicii publice de sănătate în 2010.

Evaluarea globală a calităţii serviciilor de sănătate accesate în ultimul an de către pacienţii luaţi în studiu arată că aceasta este satisfăcătoare (42,00%) şi bună (31,33%) (fig. 1).

Tot ca o evaluare globală, s-a căutat să se observe dacă sistemul public răspunde nevoilor de sănătate ale populaţiei. Pacienţii luaţi în studiu au răspuns afirmativ în procent de 77,33%.

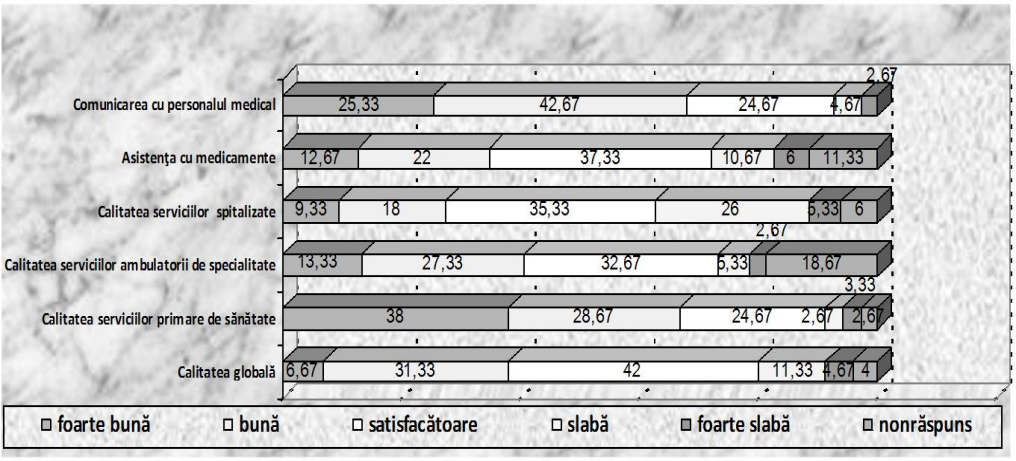

Fig. 1. Calitatea serviciilor de sănătate accesate în ultimul an de către persoanele luate în studiu

Aprecierile referitoare la serviciile primare de sănătate (medicina de familie) sunt foarte bune (38,00%), bune (28,67%) şi satisfăcătoare (24,67%); doar 3,33% din total subiecţilor le-au apreciat ca fiind foarte slabe.

În ceea ce priveşte prestaţiile oferite de ambulatoriul de specialitate, 32,67% dintre subiecţii investigaţi le-au apreciat ca fiind satisfăcătoare şi bune (27,33%), iar 5,33% ca slabe şi 2,67% ca foarte slabe.

Doar 35,33% dintre persoanele luate în studiu au apreciat serviciile secundare de sănătate de care au beneficiat în spitalele publice din Iaşi ca fiind satisfăcătoare şi 18,00% ca bune şi foarte bune. La fel se poate afirma şi despre asistenţa cu medicamente în 2010: 37,33% dintre pacienţi o apreciază ca fiind satisfăcătoare, 22,00% bună şi doar 12,67% ca fiind foarte bună, însă 16,67% o apreciază ca fiind slabă şi foarte slabă.

Referitor la uşurinţa de a obţine îngrijirile medicale de care au nevoie, 53,33% dintre subiecţii investigaţi au răspuns cu "*da, uneori*" şi 30,67% "*da, întotdeauna*". Atunci când pacienţii au avut nevoie de îngrijiri medicale, aceştia le-au apreciat ca fiind adecvate faţă de starea lor de sănătate (59,33%), dar 20,67% au recunoscut că nu au cunoştinţele necesare pentru a evalua obiectiv acest aspect.

În ceea ce priveşte nevoia de a cheltui suplimentar bani din bugetul personal pen-

tru a se trata atunci când sunt bolnavi, majoritatea subiecților (60,67%) apreciază că trebuie să folosească suplimentar bani, 26,00% afirmă că au fost suficiente asigurările de sănătate pentru a fi tratați corespunzător şi 12,67% dintre pacienți nu apelează la bani din buzunarul propriu pentru că nu îşi pot permite.

Referitor la accesibilitatea la îngrijiri de sănătate în localitatea în care domiciliază, cea mai mare parte a pacienților (45,33%) apreciază că pot obține aceste îngrijiri *uneori*, iar 23,33% afirmă că au acces *întotdeauna* la îngrijirile necesare în localitatea de domiciliu. Un procent de 2,00% apreciază că nu au încredere în serviciile de sănătate din localitatea de domiciliu.

Orarul unităților din rețeaua sanitară este apreciat ca fiind corespunzător de către mai mult de jumătate dintre subiecții investigați (50,90%) şi satisfăcător (31,80%), doar 6,60% din subiecți afirmă că acesta nu se respectă.

Aproape în majoritate (93,33%) dintre subiecții luați în studiu consideră că statul trebuie să se implice în sprijinirea asigurărilor sociale de sănătate din România. 32,67% dintre subiecții cuprinşi în cercetare consideră că atunci când au avut nevoie de servicii medicale nu au primit serviciile medicale la nivelul contribuției plătite.

În ceea ce priveşte accesul asiguraților la aceleaşi servicii medicale, indiferent de suma plătită diferit pentru contribuția la fondul asigurărilor sociale de sănătate, un procent foarte mare (62,60%) consideră că acest lucru este corect. Există şi subiecți (16,40%) care apreciază că nu îi interesează acest subiect, pentru ei fiind important să primească servicii de sănătate adecvate nevoilor lor de sănătate atunci când au nevoie.

Pentru cei care nu plătesc contribuții la fondul de asigurările sociale de sănătate este un lucru firesc să primească servicii medicale (48,00%) deoarece consideră că statul este obligat să asigure ocrotirea sănătății cetățenilor în virtutea contribuției plătite de-a lungul activității, chiar dacă în prezent nu contribuie (în cazul pensionarilor, şomerilor etc.). Faptul că anumite categorii de persoane care beneficiază de serviciilor de sănătate nu contribuie financiar la fondul de sănătate este acceptat de 34,67% din totalul subiecților luați în studiu. De asemenea, 16,67% sunt de părere că acest lucru este de acceptat pentru faptul că şi ei pot ajunge în situația de a nu mai plăti contribuția pentru sănătate (prin ieşirea din perioda de şomaj). La polul opus se află 39,33% dintre subiecți care nu acceptă acest lucru, în principal pentru că pentru a beneficia de servicii de sănătate trebuie să se plătească, dar şi pentru că salariații plătitori de contribuții de sănătate nu pot susține financiar întrega populație a țării.

Contribuția plătită de fiecare asigurat care a primit servicii spitaliceşti în 2010 a fost apreciată ca fiind în general echilibrată (20,00%) şi mare în raport cu serviciile medicale de care au beneficiat (18,00%). Unii asigurați (22,67%) apreciază drept mare în raport cu venitul lunar realizat nivelul contribuției virate la fondul de sănătate, în timp ce doar 6,00% consideră că aceasta este mică în raport cu nevoile lor de sănătate.

Referitor la disponibilitatea de a plăti suplimentar (co-plată) pentru unele servicii medicale (cum ar fi serviciile medicale de înaltă performanță, fertilizare *în vitro* sau corecții estetice), mai mult de jumătate dintre subiecți (54,00%) afirmă că sunt de acord cu aceasta, iar dintre cei care nu sunt de acord (12,00%) consideră că statutul de

asigurat ar trebui să le confere accesibilitate pentru aceste categorii de servicii.

Afirmaţia *"Toţi asiguraţii beneficiază de servicii medicale, medicamente, materiale sanitare / dispozitive medicale în mod nediscriminatoriu / necondiţionat / echitabil / egal"* este considerată adevărată sau parţial adevărată de 58,67% dintre subiecţi. Principalele motive pentru care această afirmaţie a fost considerată neadevărată/sau parţial adevărată au fost: discriminarea (pe criterii etnice, stare materială); unii asiguraţi nu au acces la serviciile medicale datorită distanţei, programului de muncă prelungit.

În ceea ce priveşte serviciile medicale preventive, pentru menţinerea unei stări bune de sănătate 40,67% dintre subiecţi consideră că nu sunt informaţi, doar 15,33% apreciază ca suficientă informarea asupra serviciilor medicale preventive. Există şi un număr relativ important (24,00%) dintre pacienţi care nu înţeleg necesitatea prevenţiei. O mare parte dintre pacienţii cuprinşi în cercetarea de faţă (48,00%) afirmă că au beneficiat de control periodic, ştiind că au acest drept. Dintre cei care nu au beneficiat de acest serviciu, 27,33% au considerat că nu au avut nevoie, în timp ce 22,00% afirmă că nu au ştiut ca au acest drept.

Circuitul bolnavului în sistemul de sănătate nu este cunoscut de 21,33% dintre subiecţi, 12,67% dintre cei care afirmă că îl cunosc, îl desemnează ca fiind următorul: medic de familie-ambulatoriu de specialitate–spital–medic de familie. Aproape toţi subiecţii investigaţi sunt pe lista unui medic de familie (97,33%); dintre aceştia, 26,00% sunt în evidenţă cu boli cronice şi 25,33% sunt în evidenţa unui medic specialist. Majoritatea (77,33%) pacienţilor afirmă că nu au schimbat până acum medicul de familie, dar 10,67% l-au schimbat din următoarele motive: schimbarea domiciliului, distanţa mare faţă de domiciliu, nemulţumirea din cauza timpului mare de aşteptare la cabinet.

Referitor la serviciile primare de sănătate de care au beneficiat, o mare parte au beneficiat de consultaţii şi tratament din partea medicului de familie (39,33%) şi de recomandarea unor investigaţii (16,67%).

Relaţia cu personalul medical este apreciată de majoritatea pacienţilor drept bună (42,67%) şi foarte bună (25,33%); numai 24,67% afirmă că aceasta este satisfăcătoare.

Funcţiile şi rolul Casei Naţionale de Asigurări de Sănătate sunt cunoscute de o parte dintre subiecţi (32,67%). Dintre cei care nu le cunosc, cei mai mulţi (20,67%) susţin că nu au cunoştinţă de existenţa unor campaniilor de informare organizate de această instituţie. Un procent îngrijorător de subiecţi investigaţi (65,33%) afirmă că nu au fost informaţi niciodată de CASJ cu privire la serviciile de sănătate, sau cu privind drepturile şi obligaţiile de asigurat. În ceea ce priveşte informarea de către CAS asupra condiţiilor de furnizare de servicii medicale şi despre schimbările în modul de funcţionare/acordare a acestora, înainte de aplicarea acestor modificări, mai mult de jumătate dintre subiecţi (57,33%) afirmă că aceasta nu a fost făcută corespunzător în 2010.

Atunci când vor să-şi exprime părerea cu privire la serviciile de sănătate primite, majoritatea subiecţilor (41,33%) afirmă că se adresează medicului de familie, urmează apoi casele de asigurări de sănătate, care au fost accesate în acest scop de 23,33% dintre pacienţi, iar la polul opus se află birourile de presă ale caselor de asigurări de sănătate (7,33%).

În ceea ce priveşte necesitatea de a fi

efectuate controale din partea CNAS/ CASSJ la furnizorii de servicii medicale, cu privire la execuţia contractelor şi îndeplinirea obligaţiilor faţă de pacienţii asiguraţi, mai mult de jumătate dintre cei chestionaţi (55,33%) cred în utilitatea acestora pentru o mai bună disciplină financiară în condiţii de criză.

Pentru creşterea performanţei şi calităţii serviciilor de sănătate, 24,00% dintre subiecţi afirmă că este nevoie de îmbunătţirea legislaţiei specifice.

DISCUŢII

Rezultate studiului sunt utile decidenţilor pentru fundamentarea unei strategii de îmbunătăţire a serviciilor de sănătate. Vom prezenta principalele obiective ale acesteia.

În primul rând creşterea calităţii, performanţei, eficienţei şi eficacităţii serviciilor spitaliceşti. Extinderea gamei de îngrijiri de sănătate furnizate de serviciile spitaliceşti cum ar fi: îngrijiri ambulatorii integrate cu cele spitaliceşti, spitalizare de zi, spitalizare de o zi. Populaţia va avea un acces local îmbunătăţit la o gamă mai largă de servicii integrate de asistenţă primară şi spitalicească, mai bine adaptate nevoilor de sănătate ale comunităţii.

Finanţarea echitabilă şi transparentă a serviciilor de sănătate va trebui să se bazeze pe responsabilitatea locală şi pe mecanisme de raportare corespunzătoare. Finanţarea adecvată şi sustenabilă a spitalelor, în concordanţă cu politicile de sănătate şi cu planificarea naţională pe termen lung a numărului de paturi de spital.

Restructurarea unităţilor sanitare în vederea furnizării serviciilor medicale adecvate se va realiza funcţie de nevoile de sănătate ale populaţiei. Închiderea/sau restructurarea unităţilor spitaliceşti subutilizate sau care nu sunt necesare, în vederea limitării pierderilor financiare şi utilizarea resurselor economisite pentru rezolvarea problemelor prioritare de sănătate.

Transferul îngrijirilor furnizate în spitale pentru cazurile sociale şi pentru vârstnicii cu boli cronice către unităţi/organizaţii locale şi/sau către unităţi private, astfel încât spitalele să se poată concentra pe îngrijirile de tip acut.

Dezvoltarea serviciilor de asistenţă primară, în special a îngrijirilor la domiciliu, crearea unor centre de sănătate multifuncţionale atât în mediul urban, cât şi în rural şi integrarea asistenţei primare cu serviciile ambulatorii de specialitate şi serviciile spitaliceşti, în concordanţă şi cu programele naţionale de sănătate.

Îmbunătăţirea managementului operaţional la nivel local, judeţean şi regional.

Revizuirea cadrului legislativ specific pentru a reformare sistemul de sănătate, descentralizarea ulterioară a managementului operaţional şi financiar şi găsirea celor mai bune soluţii pentru satisfacerea nevoilor locale, inclusiv pentru populaţia defavorizată sau vulnerabilă.

Implementarea unui sistem modern de acreditare a serviciilor de sănătate şi a unor sisteme adecvate de monitorizare a managementului calităţii.

Sistemul de sănătate trebuie să furnizeze exclusiv îngrijiri de sănătate şi să coopereze cu sectorul de asistenţă socială pentru creşterea solidarităţii sociale şi a calităţii vieţii.

CONCLUZII

Unităţile din sistemul public de sănătate vor trebui să îmbunătăţească calitatea actului medical, aceasta nu doar din pricina necesităţii de a se alinia cerinţelor europene, ci şi dintr-un alt considerent, mult mai puternic: legea concurenţei.

Aplicarea regulată a chestionarelor care să evalueze satisfacţia pacienţilor precum şi implementarea unui sistem coerent de monitorizare a calităţii creează premisele îmbunătăţirii calităţii serviciilor de sănătate oferite de instituţiile publice.

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ASPECTE CLINICE ŞI TERAPEUTICE ALE CARIEI TIMPURII ŞI ALE CARIEI SEVERE TIMPURII – SERIE DE CAZURI

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CLINICAL AND THERAPEUTICAL ASPECTS OF EARLY CHILDHOOD CARIES AND SEVERE EARLY CHILDHOOD CARIES-CLINICAL CASES (Abstract): ECC(early childhood caries) and SECC(severe early childhood caries), as a particularization of the dental caries during the early childhood (0-3 years) and the middle childhood (3-6 years), generate important oro-dental problems, with long term impact, psycho-somatic, psycho-aesthetic, and social-behaviour, in little children as in adults. **Material and methods:** 35 subjects(20 girls and 15 boys), were evaluated, aged between 3-6 years old, diagnosticated with ECC and SECC.The children were evaluated clinical, paraclinical and psycho-sociological in the Clinic of Paediatric Dentistry, Iasi, Romania. **Results**: In this article, we present 3 clinical cases that show the implication of the socio-emotional factors that produce the early childhood caries, pacient C.L., 6 years old, pacient C.F., 5, 6 years old, and pacient A.D., 6 years old. **Conclusion:** in this study we have evaluated a heterogen structure with major impact on caries risk, clinical variability of the carioactivity in decidual dentition.It is known that more than 64% non-cavitary lesions that appear in a 2 year-old child develop to cavitary lesions in less than a year. **Key words:** EARLY CHILDHOOD CARIES, SEVERE EARLY CHILDHOOD CARIES, PAEDIATRIC DENTISTRY, EARLY CHILDHOOD, SPACE MAINTAINER

Caria timpurie (ECC) şi caria severă timpurie (SECC), ca particularizări ale cariei dentare în perioada copilariei mici (0-3 ani) şi mijlocii (3-6 ani), generează profunde probleme oro-dentare, cu impact pe termen mediu şi lung psiho-somatic, psiho-estetic şi socio-comportamental atât la copii mici cat şi la populatia adultă (1,2).

După cum mentionează Sven Poulsen în 2002 (3), Dănilă I. şi Evghenikos în 2007 (4), aceste entităţi clinice reprezintă o autentică problemă de sanatate publică oro-dentară, prin debutul extrem de timpuriu şi prin riscul înalt de progresie carială, cu rezonanţă directă asupra dentiţiei permanente.

Prin complexitatea sa contextuală şi larga sa variabilitate, ECC şi SECC predicţionează dinamica şi trendul de creştere şi dezvoltare în perioadele dentiţiei mixte şi a dentiţiei permanente tinere (5), cu implicaţii asupra homeostaziei sistemului stomatognat la etapa de adult tânar.

Pe de altă parte experienţele algogene ale copilului preşcolar şi anteprescolar generate de complicaţiile periodontale ale ECC şi SECC, asociate cu tehnici de management pedodontic neadecvate şi o credibilitate discutabilă din partea practicianului faţă de durerea stomatologică a copilului, se prefigurează într-un predictor al fricii, anxietăţii şi fobiei dentare, al demodulării comportamentale pe termen lung a copilului versus cabinet şi tratament stomatologic (6, 7).

Fig. 1. Aspectul extraoral (pacienta1)

Fig. 3. Aspectul extraoral (pacienta2)

Scopul acestui studiu a fost de evaluare a factorilor predictivi în evoluţia stării de sănătate oro-dentară la copii şi optimizarea efectivă a managementului comportamental şi terapeutic preventiv şi curative al afecţiunilor oro-dentare la copilul antepreşcolar şi preşcolar.

Cei 35 de subiecţi implicaţi în studiu (20 fete şi 15 băieţi) cu vârstă cuprinsă între 3 şi 6 ani, provin dintre pacienţii care au solicitat asistentă la Policlinica de Stomatologie Infantilă. Aceştia au fost investigaţi clinic, paraclinic şi psihosociologic. Programul şi studiul au fost precedate de obţinerea consimţământului informat de către apartinator sau educator.

În lucrarea de faţă prezentăm trei cazuri clinice care pun în evidenţă prezenţa factorilor socio-emoţionali care favorizează apariţia cariei severe timpurii.

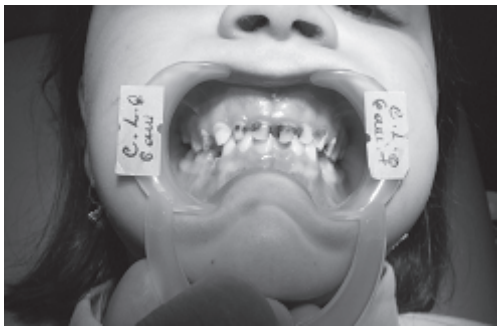

Fig. 2. Aspectul intraoral (pacienta 1)

CAZURI CLINICE

1. Pacienta C.L. în varstă de 6 ani provine dintr-un mediu familial biparental, climat armonios, copil unic, tipar emoţional carenţial, predominant extrovertit. Alimentaţia copilului a fost artificială între 2, 2-10 luni, actualmente preferând alimentele moi. Igiena oro-dentară este inconsecventă şi superficiala. Respiraţie mixtă, tipar constituţional normosom, tipar cranio-cefalic : brahi-cefal, tipar facial : leptoprosop (fig.1).

Diagnostic : carii severe timpurii, fronto--lateral maxilar şi latero-lateral mandibular, complicate la nivelul zonelor de sprijin, cu angrenaje inverse frontale, parcelare în zig--zag (fig.2).

Prognostic bun cu tratament laborios constand in tratamrntul leziunilor carioase si obturatii cu glassionomer pentru leziunile localizate la nivel frontal si latero-lateral sau coroane pedodontice pentru zona laterala.

2. Pacienta C.F. de 5,6 ani provine dintr-un cuplu biparental, cu nivel socio-economic mediu. Temperamental-comportamentul copilul prezintă tendinţe la extrovertenţă, psiho--emoţional fiind netemător, tipar comunicativ, cooperant. Copilul a fost alimentat artificial (biberon) de la vârstă de 6 luni până la 18 luni, asociativ cu suzetă înmuiată în îndulcitori(zahăr, miere de albine). Igiena oro-dentară nesatisfăcătoare, periajul dentar de seară fiind realizat sporadic. Copilul prezintă preferinţe alimentare pentru produse

Fig. 4-5. Aspecte intraorale (pacienta 2)

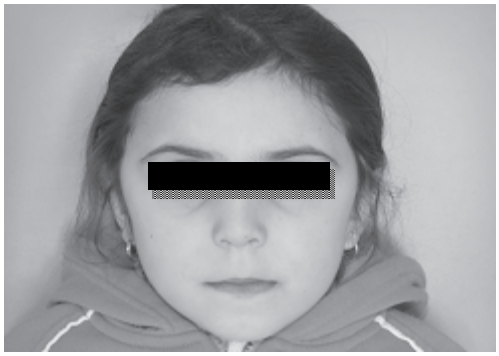

Fig. 6. Aspecte extraorale (pacienta 3)

Fig. 7. Aspecte intraorale (pacienta 3)

Fig. 8. Aspecte intraorale (pacienta 3)

făinoase şi dulciuri. La examenul extraoral se observă tiparul somatic-normosom, tipar cranio-cefalic-brahicefal, tipar facial-euriprosop, respiraţie mixtă iar intraoral deglutiţia infantilă, tendinţa la angrenaje inverse parcelare : 51, 61, 62/82, 81, 71, 72, 73, planul postlacteal în treaptă uşor mezializată (fig.3).

Diagnostic : malocluzie clasa I-Angle, sindrom de suzetă Mugnier, asociat cariei de biberon, carii la nivel incisiv maxilar simple şi complicate periodontal(52) şi la nivel maxilar bilateral, mandibular, simple şi complicate pulpar(84, 85), cu tulburări funcţionale (fig. 4, 5).

Prognostic este bun în condiţia instituirii managementului terapeutic adecvat : educaţional prin instituirea unui comportament sanogen adecvat, ortodontic interceptiv sau curativ prin decondiţionarea deglutiţiei atipice, efectuarea saltului articular la nivelul tiparelor dentare aflate în angrenaj invers incipient si tratamentul leyiunilor carioase simple si complicate.

3. AD.- 6 ani. Subiectul provine dintr-un climat biparental armonios, agreabil, nivel socio-economic mediu, copil unic. Alimentaţia a fost artificială (biberon) între 6 luni-3 ani, deprinderi şi obisnuinţe de igienă oro-dentară inconsecvente şi superficiale, preferinţă pentru alimentele moi, deglutiţie infantilă cu interpoziţie, respiraţie mixtă, emoţional-copilul este nesigur, temperamental şi cu tendinţă la introvertenţă. Tipar somatic- nor-

mosom, tipar cranio-cefalic- dolicoprosop, tipar facial- leptoprosop, sindrom protruziv anterior complet(fig.6).

Diagnostic- malocluzie clasa a III-Angle, ocluzie inversă, inocluzie verticală frontală disfuncţională şi scheletală, carii severe timpurii simple şi complicate cu pierderi premature odontale la nivel frontal maxilar (fig. 7 şi 8).

Prognostic este rezervat necesitand instituirea unui comportament sanogen adecvat, o unui tratament ortodontic interceptiv si curativ prin decondiţionarea deglutiţiei atipice, tratamentul leziunilor carioase simple si complicate cu glasionomer şi menţinatoare de spaţiu pentru pierderile odontale premature.

CONCLUZII

Sub aspectul apartenenţei socio-familiale economice şi ecologice am suprins o structură heterogenă, a lotului de studiu, cu impact major dar diferenţiat asupra riscului carios, al progresiei cariale şi a variabilităţii clinice a carioactivităţii dentare temporare. Trendul rapid ascendent devine tot mai pregnant în raport cu etapa de vârstă a copilului (3-6 ani), la nivel socio-economic scăzut sau nivel educaţional precar al aparţinătorului, carioactivitatea crescută a mamei, in cazul părinţilor foarte ocupaţi, copii crescuţi de rude. Se cunoaşte că peste 64% dintre leziunile necavitare la vârsta de 2 ani, progresează către cavităţi într-un an, aspect susţinut de clasificarea Mejare 199 (3).

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TRATAMENTUL ORTODONTIC PREPROTETIC – UN MODEL DE INTERDISCIPLINARITATE ÎN MEDICINA DENTARĂ

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PREPROSTHETIC ORTHODONTIC TREATMENT (Abstract): If, not long time ago, the simple prosthetic rehabilitation of an edentulous patient – with or without additional maxillary teeth abnormalities – was the only therapeutic solution, presently, the pre-prosthetic orthodontic treatment enabling the dental restoration treatment meets the basic requirements of any dental treatment, i.e. the (re)set-up of the morphological and functional integrity and the functions of jaws, with minimum biological sacrifice. **Key words**: MAXILLARY TEETH ABNORMALITY, PRE-PROSTHETIC ORTHODONTIC TREATMENT

Tratamentul ortodontic preprotetic a intrat în arsenalul terapeutic al medicului dentist din necesitatea de a răspunde cerinţelor tot mai elevate ale pacientului, din ce în ce mai informat, care se prezintă în clinică pentru tratament de specialitate.

Argumentele care vin în sprijinul acestui tip de tratament sunt de natură:

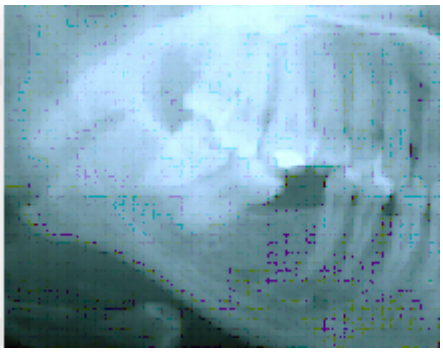

Fig. 1. Solicitarea nefiziologică a molarului doi inferior a determinat transformarea pungii parodontale false într-o pungă parodontală adevărată, care ar fi putut periclita, în lipsa unei intervenţii energice, poziţia dintelui pe arcadă (colecţia Prof. Dr. Dragoş Stanciu)

Biomecanică: odată modificată poziţia corectă de implantare a dinţilor, forţele masticatorii nu mai pot acţiona în axul lung al acestora, ceea ce determină apariţia de forţe parazite, care produc, mai lent sau mai accelerat, afectarea parodonţiului de susţinere (fig. 1), cu efecte pe termen lung asupra „persistenţei" dintelui pe arcadă;

Biologică: există situaţii, şi nu puţine, când printr-un tratament ortodontic bine condus, şi în situaţia când diagnosticul a fost pus corect (ceea ce a dus la selecţia riguroasă a cazului!), spaţiul edentat se închide complet sau aproape complet, fără a mai fi necesară protezarea ulterioară (fig. 2 a şi b);

Estetică: protezarea, de exemplu a pacienţilor cu anomalii de Clasa a II-a, diviziunea 2, fără redresarea prealabilă măcar a poziţiei patognomonice a incisivilor laterali, nu poate oferi rezultate satisfăcătoare din punct de vedere estetic nici pentru cei mai „nepretenţioşi" pacienţi (fig. 3);

Profilactică: dispozitivele ortodontice permit refacerea punctelor de contact inter-

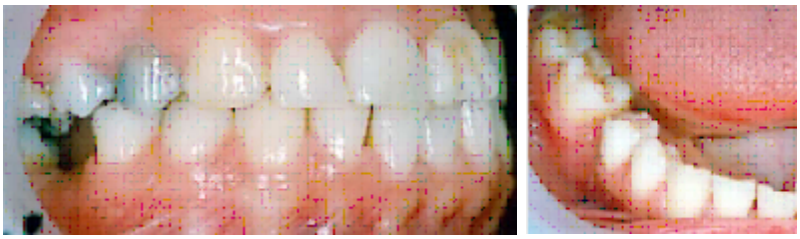

Fig. 2. Edentaţia molarului de şase ani (a) a fost rezolvată prin mijloace ortodontice, care au determinat închiderea aproape completă a breşei (b) (colecţia Prof. Dr. Dragoş Stanciu, Dr. Ileana Simion)

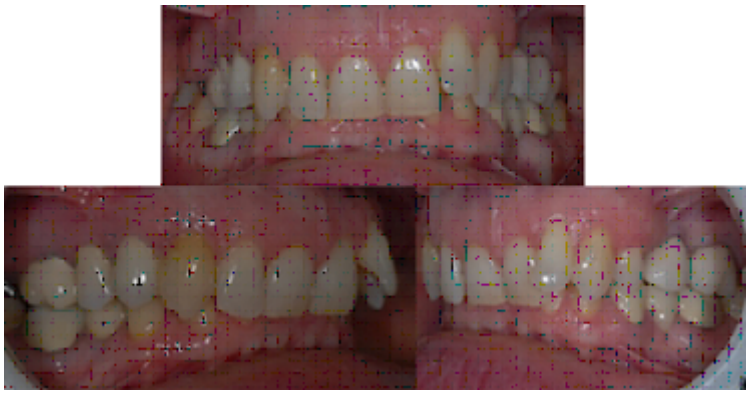

Fig. 3. Rezolvarea protetică a edentaţiilor acestei paciente care prezintă o anomalie de Clasa a II-a, diviziunea 2, înainte de rezolvarea ortodontică a anomaliei de bază s-a soldat cu rezulatate estetice cel puţin necorespunzătoare, care agravează deficitul fizionomic iniţial al malocluziei (colecţia Prof. Dr. Dragoş Stanciu)

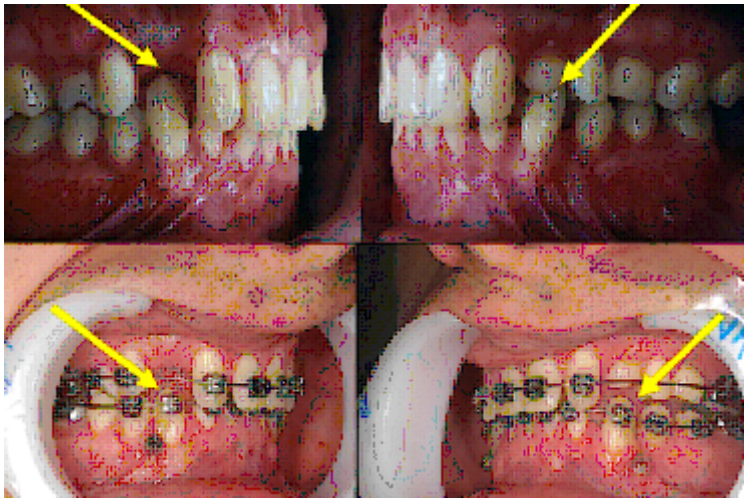

Fig. 4. Redresarea ortodontică a caninilor inferiori a permis protezarea corespunzătoare a zonelor edentate de la maxilar (colecţia Prof. Dr. Dragoş Stanciu, Dr. Ileana Simion)

dentare, cu implicaţii asupra stării de sănătate parodontală ;

Ocluzală : stabilirea / restabilirea unui ghidaj normal, graţie unui tratament ortodontic corespunzător, asigură garanţia unor rezultate protetice foarte bune pe termen lung ; mai mult decât atât, în situaţia unei ocluzii „prăbuşite", tratamentul ortodontic

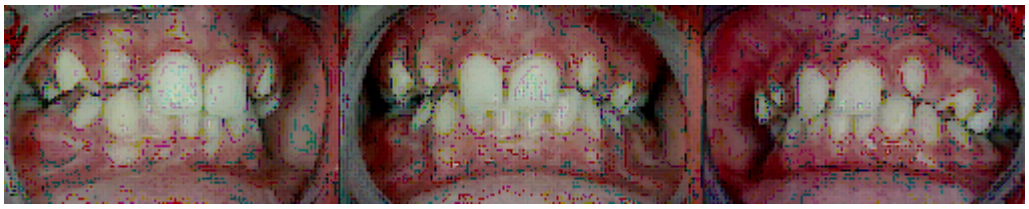

Fig. 5. Aparatul ortodontic va permite, la această pacientă cu anodonţie de incisivi laterali, alunecarea cât mai distal a breşelor edentate, evitându-se astfel protezarea zonelor frontale, cu toate inconvenientele acesteia (colecţia Prof. Dr. Dragoş Stanciu)

este singurul care poate asigura spaţiul necesar inserţiei lucrărilor protetice, în plan vertical – fără modificarea dimensiunii verticale de ocluzie dincolo de limitele fiziologice ale ATM (fig. 4).

Obiectivele tratamentului ortodontic preprotetic, schematic, sunt reprezentate de :

- repoziţionarea dinţilor stâlpi şi paralelizarea acestora ;
- distribuţia mai bună a dinţilor pe arcade, cu mărirea sau micşorarea breşelor edentate în vederea unei eventuale restaurări protetice ulterioare (fig. 5) ;
- repoziţionare radiculară corespunzătoare.

Tehnicile de tratament ortodontic preprotetic folosesc în majoritatea cazurilor aparatură fixă poliagregată, bazată pe conceptul Edgewise Standard Tweed Merrifield.

Etapele de tratament au fiecare obiective distincte, iar aceste obiective sunt aliniate scopului final al tratamentului, fie că este vorba de închiderea completă a spaţiului, şi atunci nu mai este nevoie de protezare ulterioară, fie că este vorba de redistribuirea spaţiului, în vederea unei refaceri protetice cu minim de sacrificiu biologic.

Aparatele şi forţele extraorale sunt utilizate mai ales pentru suplimentarea ancorajului, atunci când mijloacele de tip intraoral nu sunt suficiente pentru a asigura deplasările dentare dorite, pe de o parte, iar pe de altă parte, acestea pot exercita şi acţiuni ortodontice precum corectarea poziţiei molarilor primi superiori, ingresiunea sau egresiunea molarilor, distalizări dentare, modificarea planului de ocluzie, etc.

La sfârşitul tratamentului activ se instituie contenţia, care reprezintă un amalgam de tehnici şi mijloace gândite pentru a stabiliza rezultatele obţinute.

După perioada de contenţie pacientul este redirecţionat către un serviciu de protetică dentară, pentru a se institui, cât mai curând, un tratament de specialitate.

Ca o concluzie putem afirma că principalele avantaje ale tratamentului ortodontic preprotetic sunt reprezentate de posibilităţile pe care acesta le oferă de a restabili funcţionalitatea aparatului dento-maxilar cu minim de sacrificiu biologic, iar principalul dezavantaj – timpul destul de lung de tratament – poate fi uşor contracarat de fundamentele solide pe care tratamentul ortodontic le oferă ca bază de plecare proteticianului.

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EVALUAREA EXPRESIEI PROTEINELOR P16^{INK4A} ŞI Ki-67 ÎN NEOPLAZIA INTRAEPITELIALĂ CERVICALĂ ŞI CORELAREA ACESTORA CU INFECŢIA HPV-HR

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EVALUATION OF P16^{INK4A} AND Ki-67 PROTEINS EXPRESSION IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND THEIR CORRELATION WITH HPV-HR INFECTION (Abstract): **Objective**: The aim of our study was to evaluate the expression of p16^{INK4A} and Ki-67 in cervical intraepithelial neoplasia (CIN) and to correlate the high-risk human papillomavirus (HPV) infection with these two biomarkers. **Material and method**: Sixty patients were included in our study. All of the patient underwent colposcopy-guided biopsy to asses the grade of CIN. For the immunohistochemistry exam we used p16^{INK4A} and Ki-67 antibody and their expression was semiquantitatively classified in 4 classes: 0, 1, 2 and 3. HPV genotyping was used to detect high-risk HPV. **Results**: 7 of our cases were benign (11.66%); 18 cases presented CIN1 (30%), 14 cases CIN2 (23.33%), 16 cases CIN3 (26.66%) and 5 cases invasive squamous cell carcinoma (8.33%). We observed that the expression of p16^{INK4A} and Ki-67 were positively associated with CIN grade. p16^{INK4A} expressions increased significantly with high-risk HPV infection. We observed a positive correlation between the expression of the p16^{INK4A} and Ki-67. **Conclusion**: In our study, the expression of p16 and Ki-67 were positively related to the CIN grade. p16^{INK4A} expressions of high-risk HPV specimens significantly increased more than Ki-67. Therefore, in the diagnosis of CIN and high-risk HPV infection, p16^{INK4A} can be a useful biomarker. **Key words**: P16^{INK4A}, KI-67, CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN), HIGH RISK HUMAN PAPILLOMA VIRUS (HPV-HR)

Cancerul cervical este al doilea cancer ca frecvenţă pe plan mondial (1,2) şi pe locul trei în Europa. În Uniunea Europeană există variaţii mari ca incidenţă şi mortalitate, cea mai mare mortalitate fiind înregistrată în România (13,7/100.000 femei/an) (3). Având în vedere aceste aspecte este de înţeles interesul deosebit pentru diagnosticarea precoce a leziunilor cervicale preinvazive în vederea scăderii morbidităţii şi mortalităţii prin acest tip de cancer.

Diagnosticul de certitudine al neoplaziei intraepiteliale cervicale (CIN) impune examen histopatologic, încadrarea leziunilor realizându-se în conformitate cu terminologia utilizată la nivel internaţional (4). Detectarea şi gradarea leziunilor poate fi uneori dificilă, modificările inflamatorii şi hormonale de la nivelul colului uterin, dimensiunea redusă a probelor sau prezenţa unor focare mici de CIN putând crea dificultăţi de diagnostic (5).

Scopul acestei cercetări este de a evalua expresia proteinelor $p16^{INK4a}$ şi Ki67 în CIN şi corelaţia acestora cu infecţia HPV-HR (high risk human papilloma virus), în vederea studierii potenţialului de proliferare celulară accentuată prin prezenţa HPV-HR şi dereglarea ciclului celular urmare a activării expresiei oncogenelor virale, evenimente precoce în mecanismul carcinogenic.

MATERIAL ŞI METODĂ

Dintr-o cohortă de 393 de paciente care s-au prezentat la Spitalul de Obstetrică şi Ginecologie Cuza-Vodă Iaşi în perioada ianuarie 2008-decembrie 2009, au fost studiate 60 de paciente cu vârste cuprinse între 22 şi 67 de ani. Lotul de studiu a fost investigat prin biopsie ţintită colposcopic şi genotiparea HPV. Examenul histopatologic şi imunohistochimic au fost realizate în Laboratorul Disciplinei de Morfopatologie al UMF „Gr.T. Popa", Iaşi. Genotiparea HPV s-a efectuat în cadrul Laboratorului de Inginerie Genetică Virală a Institutului de Virusologie „Ştefan S. Nicolau", Bucureşti.

Pentru examenul histopatologic biopsiile au fost fixate în formol tamponat, incluse la parafină şi apoi colorate cu hematoxilină-eozină (H-E). Diagnosticul histopatologic a fost stabilit în conformitate cu terminologia CIN.

Pentru genotiparea ADN HPV s-a utilizat tehnica PCR (Roche, Linear Array HPV Genotyping), în fiecare rundă fiind incluse controale pozitive şi negative.

Examenul imunohistochimic a utilizat anticorpii $p16^{INK4a}$ (MTM, clona E6H4TM) şi Ki-67 (Dako, clona MIB-1). Coloraţiile imunohistochimice s-au realizat prin metoda Avidin-Biotin Complex. Vizualizarea cu DAB a permis evidenţierea $p16^{INK4a}$ şi a Ki67 sub forma unui produs de reacţie de culoare brună. În fiecare serie au fost incluse controale negative şi controale pozitive.

Imunomarcajul pentru $p16^{INK4a}$ nuclear +/- citoplasmatic, a fost interpretat utilizând un scor semicantitativ (6) :

- 0- rezultat negativ 0%-5% celule imunomarcate ;
- 1- pozitivitate focală/izolată sau pozitivitate difuză întâlnită în 1/3 inferioară a epiteliului ;
- 2- pozitivitate difuză în 2/3 inferioare ale epiteliului ;
- 3- pozitivitate difuză în toată grosimea epiteliului.

Imunomarcajul pentru Ki-67 a fost clasificat în patru clase în raport cu extinderea colorării nucleare în grosimea epiteliului, în baza următorului scor semicantitativ (7).

- 0- rezultat negativ, imunomarcarea exclusivă a celulelor parabazale ;
- 1- imunomarcaj nuclear prezent în 1/3 inferioară a epiteliului ;
- 2- imunomarcaj nuclear prezent în 2/3 inferioare ale epiteliului ;
- 3- imunomarcaj nuclear prezent în toată grosimea epiteliului.

REZULTATE

Distribuţia cazurilor în raport de examenul histopatologic a fost următoarea : 7 cazuri cu aspect normal (11,66%), 18 cazuri cu CIN1 (30%), 14 cazuri cu CIN2 (23,33%), 16 cazuri cu CIN3 (26,66%) şi 5 cazuri de carcinom scuamo-celular invaziv (CSC) (8,33%).

Rezultatele genotipării HPV au fost gru-

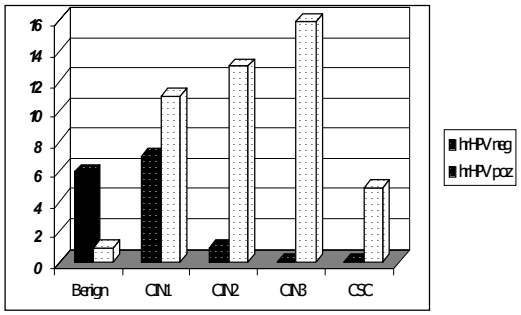

Fig. 1. Distribuţia infecţiei HPV-HR în raport cu diagnosticul histopatologic

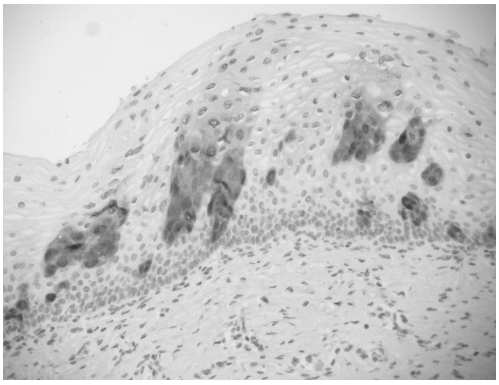

Fig. 2. CIN1. Pozitivitate focală a p16^{INK4a} în 1/3 inferioară a epiteliului (IHC, p16^{INK4a} x20)

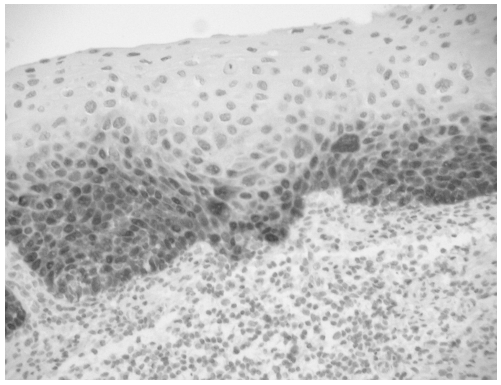

Fig. 3. CIN2. Pozitivitate difuză a p16^{INK4a} în 2/3 inferioare ale epiteliului (IHC, p16^{INK4a} x20)

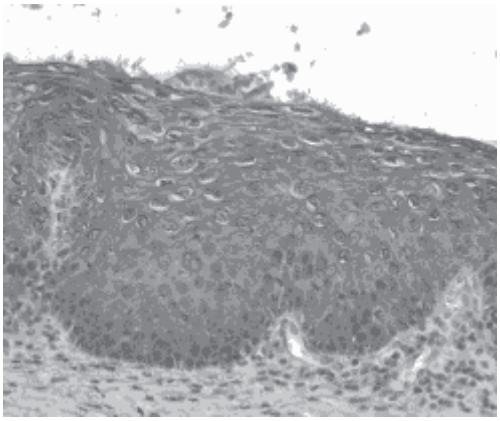

Fig. 4. CIN3. Pozitivitate difuză a p16^{INK4a} în toată grosimea epiteliului (IHC, p16^{INK4a} x20)

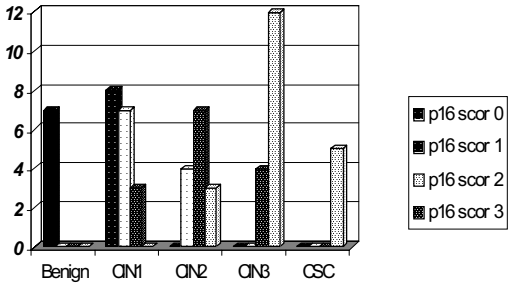

Fig. 5. Distribuţia imunomarcajului p16^{INK4a} în raport cu diagnosticul histopatologic

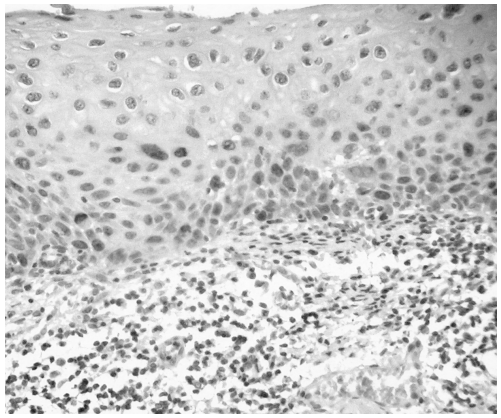

Fig. 6. CIN 1. Imunomarrcaj nuclear în 1/3 inferioară a epiteliului (IHC, Ki67 x20)

pate în raport cu grupa de risc şi diagnosticul histopatologic (fig. 1). Infecţia cu HPV-HR a fost întâlnită într-un singur caz cu col normal histopatologic (14,28%), în 61,11% din cazurile de CIN1 (11/18), în 92,85% din CIN2 (13/14 cazuri) şi în toate cazurile cu CIN3 (16 cazuri) şi CSC (5 cazuri).

Supraexpresia p16^{INK4a} a fost evidenţiată în 45 de cazuri (75%), dintre care 11 cazuri cu scor 1 (fig. 2), 14 cazuri cu scor 2 (fig. 3) şi restul de 20 cazuri cu pozitivitate difuză în întreaga grosime a epiteliului (scor 3) (fig. 4). Rezultatele imunomarcajului pentru p16^{INK4a} în raport cu diagnosticul histopatologic sunt prezentate în figura 5.

Expresia proteinei Ki-67 a fost observată în 37 de cazuri (61,66%), 9 cazuri au prezentat scor 1 (fig. 6), 13 cazuri scor 2 (fig. 7) şi 15 cazuri scor 3 (fig. 8). Distribuţia imunomarcajului pentru Ki-67 în ra-

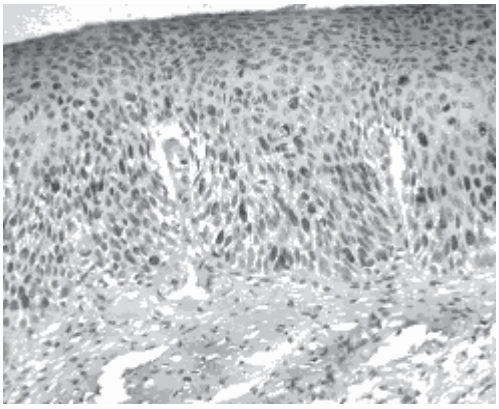

Fig. 7. CIN2. Imunomarcaj nuclear în 2/3 inferioare ale epiteliului (IHC, Ki67 x20)

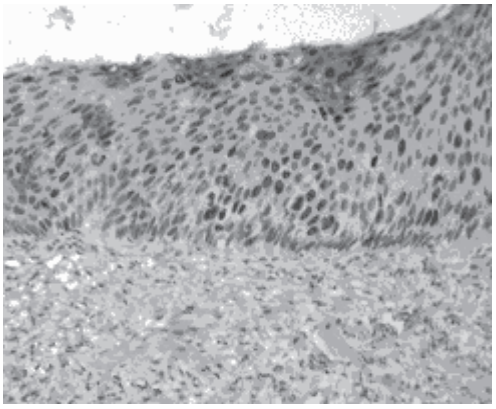

Fig. 8. CIN 3. Imunomarcaj nuclear în toată grosimea epiteliului (IHC, Ki67 x20)

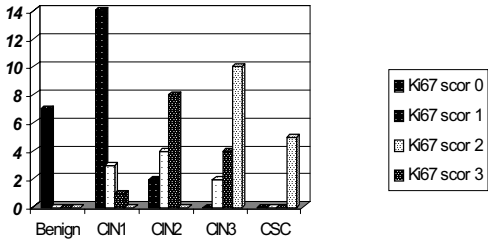

Fig. 9. Distribuţia imunomarcajului Ki-67 în raport cu diagnosticul histopatologic

port cu diagnosticul histopatologic este prezentată în figura 9.

DISCUŢII

Analiza morfologică a biopsiilor cervicale rămâne standardul de aur în diagnosticarea şi gradarea neoplaziei intraepiteliale cervicale, stabilirea conduitei terapeutice în cazul leziunilor cervicale intraepiteliale de grad înalt depinzând de confirmarea histopatologică a CIN. Aspectele histopatologice ale neoplaziei intraepiteliale de grad înalt (CIN2/CIN3) sunt bine stabilite, dar uneori dimensiunea redusă a biopsiilor cervicale, prezenţa unor focare mici de CIN sau modificările inflamatorii şi hormonale întâlnite la nivelul colului uterin pot pune probleme de diagnostic diferenţial între modificările benigne şi neoplazia scuamoasă intraepitelială, precum şi în stabilirea gradului neoplaziei intraepiteliale (8,9).

Proteina $p16^{INK4a}$, reprezintă cel mai studiat şi mai promiţător biomarker al neoplaziei intraepiteliale cervicale fiind utilizat şi ca marker surogat al infecţiei HPV-HR. Proteina $p16^{INK4a}$ este un inhibitor al ciclin dependent kinazelor (CDK), fiind supraexprimată atât în neoplazia intraepitelială cât şi în carcinomul cervical, ca rezultat al interacţiunii oncoproteinei E7 a HPV-HR cu proteina supresor tumorală pRb (10,11,12).

Numeroase studii recente au stabilit valoarea $p16^{INK4a}$ ca marker surogat al infecţiei HPV-HR şi al CIN (13,14,15,16,17). Acestea au arătat că $p16^{INK4a}$ este supraexprimată în majoritatea leziunilor cervicale, iar statusul imunoreactivităţii permite diferenţierea dintre infecţiile HPV-LR (low risk human papilloma virus) şi HPV-HR (17).

În studiul de faţă am investigat distribuţia şi intensitatea imunomarcajului pentru $p16^{INK4a}$ în neoplazia intraepitelială cervicală şi am încercat stabilirea unei corelaţii între supraexpresia $p16^{INK4a}$ şi infecţia HPV-HR.

Rezultatele noastre au arătat o supraexpresie a $p16^{INK4a}$ în 10 (55,55%) din cele 18 cazuri de CIN1. Dintre acestea 7 au prezentat scor 1 şi 3 cazuri scor 2. În cele trei cazuri cu scor 2 supraexpresia macată a $p16^{INK4a}$ poate indica un risc crescut de progresie a leziunilor. Majoritatea cazurilor

de CIN1 p16^{INK4a} negativeau fost şi HPV-HR negative, unul singur prezentând infecţie HPV-HR.

În 71.42% din cazurile cu neoplazie intraepitelială de grad înalt (CIN2/CIN3) am întâlnit supraexpresia marcată a p16^{INK4a} (scor 2, 3). Excepţie au făcut patru cazuri cu CIN2 în care marcajul pentru p16^{INK4a} a fost observat doar în treimea inferioară a epiteliului (scor 1), unul dintre aceste cazuri fiind HPV-HR negativ. În schimb toate cele cinci cazuri de carcinom scuamocelular invaziv au prezentat scor 3.

Rezultatele obţinute susţin faptul că acest biomarker poate fi util în identificarea mai precisă a gradului neoplaziei intraepiteliale pe secţiunile histologice şi în plus poate reduce variaţia în interpretarea leziunilor cervicale. Aceste rezltate susţin deasemenea faptul că p16^{INK4a} poate fi utilizat ca marker surogat al infecţiei HPV-HR, 95,65% dintre cazurile HPV-HR pozitive prezentând supraexpresia p16^{INK4a}.

Pentru creşterea acurateţii diagnosticului CIN s-au efectuat numeroase studii care au avut drept ţintă antigenul Ki-67 ca marker al proliferării celulare (18,19). Ki-67 nu este specific unei anumite faze ale ciclului celular, fiind exprimat în toate etapele active ale acestuia, exceptând faza G_0 (20,21).

Infecţia HPV activează progresia ciclului celular al gazdei ceea ce are un impact direct asupra expresiei markerilor de proliferare în sensul creşterii acestora. Studii realizate pe biopsii cervicale au demonstrat că distribuţia şi indexul Ki-67 creşte odată cu gradul leziunii, atingând un maxim în cazul carcinomului scuamo-celular invaziv (CSC) (22,23).

În leziunile intraepiteliale cervicale prezenţa celulelor Ki-67 pozitive creşte dinspre stratul parabazal (unde sunt întâlnite şi în cazul epiteliului normal) spre straturile intermediare şi superficiale odată cu creşterea gradului leziunii, conferindu-i acestui marker un rol important în diferenţierea neoplaziei intraepiteliale cervicale de epiteliul cervical normal, metaplazic, atrofic sau cu modificări reactive (22).

În studiul nostru toate cazurile cu modificări benigne şi 14 dintre cazurile cu CIN1 au fost negative la marcarea cu Ki-67. Creşterea indexului de proliferare, evidenţiat prin prezenţa marcajului cu Ki-67 a fost întâlnit în 4 (22,22%) dintre cazurile de CIN1, 12 (85,71%) dintre cazurile cu CIN2 şi în toate cazurile (100%) cu CIN3 şi CSC. Se observă o corelaţie clară între numărul de cazuri Ki-67 pozitive şi creşterea gradului leziunii, cu o mică diferenţă între cazurile cu CIN2 şi CIN3. În schimb diferenţa este clară între cazurile cu CIN1 şi cele cu CIN2. În toate cazurile cu CIN1, marcajul pentru Ki-67 a fost întâlnit în treimea inferioară a epiteliului (scor 1), în timp ce în leziunile de grad înalt imunomarcarea s-a observat în 2/3 superioare ale epiteliului (scor 2 şi 3).

Combinarea acestor doi markeri, Ki-67 şi p16^{INK4a}, alături de genotiparea HPV reprezintă instrumente utile în identificarea leziunilor CIN oculte, în verificarea şi confirmarea diagnosticului histopatologic în cazurile echivoce, crescând astfel acurateţea interpretării histopatologice a biopsiilor cervicale şi instituirea unui tratament adecvat (24).

CONCLUZII

Rezultatele noastre indică existenţa unei corelaţii directe între activitatea proliferativă (Ki-67) şi gradul displaziei cervicale, precum şi asocierea supraexpresiei p16^{INK4a} cu prezenţa unei leziuni de grad înalt şi a infecţiei HPV-HR- elemente relevante în succesiunea secvenţelor mecanismului patogenic al carcinogenezei cervicale.

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STUDIU COMPARATIV PRIVIND APORTUL DE ALIMENTE LA UN LOT DE PERSOANE CU DISLIPIDEMII FAŢĂ DE UN LOT MARTOR, DIN ZONA TÂRGU NEAMŢ

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COMPARATIVE STUDY CONCERNING FOOD INTAKE IN A GROUP OF PEOPLE WITH DYSLIPIDEMIAS *VS.* A WITNESS GROUP IN THE TÂRGU NEAMŢ AREA (Abstract): The aim of the study was to find a correlation between the consumption frequency of the main food groups and the presence of dyslipidemia as compared to a witness group without dyslipidemias. **Material and method**: For this study we have considered a number of 678 people, divided in two groups: 339 with dyslipidemias and 339 without dyslipidemias, each of the two groups comprising 137 subjects from the rural area and 201 from the urban area, through the questionnaire method. **Results**: The involved people have been questioned about the consumption of milk and related products (cheese), meat and related products (beef and pork), fish and related products and fruits. Regarding the fruit consumption, in the urban area, we have found slightly increased values in the witness group of the percentages of those who eat them daily and 4-6 times a week as compared to the group with dyslipidemias. **Key words**: DYSLIPIDEMIAS, CHEESE, FISH, FRUITS

CORRELATION AMONG CHRONOLOGICAL AGE, DENTAL AGE AND CERVICAL VERTEBRAE MATURITY IN ROMANIAN SUBJECTS

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CORRELATION AMONG CHRONOLOGICAL AGE, DENTAL AGE AND CERVICAL VERTEBRAE MATURITY IN ROMANIAN SUBJECTS (Abstract): **Aim:** The purpose of this study was to assess the correlation among the chronological age, the dental age and the skeletal maturity in adolescents and young adults. **Materials and methods**: The material consisted of panoramic and lateral cephalometric radiographs of 221 subjects, 146 girls (mean age 13.79 ±2.90) and 75 boys (mean age 13.46 ±2.82), inhabitants of Mures County (Central Romania). The chronological age was defined as the time from birth to the day the radiographs were taken. The dental age was evaluated according to Demirjian's method for third molars mineralization stage. Skeletal maturity was evaluated on cephalometric radiographs using the cervical vertebrae maturation (CVM) method. Descriptive statistical and linear regression analysis was performed and a coefficient of correlation was calculated. **Results**: The mean values obtained for cervical stages for boys and girls were significant ($p=0.0171$). The third molar mineralization seems to be highly correlated with the gender's chronological age for the whole group and for both genders as well ($p<0.0001$). The medium age of the cervical maturation showed to be younger in girls than in boys in CS1, CS3, CS5 and CS6 stages. In CS4 stages female subjects are in advance with almost 12 months, these subjects start (CS1) and end (CS6) the cervical maturation at a younger age. **Conclusions:** The results indicate that significant correlation exist between the chronological age and the dental age when crown development ends and between the chronological age and CVM (CS1 and CS6 stages for girls and CS2 and CS5 stages for boys). Keywords: DENTAL AGE, CERVICAL MATURITY, DEMIRJIAN INDEX, CHRONOLOGICAL AGE, MOLAR MINERALIZATION.

The assessment of the developmental stage of children, adolescents or young adults can be useful in many fields of medicine, even though it is difficult to do it because of the individual variation of overall developmental processes. Children with the same chronological age may show differences in the developmental stages of different biological systems (1). Assessing maturational status, whether the pubertal growth spurt of a patient has been reached or completed, can have a considerable influence on diagnosis, on treatment goals, on treatment planning, and on the eventual outcome of the orthodontic treatment (2).

Considerable variations in the development among children of the same chronological age have led to the concept of bio-

logic or physiologic age. The physiologic age is the registry of progress towards maturity that can be estimated by somatic, sexual, skeletal and dental maturity (3).

The dental age has been defined in the literature as "the subject's estimated age based on the level of tooth mineralization during the developmental process or on the eruption stage" (4). There were proposed many methods to asses this parameter: the stage of tooth eruption, the radiographic assessment of the length of the tooth crown and of the root (5). The mineralization of permanent teeth has been studied by a number of researchers (6). This type of examination can be easily done in everyday practice because the panoramic radiographs are usually a part of dental examination and there is no need of additional investigations. The dental age assessment based on tooth calcification visible on a radiograph was described by Demirjian et al. (7). Although this one is the most widely used method, it was formulated for the French Canadian population. The method evaluates the development of seven mandibular teeth from a panoramic radiograph and calculates dental age. Various researchers have tested the applicability of this method on different populations and certain correlations in patients with other racial background than the one this method was described for were found (8, 9). On the other hand, some authors reported significant differences between the dental age of French Canadian population and other populations (10, 11).

However, the study of the dental development remains a reliable method, because the development of a tooth is much less affected than other tissues by endocrine disorders or other developmental problems. Several studies concluded, that children with sexual, systemic or congenital disorders show small deviations in timing of dental development compared with healthy subjects (12, 13).

The degree of skeletal development is a reflection of the degree of physiological maturation of a subject (14). The bone age was shown to be as important as the chronological age in evaluating a patient's physical development (15). The skeletal maturation can be assessed usually using radiological methods; the classical one is based on the analysis of the hand-wrist radiographs (16, 17).

A disadvantage of this method is that it requires additional radiographic images and exposure to radiation beside those used for an orthodontic diagnosis. A commonly used method nowadays is based on the radiological assessment of cervical vertebrae outlines, the so called cervical vertebrae method (CVM). The size and shape of C2, C3 an C4 vertebrae are analyzed and the skeletal age is divided in six stages (18). This method of assessment of skeletal maturity does not require extra radiation exposure for the patient, because the analysis can be performed on lateral cephalometric radiographs, which are often used in the orthodontic diagnosis.

The aim of this study was to find a correlation between the third molar mineralization stage and the cervical vertebral maturity in Romanian subjects and to evaluate if radiographic evaluation of the third molar's development can be useful in the chronological age determination.

MATERIAL AND METHODS

After the research protocol was established, it was approved by the Ethical Committee of Scientific Research of the University of Medicine and Pharmacy from Tirgu Mures. A written consent was ob-

tained from each patient or their parents/legal representative in order to use patient's radiographs for this study.

Thus, two hundred twenty-one subjects, 146 girls (mean age 13.79 ±2.90) and 75 boys (mean age 13.46 ±2.82) were selected from patients referred for an orthodontic treatment at the Orthodontic Department of Faculty of Dentistry, University of Medicine and Pharmacy from Tîrgu Mureș, Romania. For each subject the digital panoramic and the lateral cephalometric radiographs were analyzed. The digital radiographic images were performed with Pax Flex 3D, Vatech X-ray machine, the exposure time was 12,9 seconds, at 80kVp and 9.0mA. The dental imaging software used was EasyDent. By using digital radiographs, it allowed us to enlarge certain parts of the images and perform a clear tracing of the bones or of the intraosseous dental structures.

The inclusion criteria were: (1) children of Romanian origin, (2) no history of orthodontic treatment before this radiographic examination, (3) good general health (4) no growth affecting hormonal or systemic disease, (5) good quality of lateral cephalometric and panoramic radiographs, (6) no history of trauma or surgery in cephalic or cervical region.

The chronological age was calculated based on the date of birth and the amount of time that passed from birth to the day of the radiography. The obtained values were rounded down and noted in years and decimal points.

The dental age was described on panoramic X-rays, using the Demirjian's method. The development of the four third molars was assessed on panoramic radiographs by means of the eight-grade scale according to Demirjian's system (fig. 1). Every determination was performed once by every author and if the difference exceeded one developmental stage, a lower stage was chosen. The tooth calcification stages were rated from A to H and a numeric value was assessed for each stage from 0 to 8 (A=0 to H=8). For each molar a stage was determined and from the obtained values a medium value was calculated for every subject (19). When the third molars mineralization stages were statistically analyzed and correlations were defined, cases with agenesis and molars in the first stage (bud-stage) mineralization were not included in statistical analysis.

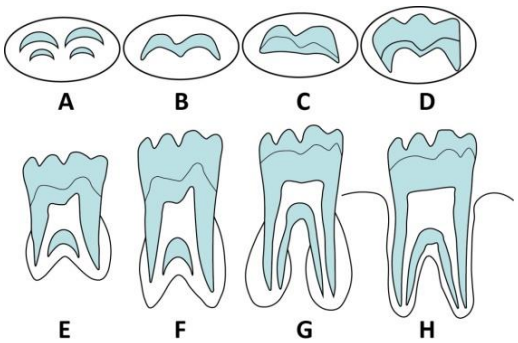

Fig. 1. The classification of the degree of molar tooth mineralization according to Demirjian et al.

The skeletal maturation stage was evaluated using the skeletal vertebrae method (CVM), by tracing the outlines of three cervical vertebrae (C2, C3 and C4) on lateral cephalometric radiographs. The tracing was performed visually by enlarging the cervical region on digital radiographs. The presence or the absence of concavity at the lower border of C2-C4 vertebrae as well as the shape of the vertebral bodies of C3 and C4 vertebrae (trapezoidal, horizontal, square and vertical) were analyzed. Six maturation stages were described (CS1 – CS6) and

numeric values were added for each stage (CS1=1,…CS6=6) (18, 20). (fig. 2)

All data were introduced in Excel and a statistic analysis was performed using the Excel Data Analysis package. Than a main statistic interpretation was performed; the mean values were calculated for the chronological age and for the cervical vertebrae maturation separate per genders and for the whole study group. A descriptive statistical and linear regression analysis was performed and a coefficient of correlation was calculated. A Student-test was used to compare the mean values of the chronological age and the dental development stages within different skeletal maturation stages; $p<.05$ was considered significant. A Chi test was used to study the association between the cervical vertebrae maturation and the dental calcification for girls and boys separately and for the whole group. Pearson's *r* correlation coefficient was calculated for the studied variables.

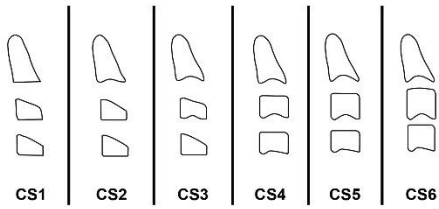

Fig. 2. The cervical vertebrae maturation stages

RESULTS

Comparing the mean values for all the studied variables between the two genders, chronological age and Demirjian index showed no statistical significance (p=0.41-0.43). The mean values obtained for cervical stages for boys and girls were significant (p=0.0171).

TABLE I

The mean values for the studied variables for boys, for girls and for the whole group (95% confidence interval)

Variable/group	Mean values			Probability of the test (p) between genders
	male	female	whole group	
Chronological age	13,46 ± 2,825	13,79 ± 2,903	13,68 ± 2,875	0.4134
Cervical stages	3,45 ± 1,445	3,95 ± 1,468	3,78 ± 1,476	0.0171
Demirjian's index	2,94 ± 1,348	3,08 ± 1,088	3,03 ± 1,186	0.4342

The mean values for the chronological and the dental ages for every cervical stage (CS1-CS6; tab. II) and tooth calcification stage (B-H; tab. III) were also calculated. As agenesis of third molar has an increasing tendency, we have included the modifications of Demirjian's method assigning scores of "0" in case of the absence of the third molar and "1" in case of the presence of a radiolucent bud (21). This is the reason why we excluded from our further statistical interpretation the subjects with agenesis of the third molar and those who had molars in a bud-stage.

A linear regression analysis and correlation was used to study the association between the chronological age and different cervical maturation stages (tab. IV) for girls and boys separately and for the whole group as well.

TABLE II

The mean values of the chronological age for different cervical maturation stages

Cervical stage/gender	n=75	n=146	Chronological age	
	males	females	males	females
CS1	7	9	12,00 ± 2,02	10,12 ± 1,85
CS2	16	20	11,23 ± 1,80	11,33 ± 2,84
CS3	12	19	13,46 ± 2,58	12,87 ± 2,78
CS4	23	46	13,19 ± 2,00	14,11 ± 2,15
CS5	10	25	15,37 ± 2,12	14,66 ± 1,49
CS6	7	27	18,13 ± 2,26	16,13 ± 2,85

TABLE III

The mean values of the chronological age and its correlation with different tooth calcification stages (* statistically strong significance)

Variable/tooth calcification stage	Mean value of chronological age		Probability of the test (p)	
	females	males	females	males
B	11,18 ± 1,89	11,76 ± 1,82	0.6085	0.1786
C	13,59 ± 1,36	11,95 ± 1,69	0.0004*	0.1476
D	14,15 ± 1,52	13,82 ± 2,18	<0,0001*	<0,0001*
E	15,11 ± 2,49	14,65 ± 1,92	0.7594	0.6482
F	18,59 ± ,91	17,13 ± 1,37	0.0004*	0.1737
G	23.4	19,10 ± 2,54		0.9653
H	20,85 ± 1,34	21,76 ± 1,82	0.7547	0.1786

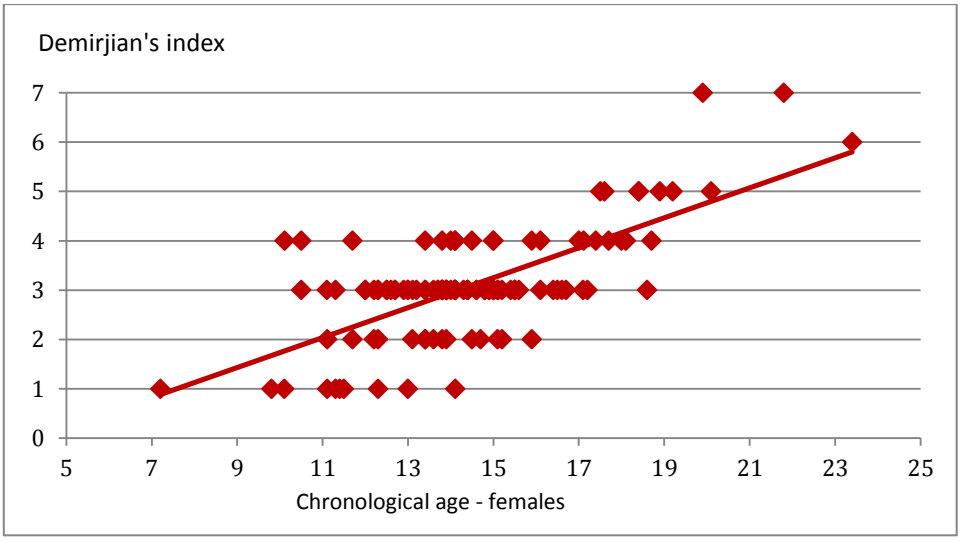

Fig. 3 a. Chronological age in females related to Demirjian's index

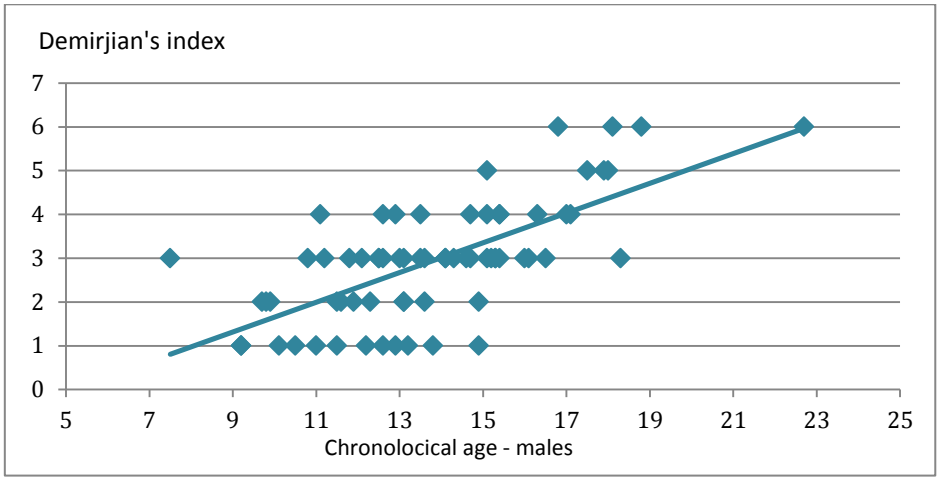

Fig. 3b. Chronological age in males related to Demirjian's index

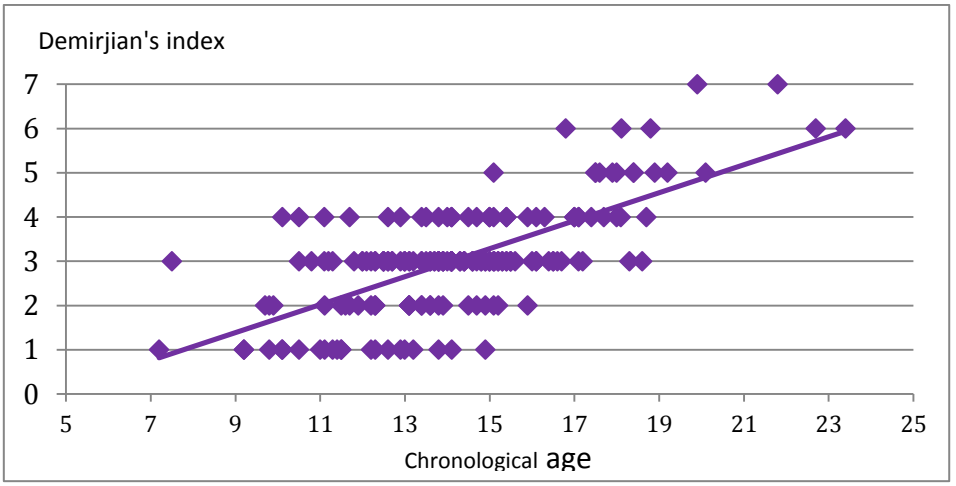

Fig. 3c. Chronological age for the whole studied group related to Demirjian's index

TABLE IV

The correlation coefficients between the chronological age and different cervical maturation stages (* statistically strong significance)

Cervical maturation stages/gender	Correlation coefficient (r)		Probability of the test (p)	
	males	females	males	females
CS1	0.22449196	0.844149912	0.633624	0.053074*
CS2	0.683256604	0.277205275	0.003905*	0.239811
CS3	0.161717393	0.406287936	0.614571	0.089749
CS4	0.517959851	0.583360207	0.011049*	0.000022*
CS5	0.622213149	0.570683236	0.051166	0.002891*
CS6	0.637864788	0.809276152	0.113476	0

TABLE V

The correlation coefficients between the chronological age and different tooth mineralization stages (B-H) in different cervical maturation stages (CS1-CS6). (*statistically strong significance)

Cervical maturation stages /gender	Correlation coefficient (r)		Probability of the test (p)	
	males	females	males	females
CS1	0.2245	0.8441	0.7103	0.2561
CS2	0.6833	0.2772	0.0091*	0.3812
CS3	0.1617	0.4063	0.6146	0.166
CS4	0.518	0.5834	0.0157*	<0,0001*
CS5	0.6222	0.5707	0.0512	0.0034*
CS6	0.6379	0.8093	0.1134	<0,0001*

TABLE VI

The correlation coefficients between the chronological age and different tooth mineralization stages (B-H) for genders (*statistically strong significance)

Subjects	Correlation coefficient (r)	Probability of the test (p)
Whole group (n=221)	0.7065	<0.0001*
Male subjects (n=75)	0.7178	<0.0001*
Female subjects (n=146)	0.7078	<0.0001*

DISCUSSION

When the age determination is required, correlation amongst the chronological age, the skeletal maturity and the tooth development related dental age can be helpful. The evaluation of the chronological age becomes simple if we are aware of patient personal data. The intraosseous tooth development is mostly genetically coordinated and seems to be independent of patient's general conditions and nutrition (22). After the young permanent dentition is complete, in adolescent period, the only unerupted permanent tooth remains the third molar. Even though the age estimation using dental methods have shortcomings and the third molars can have individual pattern of calcification rhythm, formation or intraosseous position, it remains the only tooth which can help in the dental age evaluation in post-pubertal age and for young adults (23).

The determination of skeletal maturation is extremely important in many fields of medicine. In pediatric dentistry and orthodontics, it will help in right the timing of treatment and it will make possible an individualized treatment planning. In forensic sciences it may help in emerging or young adult's corps or skeletons age identification. Beside the hand-wrist method, CVM is a valid indicator of skeletal growth during the pubertal period (24).

Although there is a strong relationship between the skeletal and the somatic maturation, this correlation seemed to be inconclusive between dental age and skeletal maturity (25). Studies revealed different concordance between dental age and skeletal maturity, which can be explained by differences in the evaluation methods, age and racial background of the studied subjects (26, 27, 28).

The selected subjects in our study were referred for orthodontic treatment; the

mean age of subjects of different gender was almost the same and the mean age of the studied group was 13,68 ± 2,875, an age when the permanent dentition is about to be completed (tab. I). When the correlation between the mean age and the skeletal maturity was assessed, the medium age of the cervical maturation showed to be younger in girls than in boys in CS1, CS3, CS5 and CS6 stages, in CS4 stages female subjects are in advance with almost 12 month. The female subjects start (CS1) and end (CS6) the cervical maturation at a younger age (tab. II)

Spearman's rank correlation coefficient (tab. IV) revealed a good relationship between the chronological age and the cervical stages in males (r=0.68, p=0.00) for CS2 and in females (r=0.80, p=0.00) for CS6. The lowest correlation was observed in CS1 for males (r=0.22 p=0.63) and in CS2 for females (r=0.27 p=0.23).

Many authors proved that the *cervical vertebrae maturation* (CVM) has a high level of correlation with individual skeletal maturation (29, 30) and the greatest increase in mandibular length occurred during the pubertal growth spurt and that this could be assessed during the interval between CS3 and CS4 (31, 32). Based on our findings, the pubertal spurt appears with almost one year earlier in female subjects and the whole skeletal maturation is more accelerated in girls.

Correlations between the chronological age and the cervical vertebrae maturation in different studies revealed that this depends on the subject's racial background. Most of the studies found correlation coefficient: a low correlation was found in Chinese (33) and Brazilian (34) subjects, high correlation was found by other several studies (35, 36, 37, 38).

Analyzing the correlation between the chronological age and the different stages of the cervical maturation in both genders, we concluded, that the cervical vertebrae maturity increased as the chronological age increased. The cervical maturation starts earlier in girls and the pubertal growth spurt (CS3 stage) takes place earlier in girls, too: the difference between the medium ages is around 6 months. Another interesting information is that the cervical maturation ends (CS6 stage) with almost two years earlier in female subject, which means that the onset of puberty is related to gender. Statistically strong significance was found in the CS1, CS4 and CS5 stages in female and CS2, CS4 stages in male subjects. The same results were found by several other studies (39, 40, 41).

Analyzing the mean ages for the different CVM stages and the third molar mineralization stages we observed that crown formation of the third molars (D stage) became completed around the pubertal growth spurt (CS3 stage) in males, meanwhile in female subjects this was observed in a later stage (CS4 stage) of cervical maturation. In the last stage of the cervical maturation (CS6 stage) where the skeletal maturation comes to end, two-third of the third molar root length (F stage) is developed in both genders.

Analyzing the significance of statistical data correlation between the chronological age and the third molars mineralization in different skeletal maturation stages, we found that the female subjects present stronger correlations in the second part of cervical maturation (CS4-CS6 stages), meanwhile for male subjects' significant correlations were observed in CS2 and CS4 stages. The third molar mineralization seems to be highly correlated with the gen-

der's chronological age for the whole group and for both genders as well ($p<0.0001$).

Studying the mean values for Demirjian's index (tab. III), there were slightly differences between the two genders at the commencement of the third molar mineralization (B stage) or at the age of complete crown formation (D stage), but the root development (G stage) ended earlier in male subjects. A significant statistical probability was found in D stage ($p<0.0001$) of mineralization for both genders and in F stage ($p=0.0004$) for the female subjects. The assessment of dental mineralization in order to evaluate the dental age seems to be a more accurate method than the study of sequence and of the phenomenon of eruption (42).

Studying the relationship between the third molar development and the chronological age, several studies found statistically significant correlations in the third-molar development between males and females especially in stages D and G (43) or in stages E-G (44). Regarding the age of tooth development, as most of the studies concluded, the female subjects present a delayed development of both the upper and lower third molars compared to the boys (45, 46, 47).

CONCLUSIONS

The number of the studied cases and the fact that they all come from one county can be considered limitations of our study. In order to evaluate the correlation among the chronological age, the dental development and the skeletal age for Romanian subjects, a higher number of subjects from other parts of the country should be selected.

Based on our findings, we conclude the following:

- The root development ended earlier in female subjects, D and F stages of tooth calcification showed significant correlation with the chronological age.
- A strong significant correlation between the chronological age and the tooth mineralization stages was found in D stage of Demirjian's classification (crown formation is complete), for both genders
- The crown formation of the third molars is completed at the age of pubertal growth spurt (CS3) in males and in a later stage of cervical maturation (CS4 stage) in females
- At the age of complete skeletal maturation (CS6 stage) two-third of the third molars roots are developed
- The commencement (CS1) and the end (CS6) of the cervical vertebrae maturation happens earlier in female patients, the last stage of the cervical maturations ends (CS6 stage) with almost two years earlier in female subject.
- A significant correlation between the chronological age and CVM was found in CS1 and CS6 stages for girls and CS2 and CS5 stages for boys.

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EVALUATION OF THE SYNTHESIS AND STRUCTURE OF NEW AZETIDIN-2-ONES OF FERULIC ACID

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EVALUATION OF THE SYNTHESIS AND STRUCTURE OF NEW AZETIDIN-2-ONES OF FERULIC ACID (Abstract): **Aim:** To synthesize some new azetidin-2-ones of ferulic acid and to evaluate them from physicochemical and spectral point of view. **Material and methods:** The synthesis was carried out in several steps: (i) obtaining the ferulic acid chloride; (ii) obtaining the ferulic acid hydrazide with hydrazine hydrate (98%); (iii) condensation of ferulic acid hydrazide with different benzaldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde) in order to obtain the corresponding hydrazones; (iv) cyclization of ferulic acid hydrazones with chloroacethyl chloride in freshly distilled toluene medium and in the presence of triethylamine, resulting in the corresponding azetidin-2-ones. **Results**: Six new azetidin-2-ones of ferulic acid were synthesized. They were characterized in terms of their physicochemical properties and their structure was confirmed by IR and ^{1}H-NMR spectroscopy. **Conclusions:** Six new azetidin-2-ones of ferulic acid were synthesized, physicochemically characterized and validated spectrally. **Keywords:** FERULIC ACID, AZETIDIN-2-ONES, SPECTRAL VALIDATION.

In the present days we are witnessing to a growing interest of researchers in the use of antioxidants in reducing oxidative stress.

Development of new compounds including in their structure biologically active entities, especially heterocyclic systems is a major concern for researchers. In this context, special attention is directed to azetidin-2-one derivatives.

It is known that the azetidin-2-one ring is the common structure of beta-lactam antibiotics such as penicillins, cephalosporins, carbapenems, monobactams and nocardicine used as chemotherapeutic agents in the treatment of infectious diseases (1). Recent studies assign to this heterocyclic ring many other biological effects: hypoglycemic, antitumor, anti-HIV, anti-inflammatory, analgesic and antioxidant actions (2, 3).

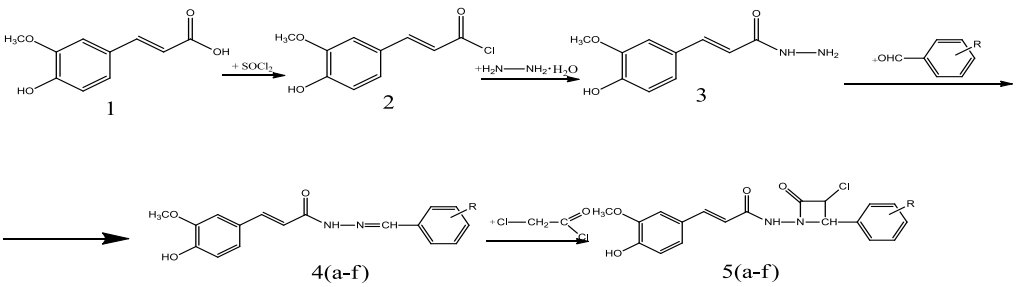
On the other hand, ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a phenolic compound widely distributed in the plant world. Due to its antioxidant effect, ferulic acid may protect DNA and lipids from oxidation by reactive oxygen species and could be useful in the prevention and treatment of several oxidative stress-related disorders including Alzheimer's disease, diabetes mellitus, cancer, hypertension, and atherosclerosis (4, 5).

The aim of this study was to develop new compounds with heterocyclic structure and improved antiinflammatory and antioxidant effects. In order to achieve this goal new azetidin-2-one derivatives of ferulic acid there were synthesized.

MATERIAL AND METHODS

Ferulic acid, thionyl chloride, hydrazine hydrate, aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde), chloroacetyl chloride, triethylamine, organic solvents (*p.a.* quality) were purchased from Sigma Aldrich Company and Fluka Company. All reagents and solvents were used without previous purification. For monitoring the reaction, TLC silica gel 60 F_{254} plates, purchased from Merck Company, were used.

Synthesis of the azetidin-2-one derivatives of ferulic acid: Ferulic acid was reacted with thionyl chloride to obtain the ferulic acid chloride that was next treated with hydrazine hydrate 98% to obtain ferulic acid hydrazide. By condensation with different aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde) the corresponding hydrazones (4a-f) were obtained. These hydrazones were cyclized with chloroacethyl chloride in freshly distilled toluene medium and in the presence of triethylamine to obtain the corresponding azetidin-2-ones (5a-f) (fig. 1) (6, 7, 8).



Compound	-R	Compound	-R
5a	-H	**5d**	-Cl(4)
5b	-OH(2)	**5e**	-F(4)
5c	-NO_2(2)	**5f**	-Br(4)

Fig. 1. Azetidin-2-one derivatives of ferulic acid synthesis

General procedure for azetidin-2-ones of ferulic acid synthesis. Over 0.025 moles (5 g) of ferulic acid and under continuous stirring 0.15 moled os thionyl chloride were added. The reaction mixture was maintained at 50^0C for 4 hours and the excess of thionyl chloride was removed by rotary evaporation. In the second step, over the ferulic acid chloride (0.05 moles) 0.25 moles of hydrazine hydrate 98% were added. The reaction mixture was maintained on ice bath for 2 hours and then at room temperature for another 2 hours, under continuous stirring. Then 15 mL of cold water were added and the precipitate was dried at room temperature. Finally, 0.015 moles (3 g) of ferulic acid hydrazide with a few drops of glacial acetic acid and 0.015 moles of aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benz-

aldehyde) were refluxed for 13-20 hours in absolute ethanol. The reaction was monitored by TLC, using ethyl acetate:methanol:acetone:water (5:2:2:1) as solvent system. In the last step, the 0.0015 moles (0.5 g) of hydrazone with 0.0022 moles of chloroacethyl chloride, in the presence of triethylamine, were refluxed for 15-20 hours in toluene. The reaction was monitored by TLC, using dichloromethane:methanol (9:1) as solvent system. The obtained precipitate was washed with 1M hydrochloric acid, and the organic layer was dried on anhydrous magnesium sulphate and then washed with petroleum ether.

***Physicochemical characterization*:** The azetidin-2-one derivatives of ferulic acid were physicochemically characterized: melting point, output, molecular formula, relative mass, solubility in water and other organic solvents. The general synthesis was optimized in order to gain great output and advanced purity for the obtained derivatives. The reaction was monitored by TLC on 60 F254 silica gel plates, and spots were visualized by UV light at 254 nm. The melting point was determined by using Buchi M 565 (Buchi, Switzerland).

Spectral characterization: The IR spectra of the synthesized derivatives were recorded using a Fourier transform spectrometer, ABB-MB 3000 FT-IR MIRacle^{TM} Single Bounce ATR-crystal ZnSe, and the studied spectral range was between 4000–500 cm^{-1}, at a resolution of 4 cm^{-1}, 16 scans being carried out for each sample. The spectra were interpreted using the Horizon MB^{TM} FT–IR software (9, 10). For ^{1}H-RMN, the samples were dissolved in hexadeuterio-dimethyl- sulfoxide (DMSO-d_6) and the Bruker 400 MHz NMR spectrometer (Germany) was used. The spectral signals were automatically integrated and the chemical shift values were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The spectra were interpreted using MestReNova software (11).

RESULTS AND DISCUSSION

The azetidin-2-one derivatives of ferulic acid are crystalline powders, colored in different shades of brown, very slightly soluble in dimethylsulfoxide (DMSO) and dimethylformamide (DMF), partially soluble in absolute ethanol, methanol, chloroform, acetone, dioxane and insoluble in distilled water, benzene and ethyl ether. The physicochemical and spectral characteristics are listed (tab. I, II).

TABLE I

Physicochemical characteristics of azetidin-2-one derivatives of ferulic acid (5a-f)

Comp.	R	Physicochemical characteristics			
		Molecular formula	Molecular weight	Yield (%)	Melting point (ºC)
5a	-H	$C_{19}H_{17}ClN_2O_4$	372.80	24.00	72
5b	-OH(2)	$C_{19}H_{17}ClN_2O_5$	388.80	25.93	107-109
5c	- NO_2(2)	$C_{19}H_{16}ClN_3O_6$	417.80	17.46	123
5d	-Cl(4)	$C_{19}H_{16}Cl_2N_2O_4$	407.25	39.90	176
5e	-F(4)	$C_{19}H_{16}ClFN_2O_4$	390.79	39.10	150
5f	-Br(4)	$C_{19}H_{16}BrClN_2O_4$	450.00	31.12	89

TABLE II

Spectral characteristics of azetidin-2-one derivatives of ferulic acid (5a-f)

Comp.	FT-IR characteristic band (cm^{-1})	^{1}H-RMN (400 MHz, DMSO-d_6, δ ppm)
5a	3190 (NH), 2966, 1587, 839 (aromatic ring), 2839, 2773, 1447, 1402 (CH aliphatic), 1724 (C=O cyclic), 1651 (CO-NH), 1587 (C=N), 1394 (CH-N), 1255 (C-N), 723 (CH-Cl)	3.74 (s, 3H, OCH_3), 5.04 (d, 1H, CH-Cl), 5.34 (d, 1H, Ar-CH-N), 6.79-6.82 (m, 1H, Ar-H), 6.98-7.12 (m, 2H, Ar-H), 7.39-7.43 (m, 1H, Ar-H), 7.48-7.52 (m, 1H, Ar-H), 7.54-7.60 (m, 3H, Ar-H), 7.65-7.68 (m, 2H, Ar-H)
5b	3065 (NH, OH), 2974, 1627, 1524, 893 (aromatic ring), 2845, 2754, 1487, 1387 (CH aliphatic), 1732 (C=O cyclic), 1572 (CO-NH), 1524 (C=N), 1380 (CH-N), 1269 (C-N), 1196 (C-O), 690 (CH-Cl)	3.81 (s, 3H, OCH_3), 5.02 (d, 1H, CH-Cl), 5.32 (d, 1H, Ar-CH-N), 6.55-6.59 (m, 1H, Ar-H), 6.80-6.87 (m, 1H, Ar-H), 7.00-7.08 (m, 1H, Ar-H), 7.20-7.29 (m, 2H, Ar-H), 7.33-7.35 (m, 1H, Ar-H), 7.60-7.65 (m, 1H, Ar-H), 7.72-7.79 (m, 1H, Ar-H), 7.94 (s, 1H, Ar-H)
5c	3250 (NH), 2943, 1724, 1510, 851 (aromatic ring), 2895, 2856, 1471, 1419, (CH aliphatic), 1728 (C=O cyclic), 1628 (CO-NH), 1607 (C=N), 1387 (CH-N), 1232 (C-N), 1570, 1342 (NO_2), 709 (CH-Cl)	3.83 (s, 3H, OCH_3), 5.32 (d, 1H, CH-Cl), 5.54 (d, 1H, Ar-CH-N), 6.69-6.75 (m, 1H, Ar-H), 6.88-6.93 (m, 1H, Ar-H), 6.99 (dd, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.35-7.43 (m, 1H, Ar-H), 7.59-7.64 (m, 1H, Ar-H), 7.91-7.98 (m, 2H, Ar-H), 8.09 (dd, 1H, Ar-H)
5d	3254 (NH), 2945, 1724, 1510, 862 (aromatic ring), 2901, 2847, 1470, 1402 (CH aliphatic), 1734 (C=O cyclic), 1595 (CO-NH), 1510 (C=N), 1396 (CH-N), 1230 (C-N), 814 (C-Cl), 703 (CH-Cl)	3.82 (s, 3H, OCH_3), 4.92 (d, 1H, CH-Cl), 5,24 (d, 1H, Ar-CH-N), 6.80-6.85 (m, 2H, Ar-H), 6.95-7.04 (m, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.37 (dd, 1H, Ar-H), 7.52 (dd, 2H, Ar-H), 7.77 (dd, 2H, Ar-H)
5e	3252 (NH), 2945, 1680, 1506, 825 (aromatic ring), 2847, 1470, 1419 (CH aliphatic), 1730 (C=O cyclic), 1634 (CO-NH), 1601 (C=N), 1384 (CH-N), 1227 (C-N), 1095 (C-F), 714 (CH-Cl)	3.83 (s, 3H, OCH_3), 4.94 (d, 1H, CH-Cl), 5.26 (d, 1H, Ar-CH-N), 6.79-6.89 (m, 2H, Ar-H), 6.99-7.03 (m, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 7.29-7.37 (m, 3H, Ar-H), 7.81 (td, 2H, Ar-H)
5f	3252 (NH), 2943, 1724, 1510, 836 (aromatic ring), 2893, 2837, 1472, 1419 (CH aliphatic), 1726 (C=O cyclic), 1583 (CO-NH), 1562 (C=N), 1386 (CH-N), 1232 (C-N), 858, (C-Br), 724 (CH-Cl)	3.85 (s, 3H, OCH_3), 4.95 (d, 1H, CH-Cl), 5.28 (d, 1H, Ar-CH-N), 6.77-6.85 (m, 2H, Ar-H), 6.93-6.99 (m, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.30-7.37 (m, 1H, Ar-H), 7.58-7.63 (m, 2H, Ar-H), 7.72-7.75 (m, 2H, Ar-H)

The azetidin-2-one derivatives of ferulic acid were characterized by IR absorption. The FT-IR spectra revealed the presence of C-O stretching band cyclic at 1724–1734 cm^{-1}. The IR spectra also showed the characteristic absorption band of CH-N at 1380–1396 cm^{-1} and the characteristic absorption band of CH-Cl in the region 690–724 cm^{-1}. These data confirm the structure of derivatives and the successful azetidine ring formation.

The structure of derivatives is also supported by NMR spectra. The apparent resonance multiplicity is described as s (singlet), d (doublet), m (multiplet), dd (double doublet), and td (triple doublet) signal.

The signals of CH-Cl protons resonate as a doublet in a range of δ 4.92–5.04 ppm

and the protons linked by the N from azetidine ring (Ar-CH-N) exhibited a doublet at δ 5.24–5.54 ppm.

CONCLUSIONS

We synthesized six new azetidin-2-one derivatives of ferulic acid. The optimal reaction conditions for obtaining high yield and high purity compounds were established. The new derivatives were characterized by their physical constants (melting point, yield, molecular formula, molecular weight, solubility in different organic solvents). The chemical structure was confirmed proved by FT-IR and ^{1}H-RMN spectroscopy. All the obtained data encourage us to further investigate *in vitro* and *in vivo* these new azetidin-2-one derivatives of ferulic acid.

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CAN THE EPIC FOOD FREQUENCY QUESTIONNAIRE BE APPLIED TO THE POPULATION IN ROMANIA?

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CAN THE EPIC FOOD FREQUENCY QUESTIONNAIRE BE APPLIED TO THE POPULATION IN ROMANIA? (Abstract): **Aim**: The application of the EPIC food frequency questionnaire to the population of Romania in order to obtain information about nutritional intake/dietary pattern. **Material and Methods**: EPIC Norfolk questionnaire (translated and adapted) was administered by an interviewer to 125 people, who also completed the 24-hour diet recall on two different days. In addition, data was collected in regards to anthropometry (weight, height, body mass index), as well as information related to other lifestyle factors, such as rural / urban, education, physical activity or income. Calculating the nutrients was done on the basis of tables published by the Ministry of Health. For statistical data processing the *SPPS 17.0* software was used. **Results**: The current rating refers to data obtained by analyzing the questionnaires for the 24-hour diet recall. The analysis of the kinds of food supported the correspondence with the original English questionnaire. By analyzing the energy and macronutrient intake through the both methods, depending on the area of origin, it appears that rural people consume a higher percentage of saturated fat ($p = 0.006$) and lower of insoluble fiber ($p = 0.042$) and carbohydrates ($p = 0.036$) compared to those in urban areas, which are statistically significant differences. On average, the intake of kcal/day was 1741.22, carbohydrates/day 201.66 g, respectively 56.13%, protein 88.33 g/day respectively 25.46%, fibers 17.25 g/day, fat 94.67 g/day respectively 18.28%, of which saturated fat 22 g/day (11.04%), monounsaturated 24.65 g/day (12.24%), polyunsaturated 10.48 g/day (5.3%); cholesterol 329.63 mg/day. **Conclusions**: The analysis in this phase of the study was necessary to equate the foods from the food frequency questionnaire EPIC with those consumed by the population of Romania expressed in the 24-hour diet recall and also to have a basis for comparison with food frequency questionnaires applied now and in 6-9 months in order to validate it. **Keywords**: FOOD FREQUENCY QUESTIONNAIRE (FFQ), NUTRIENTS, EPIC.

Accurately measuring of the dietary intake is considered to be the greatest challenge in nutritional epidemiology (1). Food frequency questionnaires are nutritional assessment tools, widely used in epidemiological studies since the early 90s, which investigate either the relationship between food intake and disease or the risk factors

(2). These nutritional assessment methods should be available, cheap, and easy to apply both by participants and investigators and should be able to collect information on a regular diet for an extended period of time (3). Precisely because they satisfy these conditions, food frequency questionnaires were largely used for epidemiological purposes (4). Food frequency questionnaires are retrospective and obtain information about the frequency of consumption from a specific list of foods and beverages, and may include or not estimates of the portions consumed (5). In large epidemiological studies, diet data obtained from food frequency questionnaires are compared with specific diseases such as cancer, or risk factors such as the total cholesterol and HDL-cholesterol. Food frequency questionnaires can also be used to identify food patterns associated with inadequate intake of specific nutrients (6). Because of the intra- and inter-individual variability, food frequency questionnaires covering the previous year are recommended for studies on wider populations.

Most questions are based on a selected list of foods and food groups with similar nutritional values. Lists of specific foods are based on food pattern of the population studied (7). Creating a food frequency questionnaire can be achieved either by modifying an existing questionnaire, or by creating one from scratch. Selecting the most informative food is important to prevent the establishment of long lists which would reduce the accuracy of the data obtained. The simplest method of making the list is to examine food composition tables and identify foods that contribute significantly to nutrient intake. Assessment of portion sizes is necessary to calculate the daily intake of nutrients. It is possible to specify a typical portion (100 g meat or ½ cup of rice) or to classify portion size as small, medium or large, using real models or reference units (8). The estimates derived from food frequency questionnaire data may present systematic errors and may not adequately represent the usual nutrient intake (9). Incorrect information can lead to the false association between nutritional factors and diseases and the validation of these questionnaires is therefore important.

The validity of questionnaires is generally assessed by comparing the data with a reference method (the most common method is the 24-hour diet recalls). Most validation studies have shown a strong correlation between nutrient intake estimates using the two methods (10, 11). However, other studies have reported a discrepancy between estimated energy intake and energy needs (12) and others have shown that the food frequency questionnaire cannot be recommended as a tool to assess the relationship between absolute intake of energy or protein and disease (13). Therefore it became important the use of biomarkers to assess dietary intake (14, 15, 16, 17). Age, gender, ethnicity and health status may affect the results of validation studies. It is therefore important that the population on which the questionnaire is validated to be identical to that for which it is intended (8).

MATERIAL AND METHODS

The Norfolk EPIC questionnaire is one of the most used food frequency questionnaires, created in 1988, validated and used to assess dietary intake on 25,639 participants, aged 40-79 years, and was completed between 1993 and 1997. The questionnaire consists of two parts. The first part is the food list (130 lines); each line has an attached portion size: average portion, standard unit or household unit. Food questionnaire lines are either individual or

combinations of individual foods or types of foods. Participants were asked to select a suitable frequency of food consumption for each line of nine categories of frequencies (from never or less than once a month up to 6 times per day). The second part contains further questions on the frequency or type of certain foods (18).

The process of translation and adaptation of the EPIC-Norfolk Food Frequency Questionnaire was done as directed by WHO (19). The Romanian version is conceptually equivalent to the English one. The final version was reached by following the implementation steps, forward translation from English to Romanian, back-translation from Romanian to English, pre-testing and cognitive interviewing (20).

A group of bilingual experts (expert panel) discussed each item. These experts also decided to introduce Romanian dishes similar in composition (from foods tables) and some Romanian brands similar to those in the original questionnaire. The food tables published in the Healthy eating guide on the web site of the Ministry of Health were used (21). Interviewers have organized a focus group for the application of the questionnaire in order to check that every question can be repeated in their own words.

The study began in January 2015 and continued for the next nine months. The 24-hour diet recall and the FFQ 1 were applied on 125 people. The second tranche of questionnaires was scheduled to be applied after 6 to 9 months.

Our translated and adapted questionnaire version contains 130 items (foods and drinks). These are categorized into 10 major food groups: meat - fish, bread - flavored biscuits, cereals, potatoes - rice - pasta, dairy products - fats, sweets - snacks, soups - sauce - sandwich spreads, drinks, fruits, vegetables. Certain foods from the list that are not commonly consumed in Romania have been replaced by other foods from the same food group category and with the same nutritional value (e.g. *beef burger* - minced meat croquette, or *halibut*, a fatty fish - trout); also food brand names were replaced with their Romanian counterparts.

Subjects were asked to specify the frequency for the consumption of these foods in the previous year. There are nine levels of frequency: never or less than once a month, 1-2 times per month, once a week, 2-4 times a week, 5-6 times a week, once a day, 2-3 times a day, 4-5 times a day, 6 times per day. Seasonal variability of dietary intake of fruits and vegetables has to be also taken into account, which is a complex issue in terms of reporting, given that some of them are consumed throughout the year and others in a greater degree during the summer. Therefore the 2ndfood frequency questionnaire application will be made in another season. The last section of the questionnaire contains questions that ask more details about certain foods (mainly those containing fats or cereals), vitamin and mineral supplements or brand names. We used the drawings from the healthy eating guide for estimating the portions.

In our study the questionnaire was applied on 125 adults (between 20-70 years), both sexes, in order to demonstrate its ability to estimate general dietary intake. The questionnaire was administered by an interviewer, face-to-face, after signing the informed consent. The 125 people completed a 24-hours diet recall on two different days. In addition, it obtained data linked to anthropometry (weight, height, body mass index), as well as information related to other lifestyle factors, such as environment rural / urban, education, physical activity or income. The data were imported into SPSS for analysis and descriptive statistics were

calculated to describe the general characteristics of the participants.

RESULTS

The analysis of macronutrient intake from both days of 24 hours diet recall, by area of origin shows that rural people consume a higher percentage of saturated fats (p=0.006) and lower insoluble fibers (p=0.042) and carbohydrates (p=0.036) compared to those in urban areas, which represent statistically significant differences (Table I).But in terms of physical activity, education level or average income, there have not been observed any statistically significant differences.

TABLE I

Estimated intake of macronutrients according to the area of origin

	Area of origin	N	Mean	Std. Deviation	Std. Error Mean	p
kcal/day	U	170	1726,4764	564,59504	43,30246	0,669
	R	73	1784,0367	676,39150	79,16564	
kcal/kg	U	164	21,1565	7,83441	,61176	0,751
	R	69	22,1390	9,73671	1,17216	
carbohydrates/day	U	169	204,4997	70,76345	5,44334	0,309
	R	73	196,1807	76,64333	8,97042	
proteins/day	U	170	87,7769	45,70067	3,50508	0,938
	R	73	87,4332	37,28958	4,36442	
fat/day	U	170	104,5452	541,15417	41,50463	0,082
	R	73	72,4982	42,50945	4,97535	
saturated fat/day	U	170	20,9110	13,45397	1,03187	0,019
	R	73	24,7040	14,06594	1,64629	
% saturated fat	U	170	10,5725	4,93338	,37837	0,006
	R	73	12,1770	4,78666	,56024	
monounsaturated fat/day	U	169	23,5421	16,56792	1,27446	0,253
	R	73	27,3596	24,35051	2,85001	
% monounsaturated fat	U	170	11,8684	6,70859	,51453	0,205
	R	73	13,1173	8,12536	,95100	
polyunsaturated fat/day	U	170	10,4314	7,42569	,56952	0.859
	R	73	10,6632	9,42299	1,10288	
% polyunsaturated fat	U	170	5,3386	3,28614	,25204	0,720
	R	73	5,2156	3,23187	,37826	
cholesterol/day	U	170	334,9789	261,04653	20,02135	0,993
	R	73	316,4581	203,68285	23,83927	
insoluble fibers/day	U	170	4,8546	4,00811	,30741	0,042
	R	72	4,4886	6,77766	,79876	
total fibers /day	U	170	17,6537	9,34867	,71701	0,126
	R	73	16,3741	11,10093	1,29927	
carbohydrates %	U	170	57,3475	11,17111	,85678	0,036
	R	73	54,0282	11,49474	1,34536	
proteins %	U	170	24,5063	8,27436	,63461	0,208
	R	73	26,7452	10,79673	1,26366	
fat%	U	170	17,9969	7,11419	,54563	0,118
	R	73	19,1890	7,72308	,90392	
alcool%	U	160	,2156	1,02451	,08099	0,125
	R	71	,8338	3,32140	,39418	

The analysis of macronutrient intake according to weight (normal weight, overweight, obese) shows there were statistically significant differences in terms of total daily fiber intake and polyunsaturated fats; in obese participants there was found a

higher intake of polyunsaturated fats and low total fiber per day, compared to other categories.

On average, the intake of kcal/day was 1741.22, carbohydrates 201.66 g/day, respectively 56.13%, protein 88.33 g/day respectively 25.46%, fibers 17.25 g/day, fat 94.67 g/day respectively 18.28%, of which saturated fat 22 g/day (11.04%), monounsaturated 24.65 g/day (12.24%), polyunsaturated 10.48 g/day (5.3%); cholesterol 329.63 mg/day (Table II).

TABLE II
The average of estimated intake

	Nr.	Media	Median	Dev. Std.	Min.	Max.
kcal/day	244	1741,22	1641,41	599,60	694,61	4698,00
Kcal/kc/day	234	21,41	19,64	8,42	7,57	54,14
carbohydrates g/day	243	201,66	197,00	72,55	65,85	479,00
proteins g/day	244	88,32	82,31	44,36	16,73	402,98
fat g/day	244	94,67	58,75	452,14	13,70	7106
saturated fat g/day	244	22,00	19,42	13,70	0,60	75,80
monounsaturated fat g/day	243	24,65	19,62	19,24	2,68	174,48
polyunsaturated fat g/day	244	10,48	8,27	8,04	1,70	60,60
cholesterol mg/day	244	329,63	268,78	244,47	,00	1604,58
insoluble fibers g/day	243	4,76	3,62	4,98	,00	47,22
fibers g/day	244	17,25	15,88	9,88	,00	69,80
carbohydrates %	244	46,38	57,25	11,79	4,70	80,20
proteins %	244	20,46	23,45	10,12	10,50	93,90
fat %	244	33,16	17,40	7,37	1,40	47,30
saturated fat %	244	11,04	10,10	4,92	,35	26,15
monounsaturated fat %	243	12,24	11,39	7,15	,00	54,93
polyunsaturated fat %	244	5,30	4,48	3,25	1,20	22,56
alcohol %	232	0,40	0,00	2,03	0,00	19,70

The subjects were categorized according to the distribution of the intake (quartiles), as follows: carbohydrates <45%, 45-65%,> 65%, proteins <10%, 10-35%,> 35%, fats <30%,> 30%, fibers, <14 ‰ cal,> 14 ‰ cal and showed statistical significance according to BMI (obese) for carbohydrates and fibers (table III), but not according to area of origin. Most obese subjects fall within the 45-65% carbohydrates intake and fibers <14 ‰ cal, while most normal weight subjects consume > 65% carbohydrates and> 14 ‰ cal fibers.

As to the energy intake, it was found to be higher in normal weight versus overweight and obese patients, with statistically significant results (p = 0.001), but no differences were observed in terms of area of origin, physical activity or income.

TABLE III

The intake of macronutrients, according to BMI

		IMC						
		Normal weighted		Over weighted		Obeses		p
		Nr.	%	Nr.	%	Nr.	%	
carbohydrates	**<45%**	**8**	**24,2**	**11**	**33,3**	**14**	**42,4**	0,01
	45-65%	**29**	**19,1**	**35**	**23**	**88**	**57,9**	
	>65%	**15**	**29,4**	**21**	**41,2**	**15**	**29,4**	
proteins	<10%	0	0	0	0	0	0	0,477
	10-35%	48	23,2	57	27,5	102	49,3	
	>35%	4	13,8	10	34,5	15	51,7	
fat	<30%	49	22,3	61	27,7	110	50	0,704
	>30%	3	18,8	6	37,5	7	43,8	
saturated fat	<7%	12	23,1	21	40,4	19	36,5	**0,058**
	>7%	40	21,7	46	25	98	53,5	
monounsaturated fat	<15%	42	23,1	52	28,6	88	48,4	0,916
	15-20%	6	18,8	8	25	18	56,3	
	>20%	4	18,2	7	31,8	11	50	
polyunsaturated fat	<10%	50	22,6	62	28,1	109	49,3	0,693
	>10%	2	13,3	5	33,3	8	53,3	
fibers	**<14‰cal**	**32**	**17,9**	**51**	**28,5**	**96**	**53,6**	**0,016**
	≥14‰	**20**	**35,1**	**16**	**28,1**	**117**	**49,6**	

DISCUSSION

Measurement of the usual food intake is necessary to establish the food pattern. The food frequency questionnaire is a useful tool in epidemiological studies because it allows a long-term assessment of diet and

investigating the diet-disease relationship. The questionnaire which this study is based on is EPIC Norfolk, which was translated and adapted for the people of Romania. To estimate nutrient intake it is necessary to use the tables with the nutrient composition of the foods reported by the participants, in two different ways (24 hours diet recall and FFQ).

In order to convert the estimate of food intake frequency to the nutritional values, there has to be constructed a database suitable for nutrients. Databases available in our country do not contain complete information. The 24 hours diet recall helps us to compare the English questionnaire to the Romanian one. The health eating guide published on the site of Ministry of Health helped us to visualize the portion size.

In this study there has been observed a higher consumption of saturated fat and cholesterol and a lower consumption of fiber as opposed to the recommendations from nutritional guides, and a higher consumption in subjects from rural areas compared to those in urban areas.

CONCLUSIONS

Although there are difficulties in calculating the absolute individual intake of nutrients using food frequency questionnaires, they are useful in obtaining information on population groups and to assess the usual intake of a range of foods. This preliminary study demonstrated the applicability of the EPIC questionnaire to a population of Romania, but the validation can be done after 6-9 months when the second FFQ is applied.

ACKNOWLEDGMENTS

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A PRELIMINARY EVALUATION OF RESIDENTS' PERCEPTION OF THEIR TRAINING AND KNOWLEDGE ON THREE-DIMENSIONAL IMAGING

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PRELIMINARY STUDY OF MEDICAL RESIDENTS' EVALUATION OF THEIR TRAINING PROGRAM REGARDING THREE-DIMENSIONAL IMAGING TECHNIQUES (Abstract): The **aim** of this preliminary study was to assess the residents' perception on their training program and on their knowledge regarding the use and interpretation of three-dimensional imaging techniques in their clinical practice. **Material and methods:** A multi-item questionnaire was distributed among 104 residents training in four medical specialties at the University of Medicine and Pharmacy "Grigore T. Popa" in Iaşi. **Results:** Fifty-five residents agreed to fill in the questionnaire, achieving a participating rate of 52.88%. The majority of the respondents (74.5%) declared to indicate classic CT scans to their patients. A significantly lower fraction of the participating residents (32.7%) affirmed to indicate CBCT scans. Electronic literature was the most common information source on the use of three-dimensional imaging (52.7%). Most of the residents declared that they cannot interpret correctly CT and CBCT scans (78.2%, respectively 83.6%). A significant percentage of the respondents considered that the level of training provided on the interpretation of CT and CBCT results is very low (49.1%, respectively 60.0%). **Conclusions:** The residents' perception of the quality of their education regarding 3D imaging modalities, along with their perception of their knowledge in this area, indicates the necessity to improve their didactic and practical training. A further extended survey will be conducted to evaluate the educational experience of residents from all medical specialties that use 3D imaging techniques in neck and head imaging. **Keywords:** THREE-DIMENSIONAL IMAGING, CT, CONE BEAM CT, RESIDENCY TRAINING PROGRAM.

During the last decades, three-dimensional (3D) imaging modalities have continually contributed to diagnostic purposes and treatment planning of various head and neck pathologies, providing non-distorted and non-overlapping visualization of the anatomy, with high resolution (1). Computed tomography (CT) rapidly became a first-line diagnostic test for numerous clinical conditions. Its utilization increased as its diagnostic capability expanded, raising concern about imaging-related financial costs and radiation risks (2, 3). Cone beam computed tomography (CBCT) was introduced as a new 3D imaging technology in the late 1990's (4, 5), offering a

lower dosage of radiation exposure compared to the classic CT, a low cost, and a quick scanning time (6-9). CBCT has been recently available in Romania, although not widely accessible yet, except for private radiology clinics. The availability of new technology requires physicians to be aware of its applications, impacting the quality of medical care they offer to their patients. The indication of three-dimensional imaging modalities should be performed based on a sound knowledge of its potential, benefits, and possible risks, hence the appropriate education and training becomes imperative for the physicians who will utilize and interpret the acquired data.

Therefore, the aim of this preliminary study was to assess the residents' perception on their training program and on their knowledge regarding the use and interpretation of 3D imaging techniques in their clinical practice.

MATERIAL AND METHODS

A questionnaire was applied to residents training in four medical specialties at the University of Medicine and Pharmacy "Grigore T. Popa" in Iaşi: Orthodontics and dentofacial orthopedics, Oral and maxillofacial surgery, Pediatric surgery, and Plastic surgery and reconstructive microsurgery.

The questionnaire contained 11 items which, aside from personal data (age, sex, and medical specialty), consisted in single response questions (Yes/No), Likert scale response questions and one multiple answer question. The items aimed to assess the use of 3D imaging techniques, the information sources in this area, the respondents' perception on their current knowledge and on the quality of the residency program regarding the topic.

All respondents were encouraged to answer all items honestly and they were assured of the preservation of their anonymity.

Collected data was analyzed using SPSS software version 16 for descriptive statistics and for correlations between variables.

RESULTS

Out of 104 residents training in the selected medical specialties, 55 agreed to participate in the present study, achieving a response rate of 52.88%. The participants' ages ranged from 25 to 41 years, with a balanced male/female distribution (about 4/5).

The majority of the respondents (74.5%) declared to indicate classic CT scans in their clinical practice. A significantly lower fraction of the participating residents (32.7%) affirmed to indicate CBCT scans. Fewer Plastic surgery and reconstructive microsurgery residents opted for CBCT, compared to the other three medical specialties (pairwise t-test, 95% confidence interval).

Most of the residents considered that they cannot interpret correctly CT and CBCT examinations (78.2%, respectively 83.6%).

The most common source of information regarding the use of 3D imaging modalities was electronic literature (52.7%), followed by printed literature (40%), congresses (40%), and courses (38.2%) (fig. 1).

A significant percentage of the respondents considered that the level of training provided by the residency program on the interpretation of CT and CBCT examination is very low (49.1%, respectively 60.0%) (tab. I).

The calculated mean scores for the level of training provided by the residency program are showed in Table II. The score

regarding CT results interpretation was higher when compared to CBCT, but the difference is not statistically significant (pairwise t-test, 95% confidence interval).

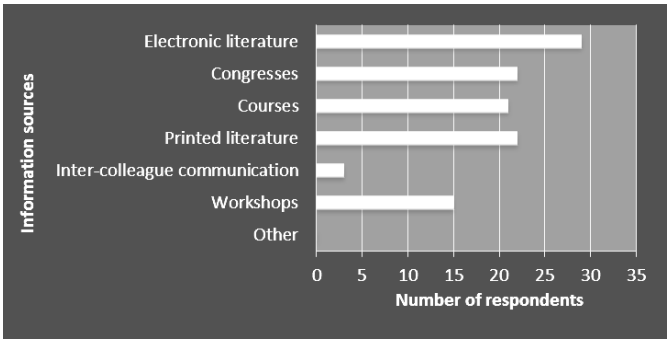

Fig. 1. Sources of information regarding the use of three-dimensional imaging modalities

TABLE I

Residents' perception on the level of training provided by the residency program regarding 3D imaging techniques

		CT	CBCT
Total	***N***	***55***	***55***
	%	100.0	100.0
1-Very low	N	27	33
	%	49.1	60.0
2-Low	N	13	9
	%	23.6	16.4
3-Medium	N	11	8
	%	20.0	14.5
4-High	N	3	4
	%	5.5	7.3
5-Very high	N	1	1
	%	1.8	1.8

TABLE II

Calculated mean scores regarding the level of training provided

	CT	CBCT
Valid N	33	33
Mean	***1.87***	***1.75***
Minimum	1	1
Maximum	5	5
Std. Dev.	1	1

DISCUSSION

The results show a more pronounced lack of knowledge when referred to CBCT imaging, compared to classic CT. A noticeable preference of CT usage over CBCT can also be observed. Smith *et al.* (2011) report a consistent CBCT accessibility and training in orthodontic residency programs in the U.S (10), while Parashar *et al.* (2012) report a general trend of providing CBCT equipment and training in the U.S., British, and Australian dental education (11). Recently, the European Academy of Dento Maxillo Facial Radiology published a paper regarding the importance of proper training of dentists in the usage and interpretation of CBCT examination (12). Most dental and medical schools in Romania have recently adopted CBCT image acquisition and interpretation in oral and maxillofacial radiology courses. Nevertheless, while CT devices have been available in university hospitals in the past decades, CBCT equipment has only recently been introduced in Iaşi through private radiology clinics. To our knowledge, this new technology is yet not available in Romanian university hospitals, contributing to the limited knowledge of its applications and the lack of practical training among residents.

A proper education of medical residents regarding 3D head and neck imaging modalities should include information on acquisition of data, on the interpretation of the detailed information provided, a thorough knowledge of the anatomical structure as they appear on 3D scans, and how to analyze the findings (11-13). We consider that this will become a more prominent component of the residency curricula in the coming years, equipping residents to use, understand and interpret correctly 3D imaging techniques.

While this preliminary study provides insight into the nature of residents' perception of their knowledge and training program regarding 3D imaging procedures, there are certain limitations to it. Neither one of the medical specialties does include a radiology module in its program (as published on the official site of residency program trainings). However, their curricula include some lectures on imaging, mostly during surgical modules. The design of the present study did not take into account the timing of these lectures, and while a part of the respondents had already attended them, the rest of the participating residents had not. This may produce bias to the results. Our research did not reveal previous similar studies conducted in Romania, that preventing us for comparing the obtained results to other findings.

On personal discussions with the respondents, a clear interest in learning more about the latest trends in 3D imaging was observed, and also willingness to share information to improve their educational experience.

CONCLUSIONS

The residents' perception of the quality of their education regarding 3D imaging modalities, along with their perception of their knowledge in this area, indicates the necessity to improve their didactic and practical training. A further extended survey will be conducted to evaluate the educational experience of residents from all medical specialties that use 3D imaging techniques in neck and head imaging.

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THE INFLUENCE OF PREMATURE LOSS OF TEMPORARY UPPER MOLARS ON PERMANENT MOLARS

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THE INFLUENCE OF PREMATURE LOSS OF TEMPORARY UPPER MOLARS ON PERMANENT MOLARS(Abstract): **Aim:** Premature loss of primary molars due to dental caries and their complications has been associated with space loss and eruptive difficulties, especially when the loss occurs early. The aim of our study was to determine the impact of premature loss of temporary upper molars upon the longitudinal axis of the first and second upper permanent molar. **Material and Methods:** The study group included 64 patients 6 -9 years old with premature loss of primary molars and a control group of 48 patients with intact temporary teeth. It was evaluated the angle between longitudinal axis of first and second upper permanent molars and occlusal plane. The sofware used is *Easy Dent 4 Viewer*®.The data were analyzed by using the Statistical Package for the Social Sciences (version 20.0; SPSS, Chicago, III). **Results:** It was observed that premature loss of upper second deciduous molars modifies greater the vertical axis of the permanent molars than the premature loss of first upper primary molar. First upper primary molar loss cause an acceleration eruption of first premolar, which will produce a distal inclintion of the both permanent molars. **Conclusions:** The use of space maintainers after premature loss of the second upper temporary molar is a last solution in preventing tridimensional lesions in the dental arch and occlusion. **Keywords:** PREMATURE LOSS, AXIAL MODIFICATION, PERMANENT UPPER MOLARS , DENTAL CARIES, TEMPORARY TEETH.

Premature loss of temporary teeth, as a consequence of untreated dental cavity of temporary teeth, may determine serious tridimensional dental, dento-dental, dento-alveolar, occlusive and variably skeletal disorders. We thus witness a migration of permanent teeth, during intra-maxillary or oral eruption, leading to a dento-alveolar incongruence. Over-eruptions of opposite teeth, narrowed arch formation, reversed toothing, premature contacts and occlusal interferences may also occur (1).

Studies have shown that premature loss of temporary first molars (m1t) mainly determines the distal migration of temporary canines. In this case, the mesial displacement of permanent molars or their mesio version doesn't occur (2). Greater effects appear at the moment of premature loss of temporary second molars, as they serve as a guide for the eruption of the permanent molars (3). The premature loss of temporary secondary molars (m2t) – mainly maxillary – determines a shortening of the arch length due to the mesial migration of permanent molars, thus compromis-

ing the „lee-way space" by affecting the posterior support area (4). The radiologic examination (orthopantomography) is a must in case of these patients, allowing the quantification of the leaning degree of permanent molars.

The main purpose of this study is to assess the impact of the premature loss of temporary upper molars on the longitudinal axis of the permanent molars. Another objective is to see whether there appear significant changes in the eruption sequence of the successor in these conditions.

MATERIAL AND METHODS

The study included 112 orthopantomographies of subjects aged between 6 and 9 who reported to the Pediatric Dental Clinic Iaşi. Out of these, 64 patients showed premature loss of a temporary upper molar and 48 had intact dentition. The condition of the subject selection was that the incisive teeth and permanent molars be intact. All the patients were given specialty treatment and their mandated, informed and motivated assent was obtained.

All orthopantomographies were conducted at a single radiologic center using the *PaX-Uni3D (Vatech)*® radiologic unit. The measurements were made by a single examiner using the *Easy Dent 4 Viewer*® software.

The processing of orthopantomographies included the identification of the following structures (fig. 1):

-*the right/left occlusion plane* – the tangent line to vestibular cuspids of the temporary molars/pre-molars or of lower temporary molars right/left (in case of loss of both temporary upper molars from the same quadrant);

- *the axis of the upper primary first permanent molar (M1)*-line passing through the center of the crown and the center of the radicular bifurcation;

- *the axis of eruption of upper permanent secondary molars (M2)*-the line passing through the middle of the crown and the center of the beginning of radicular bifurcation.

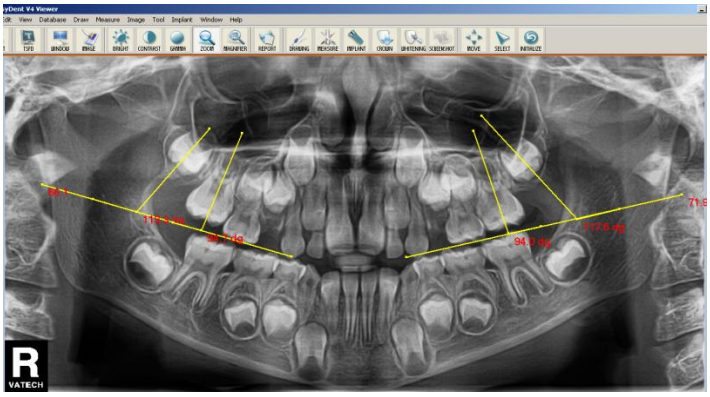

Fig. 1. Orthopantomographic aspect of premature loss of temporary upper molars

By tracing the following benchmarks, we shall evaluate the angle formed by the longitudinal axis of the primary, namely secondary permanent molar and the occlusal plane in the two upper quadrants.

The software used for the data analysis was *SPSS 20.0*. The error margins used in the statistic interpretation are of 5% and the

confidence interval was of 95%. We calculated the frequency parameters and descriptive parameters (average, standard deviation, minimum and maximum value) for each group. We used the t-student test to compare the group with premature loss with the control group. We used the t-pairs test to evaluate the correlation of the M1 upper axis with the M2 upper axis (right/left).

RESULTS

Of the 112 orthopantomographies conducted, 61 (54%) patients were males and 51 (46%) patients female (fig. 2). Concerning the subjects' age distribution, there is a prevalence of patients aged 7 and 8, with a mean age of 7.29 (tab. I).

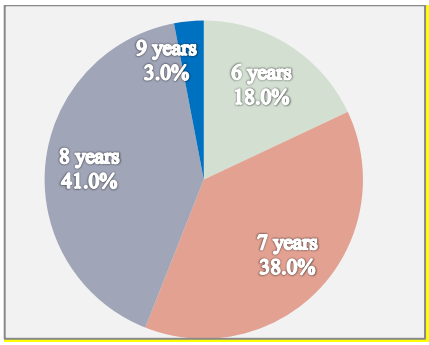

Fig. 2. Group structure on age

TABLE I

Correlative dispersion of patients depending on age and gender

Sex/group	N	Mean	std. error	std. dev	Min	Max
male	**61**	**7.25**	**0.114**	**0.844**	**6**	**9**
premature loss group	35					
control group	26					
female	**51**	**7.33**	**0.11**	**0.739**	**6**	**8**
premature loss group	29					
control group	22					
Total	**112**	**7.29**	**0.08**	**0.795**	**6**	**9**

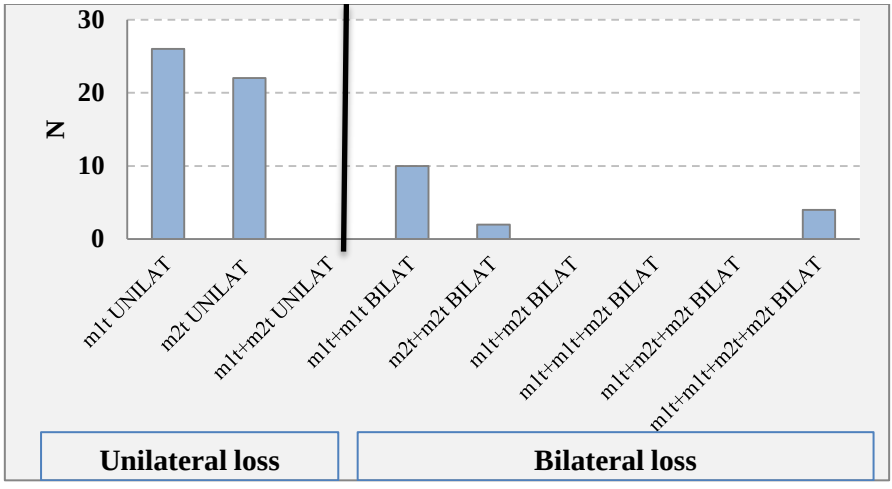

Fig. 3. Losses of m1t, m2t – comparative statistics

In our study, a total of 88 temporary upper molars were prematurely lost due to untreated dental cavity. The cumulated frequency of temporary primary molars is higher (61.36%), as compared to the secondary temporary molars (38.64%). At the same time, the frequency of the bilateral primary temporary molars is rather high, namely of 8.9% of the total cases studied (fig. 3).

From the point of view of angulations, average mesio version of the primary upper permanent molar for the control group is of 92.5° (79-110°), while for the secondary permanent upper molar of 116.5° (90-140°).

The premature unilateral loss of temporary upper molars determines a slight disto version of the primary permanent molar most likely due to the eruption spurt of first premolar (with an angular mean value of 93.9° (variation between 89.3° and 108°) compared to the mean value of the witness group of 92.26° as a compensatory phenomenon. In turn, the loss of temporary second molars has a negative influence through a mesial inclination of permanent molars (mean angular value of 90.18° for M1 and 111.86° for M2) (Fig. 4**).**

Bilateral loss of upper first temporary molars determines a distal compensation of the permanent molars axis (mean angular values of 95.13° for M1 and of 121° for M2). The greatest effect is caused by the bilateral loss of m1t and m2t which determines a strong mesio version of the primary permanent molars (mean angular values of 74.375° compared to the mean value of the witness group, of 92.26°). At the same time, one can notice a reduced influence on the eruption axis of the secondary permanent molar (fig. 5).

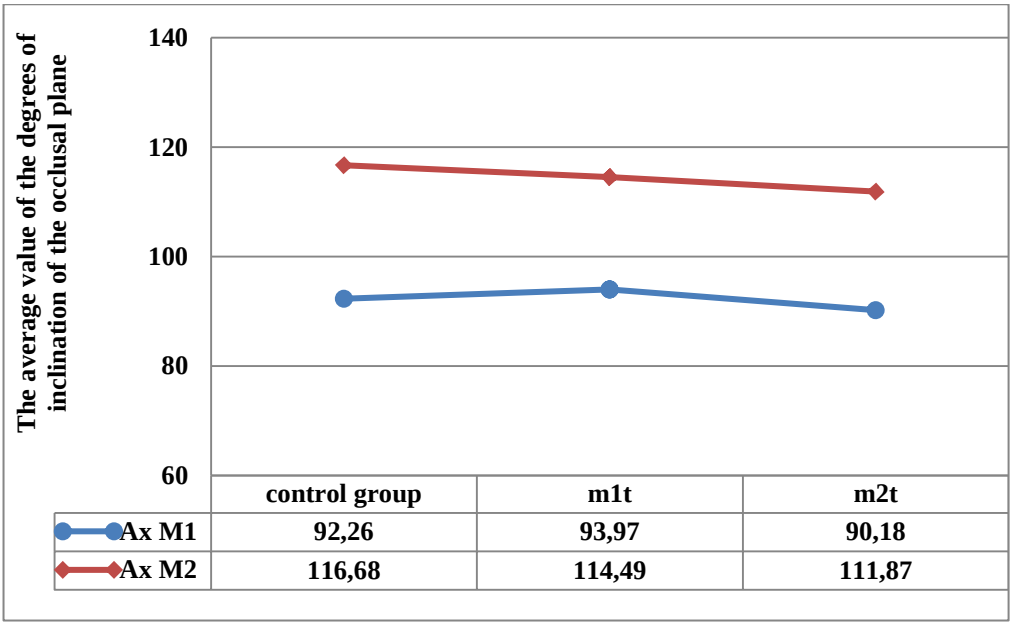

Fig. 4. Influence of unilateral premature loss of temporary upper molars on the inclination of permanent molars

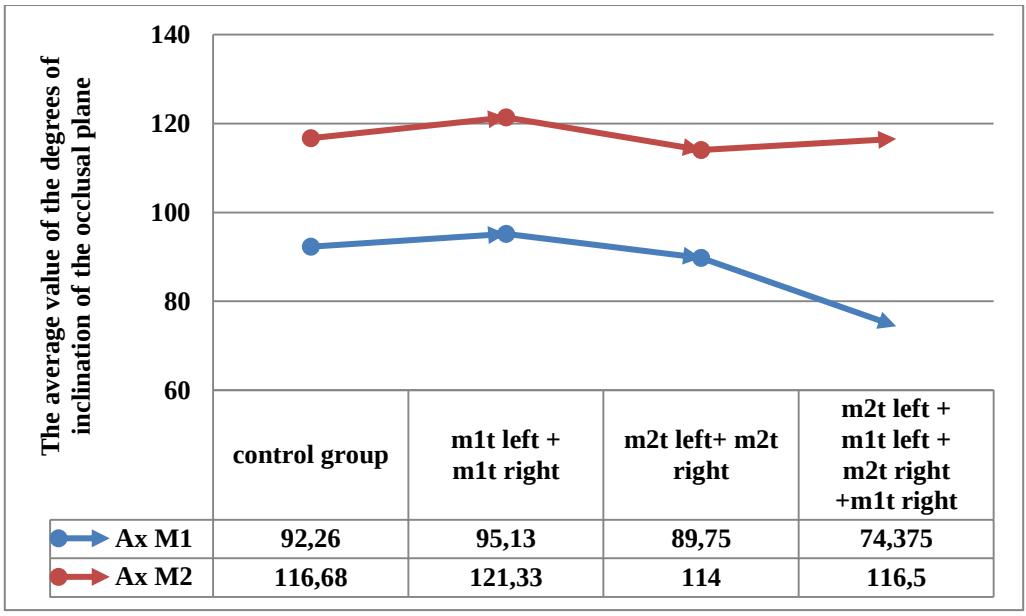

Fig. 5. Influence of bilateral premature loss of temporary upper molars on the inclination of the permanent molars

DISCUSSION

The concept of premature loss was defined as the exfoliation of deciduous teeth on the arch more than 12 months prior to the normal period of permanent teeth eruption (3,4), exceeding the normal variability limits of the exfoliation sequences of temporary teeth.

For both arches the highest space amount lost is due mainly to the mesial inclination of permanent molars. This inclination depends mainly on the dental age during extraction but also on the sequence/periodicity of eruption (5, 6).

The results obtained from the point of view of the frequency of premature loss of temporary molars due to dental cavities are similar to those obtained in other studies (7, 8). For the maxillary arch the premature losses are most frequent and are met in case of the primary temporary molar**,** namely in 61.3% of the total cases under analysis.

Based on the software analysis we could determine with higher accuracy the angle formed by the longitudinal axis of the permanent molars with the occlusion plane. These aspects are relevant under the aspect of the interrelation between the moment of premature loss of temporary molars and the moment of eruption of the primary permanent molar. The context of the premature loss of secondary temporary molar occurred after the initiation of clinical eruption of the primary permanent molar.

In the case of the *loss of the primary maxillary temporary molar* there are numerous contradicting studies. Some authors argue that there occurs a decrease of the postextractional space "within 6 months", especially through the displacement of the temporary canine, but also through a palato version of the permanent incisive teeth (9)**.** *Park et al* using a tridimensional scanning laser method of models conclude that "the

premature loss of the primary temporary maxillary molar, in a I class molar, has a limited influence on the existing space for permanent dentition"(10). *Lin* in a more recent study from 2011, states that after 12 months from the premature extraction of the primary temporary maxillary molar, the space reduction is due only to the distal displacement of the temporary canine. The same author even states that there is an increase of the arch parameter, of the intercanine length and width (2). A Brazilian study conducted by Macena B. et al in 2011 confirms the latest results (5).

The most interesting study was published by Northway in 2000, in which he proves schematically that "at the moment of the premature loss of the primary temporary maxillary molar, the primary premolar start to erupt in a mesio position, as a consequence of the mesial inclination of the temporary secondary molar and thus will consume the space for the permanent canine, which will remain included or will erupt vestibular" (11). Our results are in correlation with the study conducted by Northway, since the loss of maxillary first primary molar would determine a spurt of eruption of the first premolar which will influence the axis of the primary permanent molar by distorting it in compensation, fact proven by the increase of the mean angular value of 93.9^{o} as compared to the mean angular value of the control group, 92.26 ^{o}.

Numerous studies showed that the *premature loss of secondary temporary molars* determines a higher loss of leeway-space than in case of the loss of primary temporary molar, both due to the greater difference of the mesio distal diameter and to the fact that the permanent primary molar and the secondary one losses its contact point and follows the tendency of all teeth to displace mesially or to incline mesially (12, 13). This is also proven by our study through the much lower mean angular values for the permanent maxillary molars in case of premature loss of secondary temporary maxillary molar as compared to the mean angular values of the witness group. Nonetheless, no statistically significant situation was found in any group to influence the axis of the upper molars.

A study carried out by *Leroy R. et al* (14) in 2009 showed that at the moment of cavity affection (cavity, filling, extraction) of both maxillary temporary molars on the same half-quadrant the classic eruption sequence "4-5-3-7" will change to "3-4-5-7" or "4-3-5-7", with an accelerated canine eruption. In our study, the bilateral loss of first temporary molar and second temporary molar only determined an accentuated mesio version of primary permanent molars. The main reason for thus incongruence is the fact that the four patients who lost prematurely the upper temporary molars had a very young age (<5), and the orthopantomography was made at 6 years old. However, one can notice the low influence on the eruption axis of the secondary permanent molar.

CONCLUSIONS

This study highlights one again that the premature loss of temporary molars determines mainly a reduction of the arch length in the molar region. The space shrinking mostly appears through mesial migration and inclination of the molars, through the modification of the inclination of their axis of bone implantation and, to a smaller degree, through the distal migration of the canine.

In addition, the change of the eruption sequence following the premature loss of temporary molars is a phenomenon which

should be considered, especially when an interceptive treatment is adopted, with the preservation within normal limits of the morpho-functional occlusal complex.

ACKNOWLEDGMENTS

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DEVELOPMENT OF A MODIFIED-RELEASE PELLETS FORMULATION WITH METOPROLOL TARTRAT AND KINETIC ASPECTS OF *IN VITRO* RELEASE

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DEVELOPMENT OF A MODIFIED-RELEASE PELLETS FORMULATION WITH METOPROLOL TARTRATE AND KINETIC ASPECTS OF *IN VITRO* RELEASE (Abstract): **Aim**. The development of a new pellets formulation which is able to modulate the release of metoprolol tartrate, an active pharmaceutical ingredient very soluble in water and therefore very difficult to process. **Materials and methods**: Two types of different viscosity grade hydroxypropyl methylcellulose (Methocel K100; HPMC 15.000) and Eudragit NE30D are used as prolonged drug release agents, thus resulting in three formulations which have been prepared in form of pellets, in a conventional coating pan. The obtained pellets are characterized and compared in terms of: particles size distribution, drug loading efficiency, drug content and kinetic of *in vitro* drug release. **Results**: Lower amounts of Eudragit NE30D (7.5%) determine more uniform size distributions of particles. Drug content and charging efficiency is higher in case of fractions ranging in size from 0.80 to 1.25 mm. The combination of HPMC 15000 with 10% Eudragit NE30D leads to a prolongation for a period of 11.5 hours of metoprolol tartrate release. Release kinetics analysis was performed by fitting the *in vitro* release profile with different kinetic models. **Conclusions**: It was developed a pellets formulation that release *in vitro* the metoprolol tartrate in an extended mode, with Korsmeyer-Peppas kinetic type. **Keywords:** METOPROLOL TARTRATE, PELLETS, MODIFIED-RELEASE, *IN VITRO* KINETICS

The pellets, which are solid multi-particulates pharmaceutical forms used for oral administration in form of hard gelatin capsules or tablets, might have either an immediate release of the active ingredient or a modified (controlled) release, at different levels of the gastro-intestinal tract (stomach, intestine, colon) (1, 2, 3). During the formulation process of the pellets, the physical - chemical properties of the active substances and the mechanism of release are considered in order to choose the auxiliary ingredients and the adequate coating agents. Also, the formation process is influenced by the way the pellets are prepared. As multi-particulates pharmaceutical

forms, the pellets provide the advantage of a constant behavior during the release process of the active ingredient. Through the association in a pharmaceutical form of some pellet formulations with different profiles of active substance release it can be achieved a higher control of the active ingredient delivery, with a stronger therapeutic efficiency (4, 5, 6).

The purpose of his study was to formulate pellets with metoprolol tartrate as active ingredient. In order to obtain an optimal formulation, which is meant to enable the modulation of the active ingredient release rate, the specifications from the professional literature and previous researches in the same field were considered (6, 7, 8).

The active substance chosen to be elaborated in the form of pellets is metoprol tartrate (MT), a substance from β- blocker class. The MT is a β1 adrenolytic selective lipophilic and light quinidine, being the choice in tachyarrythmias. The antirhythmless action is caused by the negative influence of the five functions of the myocardium: has chronotropic, dromotropic, inotropic and negative tonotrop effect; due to the negative inotropic effect it takes place the decrease in the cardiac charge. It reduces the induced tachycardia of the exercise and stress, and, due to the liver cleansing, the dosage will be reduced in hepatic insufficiency. It has a short half-life (3-4h). It is recommended in HTA angina pectoris for effort, acute myocardial infarct, supraventricular and ventricular tachyarrhythimias (6).

Although this active pharmaceutical ingredient was studies in many experimental research (9, 10, 11, 12), MT still remains an interesting substance from the therapeutical perspective, this being the reason for which it was chosen in the present study. Beside this, it is assessed to establish the release mechanism of the active ingredient, which contributes to the development of a formula with maxim efficiency.

MATERIAL AND METHODS

Material

Metoprolol tartrate (Moehs Ibérica SL, Spain), sugar nonpareils 0.5-0.6 mm (Alcom SA, Romania), Kollidon K 25 (Merck, Germany), Aerosil 200 (S&D Chemicals, U.K.), talcum (S&D Chemicals, U.K.), hydroxypropyl methylcellulose Methocel K100, HPMC 15.000 (Merck, Germany), Eudragit NE30D (Degussa, Germany). All the reagents used were of analytical grade.

Methods

Pellets preparations.

Pellets were prepared in coating pan (Erveka, Germany), by powdering layers of poweder which contain metoprolol tartrate (as active ingredient) on sugar nonpareils (as inactive cores). In composition of pellets three prolonged drug release agents were used: two sorts of hydroxypropyl methylcellulose that differ in viscosity (Methocel K100, HPMC 15.000) in form of powders in the coating powder layers and a neutral copolymer based on ethyl acrylate and methyl methacrylate in form of an aqueous dispersion (Eudragit NE30D) used as binder agent too. According to the data presented in table I and II, three type of pellets has been obtained: formulation A, B and C, respectively.

Pellets size distribution: The pellets (30 g) were sieved (18 min) using a set of sieves with different apertures (2.50, 1.25, 0.80, 0.50 mm) placed into a sieve shaker equipment system. Each weight fraction was recorded (13).

TABLE I

Composition of metoprolol tartrate pellets

Ingredients / Code of formulation	Quantity (%)		
	A	B	C
Sugar nonpareils:	**33.33**		
Powder for layering:	**59.17**		
Metoprolol tartrate	26.00		
Aerosil	0.50		
Talcum	1.00		
Kollidon K25	2.00		
Methocel K100	29.67	28.17	-
HPMC 15.000	-	-	28.17
Binder aqueous dispersion:	7.5	10	10
Eudragit NE30D (dry substance)	(2.25)	(3.00)	(3.0)

TABLE II

Layering condition for pellets preparation in coating pan

	33
Charg load with powder for layering (g)	67
Binder spray rate (g/min)	0.2-0.3
Pan speed (rpm)	35
Inclination angle pan (°)	40
Binder spray time (min)	140
Drying in pan after layering (min)	15
Drying in an oven	24 h (40˚C)

Evaluation of pellets metoprolol tartrate content: Metoprolol tartrate charged in pellets (the actual content) was calculated using the following formula:

$$I\ (\%) = \frac{Q_{(g)}}{m_{(g)}} \cdot 100$$

where, *I* is the amount of MT (actual contents), *Q* is the amount of MT used in formulation (theoretical expected content) and *m* is the mass of pellets that contains 100 mg MT (experimental data determined by a spectrophotometrically official method).

$$E\% = \frac{Q_p\ (g)}{Q_t\ (g)} \times 100$$

where, *E* is efficiency of charging of pellets with MT (loading efficiency), *Qp* is the amount of MT determined in pellets (actual content) and *Qt* is the amount of MT used in formulation (theoretical expected content) (14,15).

In vitro release of metoprolol from pellets

The Erweka apparatus was used for this test buffer phosphate solution (pH= 6.8) as media, at 37±0.2˚C. The quantity of tested pellets was considered such as to correspond with 100 mg MT (16).

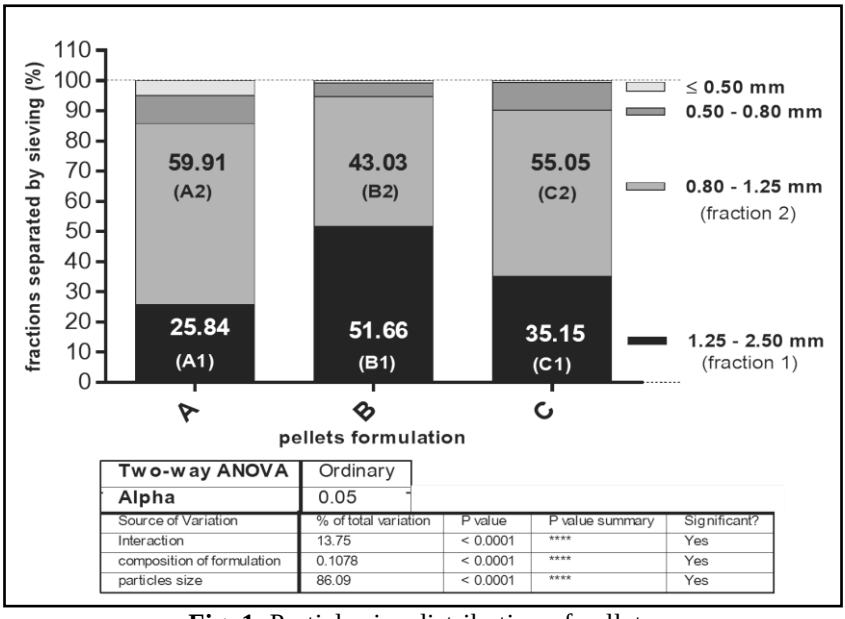
Kinetic of metoprolol release from pellets: The DDSolver software was used for fitting the data and for calculation of the statistical parameters required for comparing the dissolution profiles (17).

RESULTS

Particles size distribution

Particle size evaluation of pellets by sieving method allowed separation of two main fractions for each of the three prepared formulations (A, B and C): fraction "**1**"- consists of pellets with diameters of 1.25 to 2.50 mm and fraction "**2**"- pellets with diameters ranging from 0.80 to 1.25 mm, respectively. Variations of particle size distributions (fig. 1) are caused by the compositional differences of pellets.

In terms of pellets size distribution, it is found that more than half of the pellets of formulation A (prepared with Methocel K100 and 7.5% Eudragit NE30D) have small dimensions, fraction "**1**" (A1- 59.91%; 0.80-1.25 mm) accounting for twice of fraction "**2**" (A2- 25.84%; 1.25-2.50 mm). Size distribution of pellets in formulation B (prepared with Methocel K100 and 10% Eudragit NE30D) indicates that a small additional amount of Eudragit NE30D (2.5%) determines a significantly increasing in the size of pellets (B1- 51.66% vs. B2- 42.03%). This effect does not appear in case of formulation C (prepared with HPMC 15.000 and 10% Eudragit NE30D). Based on this last observation, the use of HPMC 15.000 in association with 10% Eudragit NE30D (C1- 35.15%/C2- 55.05%) could be more advantageous when obtaining small pellets is desired.



Two-way ANOVA	Ordinary			
Alpha	0.05			
Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	13.75	< 0.0001	****	Yes
composition of formulation	0.1078	< 0.0001	****	Yes
particles size	86.09	< 0.0001	****	Yes

Fig. 1. Particle size distribution of pellets
and statistical analysis of interaction between variables

Evaluation of pellets metoprolol tartrate content

Actual drug content (experimental data) and loading efficiency (calculated on the bases on theoretical expected data) are two parameters dependent on the size of the pellets. Data presented in table III show that the pellets contain metoprolol tartrate in range of 20% to 25%, with small differences between pellets formulations.

TABLE III

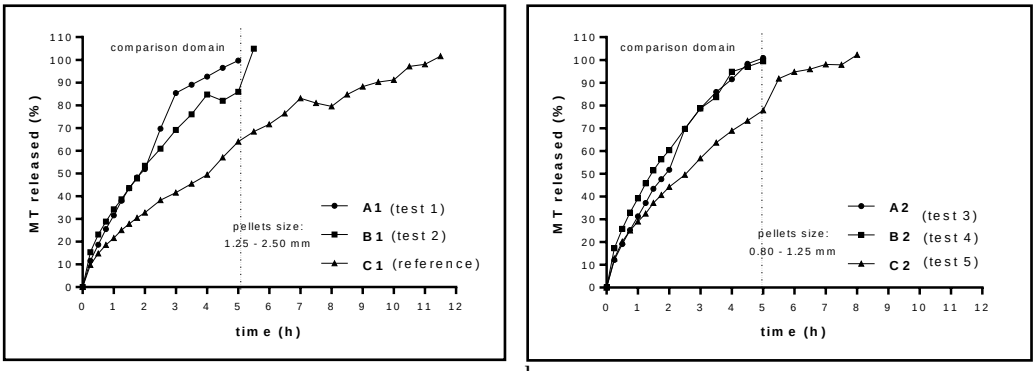
Results of evaluation of pellets metoprolol tartrate content

Formulation		Actual active content MT (%) ± SD (*n= 3)	Loading efficiency MT (%) ± SD (*n= 3)
A	**1**	19.81 ± 0.30	75.32 ± 0.67
	2	21.30 ± 0.60	82.22 ± 0.79
B	**1**	22.13 ± 0.56	86.23 ± 0.54
	2	23.85 ± 0.55	94.51 ± 0.50
C	**1**	20.38 ± 0.58	79.62 ± 0.76
	2	24.92 ± 0.65	97.14 ± 0.66
*n= number of experimental determinations			

However, in case of all three formulations, it can be noticed a higher efficiency in charging for fractions "2" (small particles: 80-97%) comparatively with fractions "1" (large particles: 75-86%). This might be due to the larger exposed surface area of the small particles in the powder layering process through which the construction of pellets occurs.

In vitro release of metoprolol from pellets

Dissolution study was performed both from the fraction "**1**" and fraction "**2**" of each pellets formulation (A, B and C). As a result, six dissolution profiles were determined by plotting the experimental data (fig. 2).



a b

Fig. 2. Release profiles of metoprolol from pellets, grouped by size (fraction "**1**"-a *vs.* fraction "**2**"-b)

The results reveal, both for fraction "**1**" and fraction "**2**", that formulations A (Eudragit NE 2.25%) and B (Eudragit NE 3.00%), based on Methocel K100, release the initial active content (100% approximately) within 5 hours, which means that there are no significant differences of the metoprolol release rates. In case of formulation C, using another combination of drug release retarding agents (HPMC 15.000, Eudragit NE 3.00%), metoprolol is released more prolonged than from the others two

formulations (8 h-C2; 11.5 h-C1). Considering C1 as reference and the others as test formulations, when comparing the release profiles for the first 5 hours of the dissolution test in terms of differences (f1) or similarity (f2), it appears that the C1 release profile is different than the others (fig. 3).

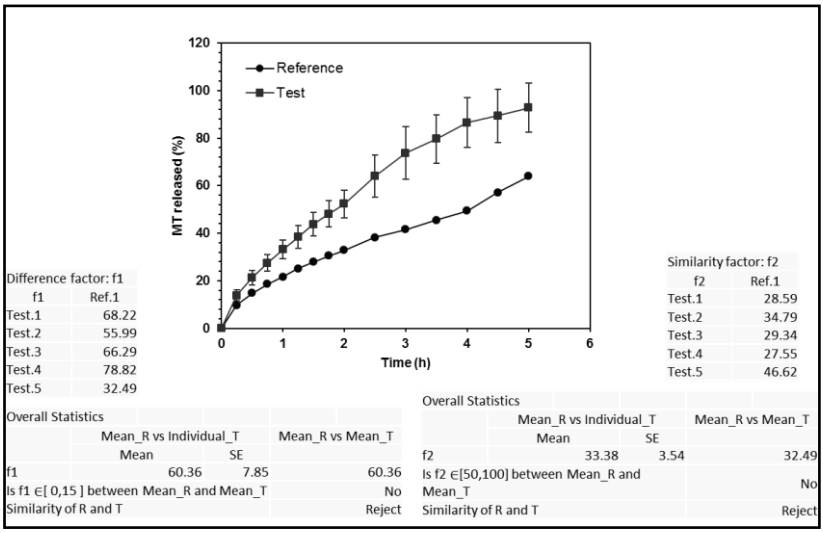


Fig. 3. Statistical comparison of metoprolol release profiles from pellets

Kinetic of metoprolol release from modified-release pellets

In order to determine the mechanism of metoprolol release kinetic from modified-release pellets, C1 release profile was fitted with various mathematical equations. Based on the values of adjusted coefficient of determination (Rsqr_adj), it was revealed that release of metoprolol occurs by dissolution and diffusion, according to Korsmeyer-Peppas equation (fig. 4).

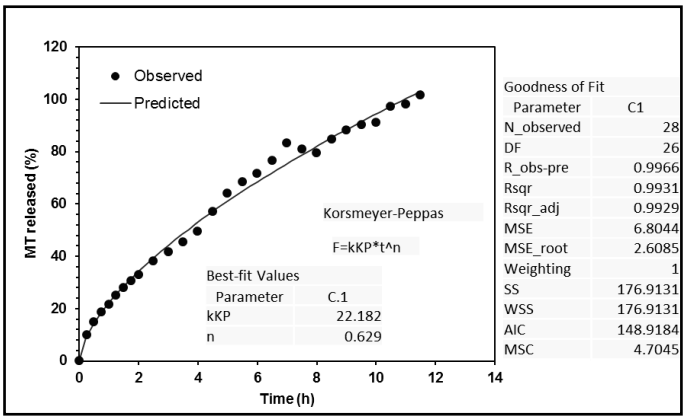


Fig. 4. Kinetic parameters calculated for release of metoprolol from C1 (Modified-release pellets)

CONCLUSIONS

The particles size distribution of pellets obtained by powder layering in coating pan method revealed the direct influence of formulation variables on the amounts of factions separated by sieving method. It has been also found that size distribution of pellets is consistent both with the metoprolol tartrate loading efficiency and the release of this active ingredient from pellets.

Although all six studied formulations of pellets showed a prolonged release of metoprolol, it seems that only formulation C1 (prepared with HPMC 15.000 and 10% Eudragit NE30D as combination of agents that modify the release) managed to prolong the release process to about 11 hours. Mechanism of kinetic release is based on dissolution and diffusion processes, according to Korsmeyer-Peppas model.

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CLINICAL-EPIDEMIOLOGICAL TRENDS OF HERPES ZOSTER: A 5-YEAR STUDY

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CLINICAL-EPIDEMIOLOGICAL TRENDS OF HERPES ZOSTER: A 5-YEAR STUDY (Abstract): Herpes zoster (shingles) is a neurocutaneous viral disease, in recent years its incidence increasing throughout the world. **Aim**: To study the incidence of herpes zoster among Iasi county patients requiring hospital admission and to analyze the clinical and epidemiological features and socioeconomic status of the study group. **Material and methods**: Retrospective study of 158 patients admitted to the largest clinical dermatology department in North-eastern Romania, the Clinical Dermatology Department of the Iasi "Sf. Spiridon" University Emergency Hospital. The study was conducted over a period of five years and included the analysis of epidemiological data, socioeconomic status, clinical forms of disease, associated diseases, pathology reports for skin biopsy fragments, administered treatment, and disease course. **Results:** This study confirms that shingles is not a sex-specific disease, the female/male ratio being 1.22: 1. Most patients belonged to the age group 70-80 years, accounting for about 35% of all patients, followed by the age group 60-70 years (24.6% of cases). A slight increase in the number of cases was recorded in autumn and summer in patients living in rural areas. 1.3% of the cases were diagnosed both with the disseminated form of disease, and complications (eczematization, bacterial superinfection, skin necrosis). 7.6% of patients presented additional skin disorders (pityriasis versicolor, impetigo, psoriasis vulgaris, mucocutaneous candidiasis), which raised the suspicion of an immune deficiency predisposing to shingles. The absence or late initiation of specific antiviral therapy correlated with prolonged hospital stay up to 4-6 days. We found an association between the erythematous form of shingles and young age, while the hemorrhagic or necrotic forms were present in the elderly and/or ill patients. The course was favorable and the length of illness was significantly shortened when the treatment adequate to the clinical form was administered. Associated comorbidities (essential hypertension 38.6%, dyslipidemia 24.6%, diabetes, mellitus 9.49%, chronic venous disease, other skin diseases) represent a factor complicating the development of herpes zoster by the cumulative stress the body is exposed to. **Conclusions:** The main statistically significant epidemiological data in the study group are: older age (over 60 years), associated diseases (which by the marked imbalances induced in the body increase the risk of varicella zoster virus reactivation) intense psychological stress. The following parameters did not change the risk of developing shingles: area of origin, sex, the season at disease onset, number of hospital days, and administered treatment. Early diagnosis and treatment of this disease is important for maintaining a good quality of life, to avoid complications, to limit the extent of the disease and its transmission to others. **Keywords**: HERPES ZOSTER, VARICELLA, IMMUNOSUPPRESSION.

Herpes zoster (shingles) is a neurocutaneous viral disease, being an acute viral infection of the skin caused by the reactivation of varicella zoster virus that has remained latent within the dorsal root and cranial nerve ganglia (1). Shingles often manifests decades after the primary infection (chickenpox), and occurs by virus reactivation and its spread to other mucosal skin surfaces through centrifugal migration. The factors triggering the reactivation of the dormant virus are not exactly known, but it is believed that reexposure to the virus, acute or chronic infectious diseases, neoplastic processes, some medications, and emotional stress play an important role. Immunocompromise increases the risk of virus reactivation, meaning that the incidence of shingles increases with aging and immunosuppression (2).

The incidence of shingles is of approximately 3.4‰ per year in the general population, increasing to 11.8‰ per year in the population over 85 years. In recent years, the frequency of this viral manifestation is increasing; it is a true public health concern since a good quality of life, avoidance of severe complications and limiting disease extension and its spread to other family members or entourage depend on early diagnosis and correct treatment of this disease (3).

The disease occurs sporadically throughout the year in all races and affects both sexes equally (6). The incidence is low in the early years of life, except for young people diagnosed with AIDS, lymphoma or other malignancies, and after bone marrow transplantation. Less than 10% of shingles patients are younger than 20 years and only 5% younger than 15 years (4, 7).

In the general population, the incidence rate is 10-20%, but increase to 50% in those who reach age 85 (8). After FDA approved the herpes zoster vaccine in 2006, a study of 38,000 adults aged over 60 years showed a decrease in the incidence of herpes zoster by 51.5% (2, 5).

The studies conducted in Romania show a sudden increase in the number of shingles cases in children aged 10-12 years (9, 10).

The aim of this study was to investigate the herpes zoster cases recorded over a 5-year period from epidemiological, clinical, therapeutic and socioeconomic status point of view.

MATERIAL AND METHODS

This study is a retrospective analysis of all cases diagnosed with herpes zoster at the Dermatology Department of "Sf. Spiridon" University Emergency Hospital during 2008-2013. The study group included 158 patients, 71 males and 87 females, aged 9 to 92 years. The medical records, patient photographs, and the histopathology reports for skin biopsy fragments were used. Epidemiological data, such as age, sex, area of origin, socioeconomic status, time of year when the disease occurred, and data related to the clinical form of herpes zoster, associated diseases, pathology reports, administered treatment and disease course were also reviewed.

RESULTS

Of all patients admitted in the interval 2008-2013 we selected a group of 158 patients with either typical signs and symptoms of various forms of herpes zoster (fig. 1) or with a totally atypical clinical picture.

Most patients presented a form of herpes zoster accompanied by neurological symptoms (56.3%). Of all patients, 29.1% developed a typical simple herpes zoster, and 1.3% were diagnosed with disseminated herpes zoster and complications (eczematiza-

tion, bacterial superinfection, skin necrosis).

Ophthalmic herpes zoster was diagnosed in 12% of cases, half of them complicated by herpetic keratitis or uveitis (fig. 2).

Fig. 1. Herpes zoster in the left intercostal zone

Fig. 2. Herpes zoster ophthalmicus

This study confirms that shingles is not a sex-specific disease. The statistical analysis of this study group showed that the balance is slightly tilted towards females (87 vs. 71), female/male ratio 1.22: 1.

By calculating patient age at the time of herpes zoster diagnosis, we found that most patients belonged to age group 70-80 years, accounting for approximately 35% of patients, followed by age group 60-70 years (24.6%) (fig. 3).

Of the 3% of patients under 20 years of age, a group of children aged 10-12 years, living in urban areas, without organ abnormalities or laboratory documented immunosuppression, but under severe mental stress, as declared by them and their parents, due to intense preparation for the 5th grade entrance exam stood out (fig. 4).

Although shingles occurs sporadically throughout the year, a slight increase in the number of cases in the autumn and summer compared to other seasons was noticed. This can be explained by the direct effect on health of weather variations, especially in the case of an elderly patient, with weakened immune system, living in a rural area where physical work is more intense during summer and autumn.

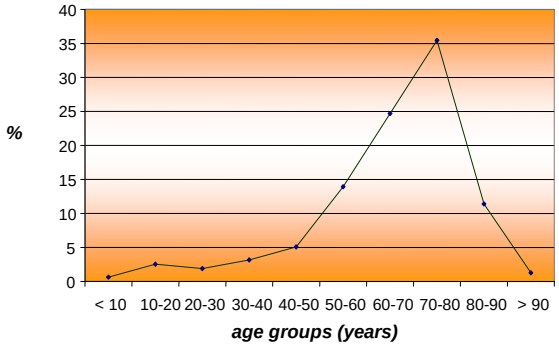

Fig. 3. Age distribution of the study group

Fig. 4. Lumbosciatic herpes zoster (L3), left side

In our study group there was a slight predominance of shingles patients coming from rural areas (53%) compared to urban areas (47% of cases).

Immunocompromised patients have a 20-100 fold higher risk of developing herpes zoster compared to the immunocompetent patients of the same age. In the study group and 7.6% of the patients also presented other skin problems (pityriasis versicolor, impetigo, psoriasis vulgaris, mucocutaneous Candida infections), which raised the suspicion of an immune deficiency predisposing to shingles.

Associated diseases are an additional stress to the body, thus making it vulnerable to reactivation of varicella zoster virus. In our study group, the most common associated disease was essential hypertension (38.6%), followed by dyslipidemia (24.6%) and diabetes mellitus (9.49%).

The average duration of herpes zoster was 10-15 days, but it took as long as a month before the skin cleared completely. In our study group hospital stay ranged between 2 and 18 days, depending on patient status upon admission, associated diseases and course under treatment (fig. 5).

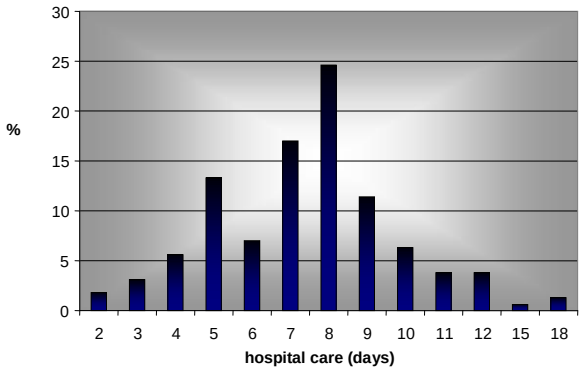

Fig. 5. Length of stay distribution of the study group

The administered treatment had an overwhelming influence on disease progression, but of great importance was the time of treatment initiation from the onset of symptoms. 83.5% of patients received full topical and systemic treatment (antiviral, antiinflammatory, analgesic, group B vitamins, ± antibiotics), but some patients

(16.5%) received no etiological treatment. Absence or late initiation of specific antiviral therapy may be correlated with prolonged hospital stay; our patients were hospitalized for a period ranging from 6 to 18 days, average 12.3 days. At the opposite pole were the patients treated correctly and on time, who were hospitalized for 2 to 12 days, average 8.1 days.

Skin biopsy followed by histopathology of skin fragments were necessary in only 3 cases for making a positive diagnosis of certainty: 1) an oligosymptomatic case with only 2-3 clusters of blisters on the dorsal and lateral external aspect of the right hand, with multiple relapses over the last 3 years (at 3-4 month intervals) that had to be differentiated from zosteriform herpes; 2) a case of eczematized shingles with atypical appearance, clusters of blisters on a poorly-defined erythematous plaque, with irregular, crumbling margins; 3) a case presenting blisters erupting in clusters, on an erythematous base, disseminated, accompanied by discrete symptoms in a young immunocompetent patients without any other disorders.

The study group included patients aged 9 to 92 years. Retrospectively, we found an association between the erythematous form of shingles herpes and young immunocompetent patients. Other associations were found between the hemorrhagic form and elderly and ill patients and between elderly ill or immunocompromised patients and necrotic shingles.

Under optimal treatment (started on time and correctly conducted) the occurrence of new clinical elements was prevented, with healing without sequels in some cases and acromial and atrophic scars in other cases. In our study group, 97.47% were discharged "improved", 2.53% "cured", none “stationary” or “deceased”.

DISCUSSION

Age distribution of the patients in our study group is similar to data reported in Europe, being slightly deflected to the right, as most patients were over 60 years of age.

Also, there was a statically insignificant predominance of female patients (55% vs. 45%). Rural/urban distribution of cases was balanced, with a slight increase in the number of rural cases (53%).

Given the limitations of a retrospective study, we can conclude that the associated diseases (essential hypertension 38.6%, dyslipidemia 24.6%, diabetes mellitus 9.49%, chronic venous disease, other skin diseases) are a factor complicating the course of herpes zoster, by the cumulative stress the body is exposed to.

Most cases of herpes zoster were accompanied by neurological symptoms (56.3%), followed by the simple classical form (29.1%) and ocular shingles (12%).

Also, an association between herpes zoster erythematous form and young patients was noted. Hemorrhagic and necrotic forms were present in the elderly and ill patients.

CONCLUSIONS

The main statistically significant epidemiological data related to herpes zoster in the study group are: older age (over 60 years), associated diseases (which by the marked imbalances induced in the body increase the risk of varicella zoster virus reactivation), and intense psychological stress. No significant change in the risk of developing shingles was found in relation with the following parameters: area of origin, sex, season at disease onset, length of hospital stay, and administered treatment.

Herpes zoster is a disease that was ig-

nored by patients for hours or days after its onset or was incorrectly diagnosed in the prodromal stage, pain preceding the appearance of skin lesions. Early diagnosis and immediate initiation of specific antiviral therapy are essential for a good quality of life and for avoiding the complications and limiting the extent of disease and its transmission to others. To this end, we suggest a thorough history and physical treatment is preventing post herpetic neuralgia. Additional diagnostic tests such as skin lesion biopsy and histopathology or PCR may be useful in atypical cases.

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THE IMPORTANCE OF CONTROL ELEMENTS IN DENTAL OFFICE MANAGEMENT

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THE IMPORTANCE OF CONTROL ELEMENTS IN DENTAL OFFICE MANAGEMENT (Abstract): In the current economic situation statistical indicators suggest a decline in global and national dental services market under increased competition. Hence, a cost-effective and strengthening market position. Thus it is recommended the office management to focus on retention and follow active patients and to create a system of quality control maneuvers. In order to achieve these objectives we must monitor certain elements of medical control, economic, management and promotion. **Keywords**: MARKETING SERVICES, CONTROL ELEMENTS, DENTAL PRACTICE, MANAGEMENT

Dental services market evolution can be interpreted using data from private health insurance companies and national professional associations of dentists and the data provided by the companies producing equipment and dental materials (1, 3, 4).

The declared values of dental materials and equipment manufacturers worldwide and European related volumes sold in each category in the last two years suggest a decrease (3, 4). The only categories where positive developments are expressed are *Cad-Cam* technology for radiology equipment and implants, but national companies distributing equipment and dental materials suggest a decline in sales while suspending contracts with C.N.A.S..

All these suggests a decrease in the number of dental treatments at national level made in a dental market with a growing number of dentists and a decrease in addressability to dental care.

In the current market of oro-dental health services proper management may facilitate praxis to new purchases and performing dentist can support multiple roles of professional, manager and entrepreneur.

For the practitioner to effectively control cabinet work certain economic elements must be taken into account, medical, marketing and management. Thus the number of patients is important in the medical act for time allocation, the scheduling, the financial aspect of the revenue they bring human resources in terms of number and their training.

The elements of medical nature are represented by the volume of patients and the number of maneuvers performed, their addressability for certain types of treatment and the quality of care.

Service quality can be measured by the implementation of therapeutic protocols and laboratory checks: X-rays, documenta-

tion patterns, general photos and intraoral camera that can capture intermediate stages, all these also covering the legal aspect of the treatment. Systematizing therapeutic measures will lead to a better control of the quality and increase the success rate. It will also lead to an efficient and predictable use of the time allocated to a particular maneuver, the necessary material and human resources involved. It is therefore recommended the formation of a constant, empathic dentist - nurse team, thus eliminating the syncope in the operating time. It is also recommended that for special, complex cases, to discuss with colleagues from the clinic or other specialists. To perfect the medical act and prevent potential complications it is recommended a review of the patients for the next day. Most people, especially the working population, one that caters to premium service want a more accurate assessment of time spent in treatment in general, and the number of each meeting. To predict this, a very accurate diagnosis should be taken into account, along with its understanding by the patient, both medically and financially. Other items which affect treatment duration are the training of the physician, the medical team, the material equipment of the cabinet and the execution time for laboratory work. Thus the office should be represented by a team of professionals with a flexible work schedule, with an adequate radiology equipment (which saves the patient trip to the radiology lab) to work with a lab, with a reasonable time of performance, which will ensure prompt shipment and provide active assistance (staff offices delegate relationship with patients to determine the color of any adjustments).

Another way to check the quality of medical care is represented by patients' opinions at the end of treatment. Their satisfaction is related to aesthetics, the time spent with them, the attention they enjoyed, all reported to the final estimate.

Patient feed-back related to the therapeutic act and the relationship with the doctor can be obtained using a questionnaire or in a phone conversation at the end of the treatment.

Patient's questionnaire should include questions about patient satisfaction, the presentation of therapeutic solution, and the relationship with the medical team. The answer may be free or quantitative, for example from 1 to 5 or from unsatisfactory to better than expected (5). There should be a section dedicated to suggestions for improving the services indicated in terms of networking, quality and types of treatment.

Patients need for certain services suggests the type of maneuvers that should be provided and hence *the tariffs* (5, 6, 7). Patients' expectations consequently dictate the level of equipment and materials supplied and also the HR element through training and number. *The qualitative* aspect is given by the professional education: specialist surgeon, orthodontist, endodontist, periodontist and *one quantitatively* reflected by the number of doctors, nurses, a receptionist, an accountant and maybe a manager. An increased quality can be achieved by continuous training of doctors and nurses and increasing communication skills within and between the medical team and the patient. This can be done by creating predictive systems, communication patterns, with answers and clear attitude that predict any case linked to the interaction of the patients, with the laboratory, suppliers. These skills can be provided in the job description and must be properly understood and acknowledged by all em-

ployees including participation in training sessions (communication and sales service) organized within the cabinet. Job descriptions should clearly state the professional obligations, continuous improvement skills, achieving periodical goals, including payment of wages, bonuses, professional advancement. Organizing regular meetings with team members will help achieve the objectives by taking certain correctional measures. Also, attention will be given to the relationship with the patients to remind them of periodic monitoring, and for any feedback.

Economic elements are represented by the value of revenue, expense, profit, cash flow. They need to be regularly assessed against monthly, quarterly, annually. Hence the recommendation to delegate tasks to specialized assistance of an accountant and manager.

Cash flow is the financial element that shows all aspects of financial activity is useful in assessing the economic performance of praxis, thus ensuring correct identification of the costs including those represented by insurance, rents, loans, rates, utilities, various contracts with certain companies (the maintenance of equipment, work safety, advertising, security) of various fees (telephone, internet, magazines, subscriptions), equipment amortization, the value of wages. Variable expenses can be represented by the stocking, bonuses, and possible unexpected expenses. To these are added investments in equipment and materials, improvement of human resources, the modernization of space, etc. in relation to inflation, changes in taxes applicable to company and employees and the purchasing power of patients.

Forecasting future investment in new technologies and the improvement of human resources is based on the addressability of patients for certain maneuvers. Acquisition of new technology can be represented by equipment, instruments, new materials, which involve improving human resources for their effective operation. These may include purchase of a radiological device, a dental microscope, a *Cad-Cam* system and an advanced system for composite materials. It can also be represented by the purchase of computer software, a camera with macro lens, to better capture the original situation, the end result, and the characteristics of the teeth and of course related software, which are intended to improve communication between practitioner and patient, between the cabinet and laboratory favoring the medical control.

Predictability of costs involved in performing maneuvers, of control over the time allotted, the addressability of patients determines the most accurate price for the treatment.

Office revenues are the revenues from patients, along with C.N.A.S. contracted amounts for physicians and laboratory examinations. A special category of income can come from contracts with private health insurance companies. A constant minimum flow of revenue can be assured by a predictable schedule of appointments, control and patient satisfaction.

Marketing elements have a role in promoting office services, both existing and new, in selling them and keeping contact with patients; advertizing can be done through the website, business cards, brochures, newsletters promotional campaigns, subscriptions.

Management involves the integration, synthesis, implementation and control of the elements mentioned above. It also in-

volves creating a business plan and implementing it. Business development will determine a delegation of a manager to closely monitor the business parameters. So, the doctor will be able focus on the role of professional and entrepreneur. This is because the national dental services market is still suffering from a lack of training and successful business models. This leads to hesitant evolving and expanding of offices and in purchasing new equipment and technologies.

The manager has to identify the elements that can measure clinical and financial development that, together with the patients determines the office politics.

A performance management involves implementing protocols, predictable system of networking and communication.

They must provide control elements, easy and accurate forecasting (8, 9). Planning and delegation processes involving office work should enable the physician to focus on the professional aspect.

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EFFECTS OF PERFLUOROCARBON EMULSION IN RHEOLOGY

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EFFECTS OF PERFLUOROCARBON EMULSION IN RHEOLOGY (Abstract): **Aim**: To study effects of a perfluorocarbon emulsion on plasma and whole human blood viscosity in the presence of albumin or modified fluid gelatin. **Material and methods:** We investigated the effects of several PFC concentrations on plasma and whole blood viscosity in the presence of human albumin solution (HAS) or modified fluid gelatine (MFG; Gelofusine) to obtain three PFC emulsion concentrations (4, 8 and 15 g/dL). Three hematocrit levels (Hct) were investigated: 30, 20 and 13%, corresponding to different clinical situations. Plasma and whole blood viscosity was measured at 37°C, using a Couette viscometer for shear rates ranging from 0.2 to128 s^{-1}. **Results and discussion:** All PFC concentrations increased plasma and whole blood viscosity for the same Hct. Viscosity values similar to physiological ones were observed at Hct 13%, with MFG – PFC 4, 8 g/dL and HAS – PFC 15g/dL; at Hct 20%, with MFG – PFC 4g/dL and HAS - PFC 15g/dL; at Hct 30%, and HAS – PFC 4, 8 g/dL. **Conclusions:** We conclude that this PFC emulsion increases plasma and blood viscosity and that among the three studied volume expanders, the interaction with MFG can result in viscosity values above the physiological one even at low Hct values. Our results suggest that such increased blood viscosity could decrease skeletal muscle oxygen pressure. **Keywords:** PFC EMULSION, WHOLE HUMAN BLOOD, VISCOSITY, HEMATOCRIT.

Severe acute blood loss resulting in hemorrhagic shock (HS) can lead to severe tissue damage responsible for high morbidity and mortality (1). The recovery of physiological macrohemodynamics parameters such as arterial pressure and cardiac output, as well as the fast restoration of microvascular perfusion and oxygen supply to vital organs, are the major aims of primary therapy of HS. Intravenous volume replacement with an acellular fluid may restore cardiac output but fails to promote microvascular perfusion (2). Severe hemodilution, due to massive infusions of volume expanders, may further impair tissue oxygenation because of decreased oxygen transport. Survival from shock is directly dependent on the restitution of adequate blood oxygen carrying capacity once the hematocrit (Hct) falls below a critical range of 20-25% (3).

It has been shown that stored as opposed to fresh RBCs have a decreased capacity to deliver oxygen in the microcirculation.

Artificial oxygen carriers based on PFC emulsions have been developed because they can increase oxygen transport in the initial stages of resuscitation (4). Indeed, in the case of acute severe anemia, or acute

normovolemic hemodilution (ANH), these artificial oxygen carriers can increase the oxygen carrying capacity of hemodiluted fluid. PFCs are chemically and biologically inert synthetic compounds (perfluorinated hydrocarbon compounds). PFCs have high oxygen solubility through weak van der Waals forces (4).

The rheological properties of blood regulate many physiological and pathophysiological parameters of the cardiovascular system, such as arterial blood pressure, blood flow in the microcirculation, hemostasis, inflammation, etc. Viscosity contributes to endothelial shear stress (5, 6).

The interactions among PFC and the different volume expanders used in clinical practice have not been studied.

We made the hypotheses that the PFC emulsion interacts with the three above-cited types of colloids and that these interactions modify plasma and blood viscosity; the interactions depend on the value of Hct.

MATERIAL AND METHODS

Human whole blood was diluted with the PFC emulsion mixed with different volume expenders. Three PFC concentrations were tested: 4, 8 and 15 g/dL each combined with Hct values of 13, 20 and 30%. Controls used were human whole blood at 40% Hct and diluted with autologous plasma or the volume expenders at Hct 13, 20 and 30%.

The volume expenders used were: human albumin solution 20% (HAS) diluted in Ringer solution at 50 g/L; gelofusine 4%: a modified fluid gelatin (MFG).

The viscosity of plasma, PFC and volume expanders was measured at 37°C with an automatic capillary viscometer, and expressed in $mPa.s^{-1}$. The viscosity of the different compounds: plasma, volume expanders and whole blood at different Hct levels and of mixtures of each compound with PFC at different concentrations, was determined at 37°C, using a Couette type coaxial viscometer at shear rates from 0.2 to 128 sec^{-1} and expressed in $mPa.s^{-1}$.

RESULTS

The intrinsic viscosities of volume expanders alone or mixed with plasma, in the absence or presence of increasing concentrations of PFC are presented in table I. Volume expanders alone (or in the presence of plasma) exhibit an inverse linear viscosity as a function of shear rate (lower shear rate values are associated with higher viscosity). When PFC emulsion was added, the viscosity increased as a function of the PFC concentration. The intrinsic viscosity was similar between plasma and HAS, but increased strongly with MFG (tab. I).

In preliminary experiments we verified that the presence of the PFC emulsion, even at high concentrations, did not interfere with the measurements of viscosity.

A total of 45 situations, according to Hct values (40, 30, 20 and 13%), volume expanders (plasma, MFG, HAS) and PFC concentrations (0, 4, 8, and 15 g/dL) have been studied. The control situations of whole blood at Hct values of 40%, and blood diluted with autologous plasma and with volume expenders, at Hct values of 30, 20 and 13% respectively were represented in figure 1.

When blood was mixed only with volume expanders (fig. 1), whatever the final Hct, the hemodiluted blood presented always viscosities lower than whole blood at 40% Hct. In the situation where Hct value was 13%, whatever the volume expander used, the viscosity values were similar with those observed upon dilution with autolo-

gous plasma.

When PFC was added to blood hemodiluted with the volume expanders, the viscosity was statistically higher. In fact, the presence of the PFC emulsion, independently of the Hct values, always resulted in highly increased blood viscosity (fig. 2, 3).

TABLE I

Intrinsic viscosity of the human plasma, volume expenders and mixtures of volume expenders with different PFC emulsion concentrations

Solutions	PFC emulsion (g/dL)	Intrinsic viscosity (mPa.s^{-1}) at shear rates 20.4 s^{-1} (left), 128 s^{-1} (right)	
		Alone	Plus plasma
Human plasma	0	1.4	
HAS	0	1.0/1.0	
	4	1.1/1.2	1.0/1.2
	8	1.7/1.5	1.2/1.3
	15	3.0/2.8	5.2/3.1
MFG	0	1.7/1.7	
	4	2.5/2.3	1.8/1.6
	8	3.9/2.9	3.4/2.6
	15	58/off limit	20/9

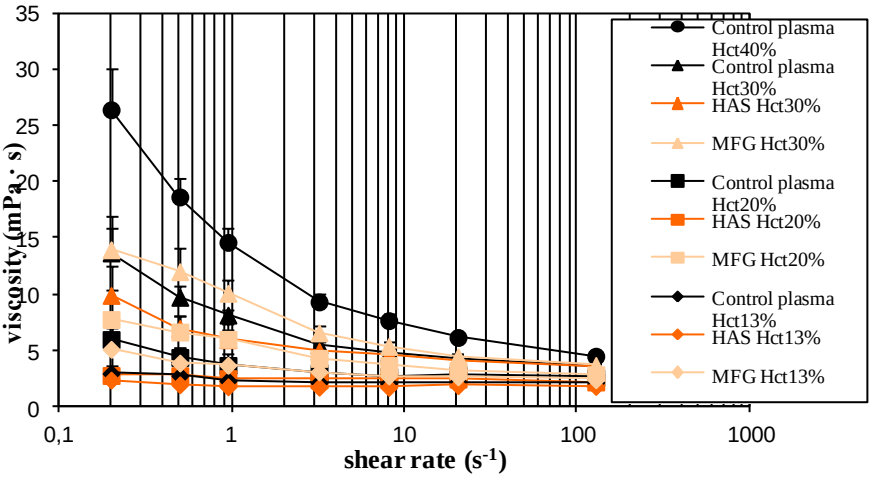

Fig. 1. The effects of shear rates on the viscosity of red blood cells diluted with autologous plasma at Hct 40, 30, 20, and 13% and with different volume expanders at Hct 30, 20, and 13% (n = 6 for each group)

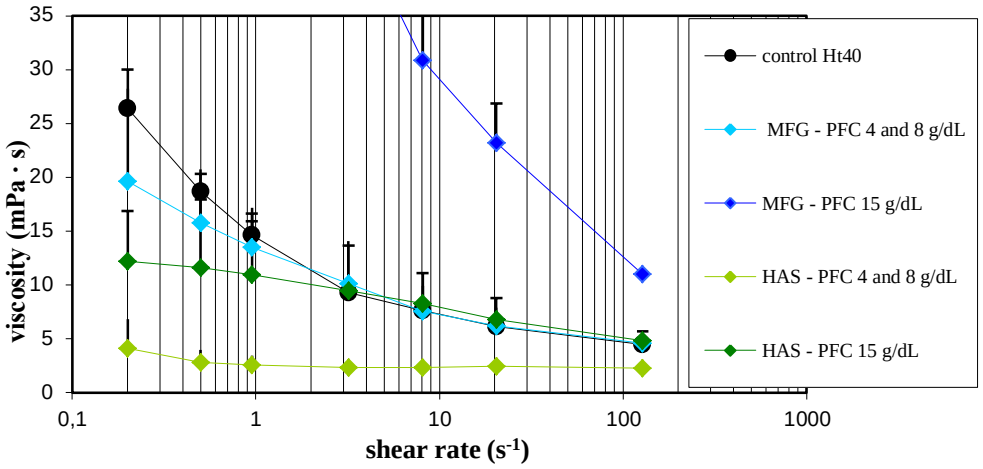

Fig. 2. The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 13%

When hemodilution reached a final Hct 13%, and was made with HAS, a strong decrease of blood viscosity was observed. When 15 g/dL of PFC emulsion was added, this restored the physiological viscosity (NS vs control at Hct 40%) (fig. 2). A similar viscosity as control 40% Hct was observed in the presence of MFG and PFC 4 and 8 g/dL. In contrast to what was observed with HAS, when all the other volume expenders studied were mixed with 15 g/dL PFC to obtain identical hemodilution, the viscosity was always higher than the physiological value (Hct of 40%). However, at these same Hct values, HAS with lower PFC emulsion concentrations (4 and 8 g/dL) led always to viscosity values below those observed at Hct 40%.

When hemodilution reached a final Hct of 20% (fig. 3), blood viscosity was significantly higher than the physiological viscosity with MFG and PFC of 8 and 15 g/dL respectively. Viscosity values were similar to physiological values for MFG PFC 4 g/dL and HAS PFC 15 g/dL. All other combinations of volume expanders and PFC at different concentrations resulted in viscosity values below the physiological ones (fig. 3).

When hemodilution reached a final Hct of 30% (fig. 4), blood viscosity was significantly higher than the physiological viscosity with MFG and PFC of 4 and 8g/dL respectively. Viscosity values were similar to physiological values for HAS PFC 4 and 8g/dL. All other combinations of volume expanders and PFC at different concentrations resulted in viscosity values below the physiological ones (fig. 4).

DISCUSSION

The effects of PFC on blood rheology are more difficult to analyze because in clinical practice, the final results of resuscitation with volume expanders and oxygen carriers is a complex mixture of native red

blood cells, plasma, volume expanders and eventually PFC. These complex mixtures could have unpredictable effects on blood rheology.

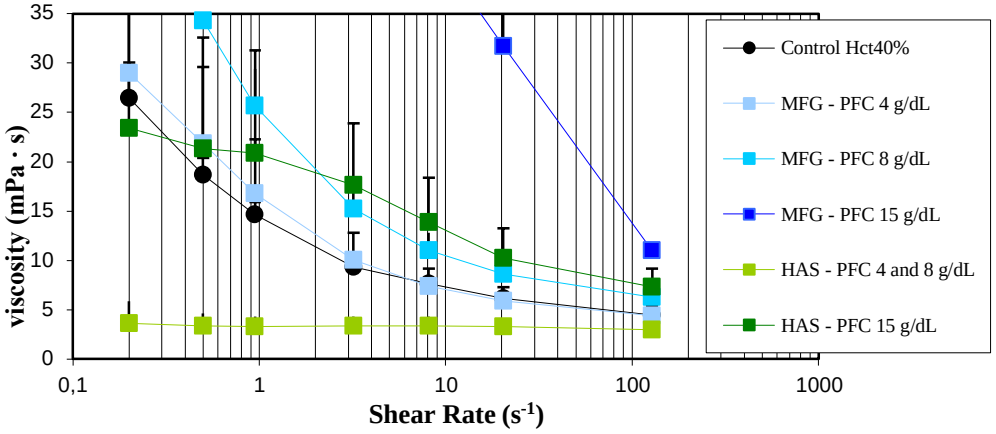

Fig. 3. The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 20%

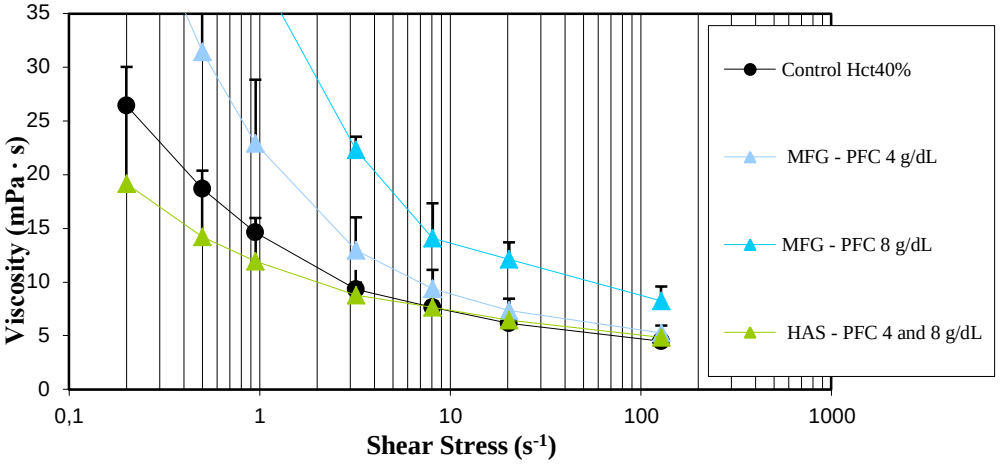

Fig. 4. The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 30%

We measured *in vitro* the viscosity of human whole blood diluted with the PFC emulsion mixed with different volume expanders. Three PFC concentrations were tested: 4, 8 and 15 g/dL each combined with Hct of 13, 20 and 30%. Controls used were human whole blood at 40% Hct (physiological blood viscosity values) and

diluted with autologous plasma or the respective volume expanders at Hct 13, 20 and 30%.

For this study, we have chosen different PFC concentrations: 4 g/dL, 8 g/dL and 15 g/dL. The higher doses, although not used in clinical practice could allow to magnify potential effects and to better resolve trends in the rheological behavior of mixtures. Different shear rates (2.8 to 128 s^{-1}) which represent the different blood flow inside post capillary venules and large vessels (7). Different Hct values to explore the different cases susceptible to be meeting in acute clinical situation such as HS (Hct values of 10-15%) or cardiopulmonary bypass (Hct values of 20-30%).

Freyburger *et al* have shown significant alterations on the blood rheological properties *in vivo* hemodilution situation (8). MFG has been shown to facilitate red blood cell aggregation *in vitro* by reducing the electrostatic repulsive forces after adsorption on the cell membrane. MFG has been shown to interact specifically with plasma proteins such as fibronectin, and also may enhance the formation of bridges between red blood cells (9).

The effect of PFC on the rheological parameters of blood in a living organism at the Hct twice lower than the normal values was studied by Kuznetsova *et al*. (10). These authors showed that an increase in emulsion concentration leads to an increase in blood asymptotic viscosity and the aggregation of erythrocytes. It was concluded that only a small volume of PFC emulsion, as compared with the total amount of erythrocytes, can improve the blood fluidity upon disturbed circulation. The results suggest that PFC and MFG interact to increase viscosity and even at low doses of PFC and low Hct values, the presence of MFG results in viscosity values above those considered to be physiological.

CONCLUSIONS

The main findings of this study are that: addition of PFC, even at low clinically used concentrations, to different volume expanders, in the absence or presence of RBC, always resulted in increased viscosity values. At several concentrations, PFC increased viscosity values above the physiological values (that measured for 40% Hct in plasma). For instance, at an extreme Hct value of 13%, the addition of MFG, 4 g/dL of PFC restored the physiological viscosity values. For higher PFC concentrations, viscosity is dramatically higher than physiological values even at such low Hct values. These observed viscosities were more important when Hct was increased.

If the concentrations of PFC must be increased, the albumin solution has the smallest effect on blood rheology.

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NEWS

NEW TARGET FOR THE OLD ASPIRIN

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, a classical and authentic cyclooxygenase (COX) inhibitor, reduces the risk of developing several types of cancer. On site of inflammation, induction of COX-2 quickly results in the biosynthesis of prostaglandins leading to inflammatory response. Overexpression of inducible COX-2 and an increased prostaglandin biosynthesis play a significant role in carcinogenesis. Ogawa and coworkers investigated the effect of aspirin on regional lymph node metastasis during the development of lung cancer in mice. The carcinoma model was obtained by injecting a solution containing Lewis lung carcinoma (LLC) cells overexpressing green fluorescent protein (GFP) and BD Matrigel in the male mice lungs. Operative mortality was 10%. Single pulmonary nodules developed at the implanted site in 95% of animals, and regional mediastinal lymph node metastasis were observed 14 days after LLC-GFP cell injection in mice. Aspirin was orally administered (100 mg/kg, twice a day) after LLC-GFP cell injection. The reducing of percentage metastasis to regional lymph nodes was observed beginning with day 12 (63%), with no significant suppression of primary tumor growth in the lungs. Aspirin treatment led to a significant reduction in mortality (survival rate 70%; $P < 0.0001$). The authors suggested that COX inhibitors have potential to prevent lymph node metastasis (Ogawa F, Amano H, Ito Y, Matsui Y et al. Aspirin reduces lung cancer metastasis to regional lymph nodes. *Biomed Pharmacother* 2014; 68:79–86).

Maria Aprotosoaei

POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AN EXTRACTIVE FRACTION FROM *ABIES ALBA* BARK

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POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AN EXTRACTIVE FRACTION FROM *ABIES ALBA* BARK (Abstract): The wood of mature *Abiesalba*Mill. (silver fir) trees is used in construction and furniture industries, the bark being the main waste product. **Aim:** This study aimed to investigate possible therapeutic applications of *Abiesalba* bark waste. **Material and methods:** A raw extract and four extractive fractions were obtained from silver fir bark. They were also studied regarding their polyphenolic content (Folin-Ciocalteu assay). The ethyl acetate extractive fraction was further studied regarding its ability to scavenge free radicals (DPPH, superoxide anion and hydroxyl radicals) and to chelate ferrous ions. **Results and discussion:** The ethyl acetate fraction contained the highest amount of polyphenols (47.72 ± 0.38 g gallic acid equivalents/100 g). Its DPPH scavenging and ferrous ions chelating abilities (EC_{50} = 7.9± 0.1 and 1.56 ± 0.05μg/ml) were comparable to those of the positive controls, catechin (EC_{50} = 7.10± 0.05 μg/ml) and EDTA-Na_2 (EC_{50} = 1.27± 0.01μg/ml), respectively. It also scavenged superoxide anion and hydroxyl radicals with EC_{50} values of 53.30± 5.91 and 63.12 ± 1.78μg/ml, respectively. **Conclusions:** The ability of the ethyl acetate fraction to scavenge free radicals and chelate ferrous ions justifies further studies on the possible therapeutic applications of its antioxidant potential. **Keywords:***ABIES ALBA*MILL., POLYPHENOLS, DPPH RADICAL, SUPEROXIDE ANION RADICAL, HYDROXYL RADICAL, FERROUS IONS.

In the last years, there was a special interest for the study of polyphenols, especially proanthocyanidins – condensed flavan-3,4-diol structures (1, 2). One of the most studied extracts is Pycnogenol® (Horphag Research Ltd, Geneva, Switzerland). This is an extract isolated from the maritime pine (*Pinusmaritima* Mill.) bark containing proanthocyanidins, phenolic acids and flavonoids. Pycnogenol® acts as a scavenger for reactive oxygen species. Therefore, it reduces the incidence of mutagenic DNA damage and protects normal cells from the cytotoxic effects of oxidative stress. Pycnogenol® stimulates the activity of certain intracellular enzyme systems with antioxidant effects (superoxide-dismutase, catalase, glutathione-peroxi-

dase, glutathione-reductase) (3). The promising results of these investigations on Pycnogenol® justify the present study aiming to evaluate the antioxidant potential of polyphenols from another conifer bark.

Abiesalba Mill. (*Pinaceae*, silver fir) is naturally spread in the highlands of Romania. It is also planted as forest species, its wood being used as raw material in furniture industry or as building material. The aim of this research was to explore possible medicinal applications for the bark - the main waste from the industrial processing of the wood of mature silver fir trees. For this purpose, a raw extract and four fractions were isolated from silver fir bark using a fast and inexpensive method. The ethylacetate fraction, having the highest polyphenolic content, was subjected to several *in vitro* antioxidant assays.

MATERIAL AND METHODS

Plant material. *Abiesalba* Mill. bark was collected in February 2008 in Bicaz area, Neamț district. The bark samples were dried at room temperature in the dark for two weeks.

Extraction and fractionation.Silver fir bark (150 g), dried and powdered, was extracted with 1500 ml of 80% methanol for 1 h using a magnetic stirrer (FALC F-30ST magnetic stirrer). The extraction was repeated two times. The combined extracts were concentrated under reduced pressure (Büchi R-210 rotavapor, Büchi V-850 pressure controller, Büchi V-700 vacuum pump) and then lyophilized (Unicryo TFD 5505 freeze-dryer). 17.44 g of dry raw extract were obtained. 16 g of the raw extract were suspended in 160 ml of double distilled water and successively extracted with 5×160 ml of diethyl ether, 9×160 ml of ethyl acetate and 10×160 ml of n-butanol. The extractive fractions were concentrated under reduced pressure while the remaining aqueous phase was lyophilized.

Quantification of total phenolic content.Total phenolic content was determined by the Folin-Ciocalteu method (4). Extracts (1 mg/ml in DMSO; 40 μL) were mixed with water (3.16 ml) and Folin-Ciocalteu's phenol reagent (0.2 ml). After 5 min., 20% sodium carbonate solution (0.6 ml) was added. The solutions were mixed and left standing at room temperature. After 2 h the absorbance was measured at 765 nm. The results were expressed as g of gallic acid equivalents (GAE)/100 g extract.

DPPH radical scavenging assay.Free radical scavenging activity was evaluated by testing the ability to scavenge diphenylpicrylhydrazyl (DPPH) radical (5). Different concentrations of the sample to be tested (0.31-5 mg/ml in DMSO; 50 μL) were added to a solution of DPPH in methanol (A_{517nm}=1.00 ± 0.05; 2.95 ml). After 5 min., the absorbance was measured spectrophotometrically at 517 nm. (+) Catechin hydrate was used as positive control. Percent DPPH radical-scavenging activity was calculated using the formula: $100 \times (A_{start} - A_{end})/(A_{start})$ where A_{start} is the absorbance before adding the sample and A_{end} is the absorbance measured 5 min. after adding the sample.

Superoxide anion radical scavenging assay.Superoxide anion radical scavenging activity was evaluated according to a described procedure with minor changes (6, 7). All reagents were prepared in 100 mM phosphate buffer (pH 7.4). The reaction mixture contained different concentrations of sample (20.16-322.56 μg/ml), nitrobluetetrazolium (25 μM), beta-nicotinamide adenine dinucleotide reduced (NADH, 78 μM) and phenazine methosulphate (10

μM). After 2 min. the absorbance was read at 560 nm. (+) Catechin hydrate was used as positive control. Percent superoxide anion radical scavenging activity was calculated using the formula: $100 \times (A_{control} - A_{sample})/(A_{control})$.

Hydroxyl radical scavenging assay.Hydroxyl radical scavenging activity was assayed by the deoxyribose method (8, 9). Briefly, the sample (0.62-10 mg/ml in ethanol; 95 μL) was mixed with 5 mM 2-deoxy-D-ribose (0.4 ml) and phosphate-saline buffer (0.1 M; pH 7.4) up to a final volume of 1.6 ml. After addition of 2 mM $FeSO_4 \times 7H_2O$ (0.2 ml), the mixture was incubated at 37ºC for 1 h. Then 1% thiobarbituric acid (1 ml) and 2.8% trichloroacetic acid (1 ml) were added followed by heating for 15 min. at 100ºC. The mixture was cooled and the absorbance was measured at 532 nm. (+) Catechin hydrate was used as positive control. Percent hydroxyl radical scavenging activity was calculated using the formula: $100 \times (A_{control} - A_{sample})/(A_{control})$.

Ferrous ion chelating assay.Ferrous ion chelating activity was estimated according to a literature procedure (10, 11) with minor changes. The assay was carried out in 20 mM potassium phosphate buffer (pH 7.2). The sample was mixed with a solution of 2 mM $FeCl_2 \times 4H_2O$ (0.05 ml). After 1 min., 5 mMferrozine (0.2 ml) was added followed by vigorous shaking. The absorbance of the ferrozine-ferrous ions complex was measured at 562 nm. Ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2) served as positive control. The percentage of ferrous ion chelating activity was calculated using the formula: $100\times (A_{control} - A_{sample})/(A_{control})$.

RESULTS AND DISCUSSION

*Extraction and fractionation.*In order to obtain a polyphenol-rich extract, *Abiesalba* Mill. bark was extracted with 80% methanol - a common solvent used for polyphenols extraction from vegetal sources. The hydromethanolic raw extract (AA) was fractioned with solvents of different polarities; four extractive fractions were obtained containing phytochemicals soluble in diethyl ether (AA-1), ethyl acetate (AA-2), n-butanol (AA-3) and water (AA-4). The three organic fractions were concentrated under reduced pressure leading to 1.30 g of AA-1, 2.04 g of AA-2 and 5.67 g of AA-3. The remaining aqueous phase was lyophilized and 6.84 g of AA-4 were obtained. Extracts were stored at -20°C until use.

*Quantification of total phenolic content.*Fraction AA-2 contained the largest amount of polyphenols (47.72 ± 0.38 g gallic acid equivalents/100 g). The lowest polyphenolic content was determined in fraction AA-1 (15.02 ± 0.31 g gallic acid equivalents/100 g) (tab. I).

TABLE I

Total phenolic content of raw extract and extractive fractions from*Abies alba* Mill. bark

Extract / Extractive fraction	Total phenolic content (g gallic acid equivalents/100 g extract or extractive fraction)
AA	29.86 ± 1.07
AA-1	15.02 ± 0.31
AA-2	47.72 ± 0.38
AA-3	26.92 ± 0.14
AA-4	27.20 ± 0.30

values are means of three replicates ± SD

Both normal-phase and reversed-phase HPLC-DAD analysis of silver fir bark extracts revealed the presence of low molecular phenolics in fractions AA-1 and AA-2 while fractions AA-3 and AA-4 contained proanthocyanidin-type polymers (data not shown). Highly polymerized proantho cyanidins are poorly absorbed in the gut and poorly metabolized by the intestinal microflora. In addition, polymeric proantho cyanidins bind to proteins, starch and enzymes forming complexes which cannot be absorbed. Low molecular polyphenols have a high bioavailability being absorbed in the gut (12). Fraction AA-2 contained the largest amount of polyphenols, mainly low molecular compounds and therefore it was chosen for further studies.

DPPH radical scavenging assay. Scavenging of free radicals is one of the main mechanisms by which polyphenols act as antioxidants. Polyphenols scavenge DPPH radical by donating hydrogen atoms of phenolic hydroxyls and transfering an electron from phenolic hydroxyls or phenoxide anions. Both the solvent and the redox potentials of polyphenols affect the mechanism of radical scavenging. Polar solvents (methanol, ethanol) form strong hydrogen bonds with phenolic hydroxyl groups and therefore, in polar solvents, electron transfer is the major mechanism by which polyphenols scavenge DPPH radical; in apolar solvents, hydrogen donation prevails (13). Fraction AA-2 and catechin scavenged DPPH radical in a concentration dependent manner. According to the EC_{50} values, the scavenging ability of fraction AA-2 (EC_{50} = 7.9 ± 0.1 μg/ml) was comparable to that of catechin (EC_{50} = 7.10 ± 0.05 μg/ml) (tab. II).

Superoxide anion radical scavenging assay.Despite a low reactivity and a low capacity of crossing cell membranes, superoxide anion radical is toxic due to its capacity to promote DNA damage. Superoxide anion radical does not react with DNA directly. Its DNA damaging potential is due to the conversion into highly reactive hydroxyl radical through iron-induced redox reactions (14). The effect of fraction AA-2 on superoxide anion radical was evaluated by measuring the inhibition of NADH-dependent nitrobluetetrazolium reduction. In this assay, superoxide anion radical is generated in a NADH-phenazinemethosulphate system. Once generated, superoxide radical reduces nitro bluetetrazolium to blue formazan which is measured spectrophotometrically at 560 nm. In the presence of antioxidants, the formazan production is diminished; the decrease in absorbance at 560 nm is proportional with the superoxide scavenging activity (6, 7). Both fraction AA-2 and catechin scavenging effects were concentration dependent. Fraction AA-2 scavenging activity increased from 24.13±0.69% at 20.16 μg/ml to 82.81± 0.79% at 322.56 μg/ml. At the same concentrations, catechin scavenged superoxide radical by 32.57±0.06% and 92.53±0.12%, respectively. According to the EC_{50} values, fraction AA-2 (53.30 ± 5.91 μg/ml) had a lower scavenging activity in comparison to catechin (39.53 ± 0.95 μg/ml) (tab. II).

Hydroxyl radical scavenging assay.Hydroxyl radical is considered to be the most deleterious reactive oxygen species. As it has an extremely short half-life and a low diffusion capacity, hydroxyl radical will damage the closest biomole-

cules immediately after its generation. In case hydroxyl radical is produced in immediate vicinity of nucleic acids, it may oxidize DNA directly. The effect of fraction AA-2 on hydroxyl radical was estimated using 2-deoxy-D-ribose degradation assay (8, 9). Hydroxyl radicals generated by iron (II) sulfate heptahydrate in phosphate buffer damage 2-deoxy-D-ribose with the release of thiobarbituric-acid reactive material which is spectrophotometrically quantified at 532 nm. The decrease of absorbance at 532 nm indicates hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity of fraction AA-2 increased from 17.06±0.18% at 15.62 μg/ml to 70.41±0.76% at 250 μg/ml. The scavenging effects of the positive control, catechin, were 27.14±0.97% at 15.62 μg/ml and 76.36±0.34% at 250 μg/ml. As compared by the EC_{50} values, fraction AA-2 (63.12 ± 1.78 μg/ml) showed less hydroxyl radical scavenging activity than the positive control, catechin (46.16 ± 0.72 μg/ml) (tab. II).

Ferrous ion chelating assay.Ferrous ions are involved in the generation of reactive oxygen species, mainly hydroxyl radical *via* autoxidation and Fenton/Haber-Weiss reactions. Iron-induced reactive oxygen species inhibit tumor suppressor genes increasing the risk of cancer development (14). Ferrous ions chelation is another mechanism by which polyphenols act as antioxidants. The ferrous ions chelating activity was evaluated by the ferrozine assay. Ferrozine binds ferrous ions forming red coloured complexes with a strong absorbance at 562 nm. Chelating agents reduce ferrozine-ferrous ions complex formation with a decrease in absorbance at 562 nm (10, 11). Fraction AA-2 and EDTA-Na_2 chelated ferrous ions in a concentration-dependent manner. The positive control, EDTA-Na_2 and fraction AA-2 showed comparable chelating effects (EC_{50} = 1.27 ± 0.01 μg/ml and 1.56 ± 0.05 μg/ml, respectively) (tab. II).

TABLE II

EC_{50} values (μg/ml) of fraction AA-2 in different antioxidant assays

Sample	DPPH radical scavenging activity	Superoxide anion scavenging activity	Hydroxyl radical scavenging activity	Ferrous ion chelating activity
AA-2	7.9 ± 0.1	53.30 ± 5.91	63.12 ± 1.78	1.56 ± 0.05
catechin	7.10 ± 0.05	39.53 ± 0.95	46.16 ± 0.72	n.d.
EDTA-Na_2	n.d.	n.d.	n.d.	1.27 ± 0.01

values are means of three replicates ± SD; n.d. – not determined

CONCLUSIONS

This study demonstrated that an ethyl acetate extractive fraction (AA-2) isolated from the bark of mature *Abies alba* Mill. trees effectively scavenged free radicals (DPPH, superoxide anion and hydroxyl radicals) and chelated ferrous ions. Our findings justify further studies on the identification of the active constituents and *in vivo* antioxidant activity of fraction AA-2.

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SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME NEW BENZYLIDENE-THIAZOLIDINE-XANTHINE DERIVATIVES

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SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME NEW BENZYLIDENE-THIAZOLIDINE-XANTHINE DERIVATIVES (Abstract): The International Diabetes Federation reported that 246 million adults worldwide had diabetes mellitus and the prevalence of this syndrome was expected to increase continuously. **Aim:** To design new compound with potential antidiabetic and antioxidant activity. **Material and methods:** New benzylidene-thiazolidine derivatives (BT2a-2e) were obtained by condensation of xanthine-thiazolidine-4-one (TZ-4-one) with aromatic aldehydes. The synthesized compounds were characterized by spectral method (IR, ^{1}H-NMR, ^{13}C-NMR) and their antioxidant potential has been also evaluated. **Results:** The synthesized compounds have important antioxidant effects as compared to xanthine-thiazolidine derivatives. The most active compounds were those obtained by condensation with 4-dimethylaminobenzaldehyde (BT2c) and 4-nitro-benzaldehyde (BT2e). **Conclusions:** The chemical modulations performed on the structure of TZD-4-one have a good influence on their antioxidant potential. **Keywords**: XANTHINE, THIAZOLIDINE, SPECTRAL METHODS, ANTIOXIDANT

Type 2 diabetes mellitus (T2DM) is considered to be the „epidemic of the 21st century" and the discovery of new therapies is a challanges for researchers. An inadequate glycemic control can be the leading cause of cardiovascular disorders, blindness, renal failure and amputations (1).

Due to the complex nature of this disease and decreased beta cell function, the management of glycemic control is difficult. There are currently used distinct classes of hypoglycemic agents: biguanides, sulfonylureas, meglitinides, thiazolidinediones, α-glucosidase inhibitors. The disadvantages of this therapy are the multiples side effects (weight gain, lactic acidosis, flatulence, oedema, fracture, liver and renal failure, pancreatitis) and more importantly the difficulty to obtain a good glycemic control. The new goal in T2DM management is the development of incretin modulators and dipeptidyl peptidase IV inhibitors (DPP IV).

DPP IV inhibitors are of considerable

interest to the pharmaceutical industry and intense researches in this area have resulted in the release to the market of such drugs as: sitagliptin, saxagliptin, linagliptin, alogliptin, vildagliptin (2). Linagliptin is a xanthine-based orally administered long acting drug, which at a 5 mg dose inhibits DPP IV activity more than 80% (3).

Hyperlipidemia and free radicals are among the most important factors involved in the pathogenesis of diabetes. Persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species resulting from glucose auto-oxidation and protein glycosylation (4).

This study presents the synthesis, physico-chemical and spectral characterization, and evaluation of the antioxidant activity of new xanthine scaffold benzylidene-thiazolidine-4-ones, in order to obtain new compounds with potential antidiabetic and antioxidant action.

MATERIAL AND METHODS

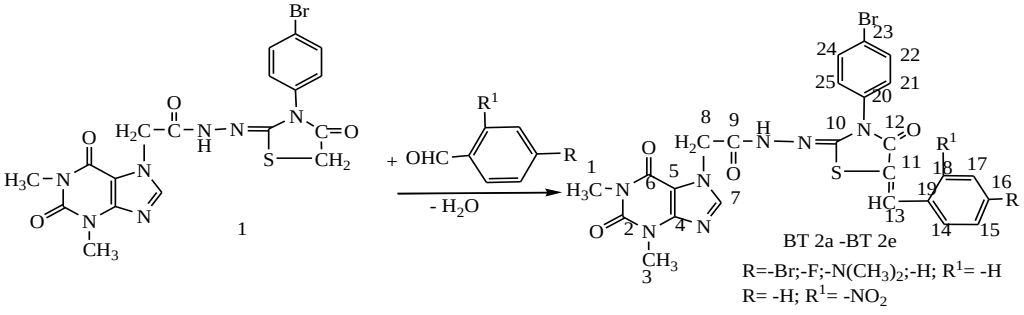
4-brom/4-fluor/4-N-dimethylamino/2-nitro-benzaldehyde; vitamin C; vitamin E; DPPH; ABTS, potassium persulfate; monosodium and disodium phosphate; ammonium molybdate; potassium ferricyanide; trichloracetic acid; iron chloride. ^{1}H-NMR and ^{13}C-NMR were recorded in DMSO-d_6 with a BrukerAvance 300 MHz instrument. The chemical shift was expressed in ppm as a function of tetramethylsilane (TMS) as internal standard. FTIR spectra were recorded on a Biorad FT-IR- FTS 575C spectrophotometer. All melting points were determined on Buchi B-540 Melting Point apparatus.

Chemistry

The compounds were obtained through condensation of 2-{2-[2-(1,3-dimethylxanthin-7-yl)acetyl]hydrazono}-3-(4-brom) phenylthiazolidin-4-one (TZ-4-one, 1) with different substituted aromatic aldehydes, by adapting similar methods described in the literature (5) (Scheme 1).

General procedure for BT 2a-2e synthesis

2-{2-[2-(1,3-dimethylxantin-7-il)acetyl]-hydrazono}-3-(4-brom)phenylthiazolidin-4-one (4g, 0.0079 mol) was dissolved in 100 ml dioxane. To this solution aromatic aldehyde (0.0079mol) and piperidine (0.00395) were added as catalyst and maintained under reflux for 6 h. The solvent was removed under reduced pressure and the final compound was precipitated in cold water. The solid product separated was filtered, washed with excess of cold water, dried and recrystallized from dioxane.



Scheme 1. Synthesis ofxanthine scaffold benzylidene-thiazolidine-4-one derivatives

2-{2-[2-(1,3-dimethy-xanthine-7-yl)acethyl]hydrazono}-5-(4-florbenzylidene)-3-(4-brom-phenyl)-thiazolidine-4-one (**BT2a**). Yellow powder; yield 69.8%; m.p. 270-271^{0}C; FT-IR (cm^{-1}): 1642.1 (-C=N), 1228.4 (-C-N), 1594.8 (-C=O amide), 1491.6, 1425.1 (-N-CH_2), 3246.5 (-NH-), 1699.9 (-C=O thiazolidine moiety), 760.7 (C-S-C), 497.5 (-C-Br), 973.8 (-C-F); ^{1}H-NMR δ/ppm (300 MHz, DMSO): 8.2 (s, 1H, CH=N-); 7.5-7.51 (m, 4H, benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH_2-C=O); 3.4-3.2 (s, 6H, 2CH_3); ^{13}C-NMR δ/ppm (300 MHz, DMSO): 165.2 (C_{12}); 154.7, 150.9 (C_2, C_6); 148.4 (C_4); 139.6, 131.7, 119.2 (C_{20} , C_{24}, C_{23}); 113.2 (C_{11}); 105.7 (C_5); 44.2 (C_8); 29.4, 27.5 (C_1, C_3).

2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl]hydrazono}-5-(4-brombenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one (**BT2b**). Yellow powder; yield 68,4%; m.p. 273-275^{0}C; FT-IR (cm^{-1}): 1643.05 (-C=N), 1228.43 (-C-N), 1594.84 (-C=O amide), 1497.56, 1426.1 (-N-CH_2), 3247.5 (-NH-), 1699.94 (-C=O thiazolidine moiety), 760.78 (C-S-C), 497.54 (-C-Br); ^{1}H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s,1H, -CH=N-); 7.57- 7.51 (m, 4H, benzylidene); 7.54-7.48 (m, 4H, phenyl); 5.8 (s, 2H, -CH_2-C=O); 3.44, 3.23 (s, 6H, 2CH_3); ^{13}C-NMR δ/ppm (300 MHz, DMSO): 165.27 (C_{12}); 154.74, 150.95 (C_2, C_6); 148.42 (C_4); 139.63, 131.76, 119.29 (C_{20}, C_{24}, C_{23}); 113.22

2-{2-[2-(1,3-dimethy-xanthine-7-yl)acethyl]hydrazono}-5-(4-N-dimethylaminobenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one (**BT2c**).Orange powder; yield 63,4%; m.p. 264-265^{0}C; FT-IR (cm^{-1}): 1643.1 (-C=N), 1228.4 (-C-N), 1594.8 (-C=O amide), 1468.5, 1426.1 (-N-CH_2), 3247.5 (-NH-), 1699.9 (-C=O thiazolidine moiety), 760.7 (C-S-C), 497.5 (-C-Br), 3107 [-N(CH_3)$_2$]; ^{1}H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s, 1H, -CH=N-); 7.58- 6.9 (d, 4H, 4CH benzylidene); 7.5-7.35 (d, 4H, phenyl); 5.79 (s, 2H, -CH_2-C=O); 3.44-3.23 (s, 6H, 2CH_3); 3.05 (s, 6H, 2CH_3benzyilidene).^{13}C-NMR δ/ppm (300 MHz, DMSO): 165.6 (C_{12}); 154.4, 150.9 (C_2, C_6); 148.4 (C_4); 140.3, 129.05, 121.9 (C_{20}, C_{24}, C_{23}); 117.37 (C_{11}); 105.77 (C_5); 44.21 (C_8); 40.32 (C_{26} and C_{27} -N(CH_3)$_2$); 29.47, 27.51 (C_1, C_3).

2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl]hydrazono}-5-benzylidene-3-(4-bromphenyl) -thiazolidine-4-one (**BT2d**).Yellow powder; yield 67%; m.p. 245-247^{0}C; FT-IR (cm^{-1}): 1642.1 (-C=N), 1228.4 (-C-N), 1592.8 (-C=O amide), 1464.6-1423.1 (-N-CH_2), 3245.5 (-NH-), 1697.9 (-C=O thiazolidine moiety), 758.7 (C-S-C), 497.5 (-C-Br); ^{1}H-NMR δ/ppm (300 MHz, DMSO): 8.2 (s,1H, -CH=N-); 7.5-7.4-7.3 (m, 5H, 5CH benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH_2-C=O); 3.4-3.3 (s, 6H, 2CH_3); ^{13}C-NMR δ/ppm (300 MHz, DMSO): 165.2 (C_{12}); 154.7, 150.9 (C_2, C_6); 148.42 (C4); 139.6, 131.7, 119.2 (C_{20}, C_{24}, C_{23}); 113.2 (C_{11}); 105.7 (C_5); 44.2 (C_8); 29.4, 27.5 (C_1, C_3).

2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl]hydrazono}-5-(2-nitrobenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one(**BT2e**).Yellow powder; yield 77%; m.p. 253-255^{0}C; FT-IR (cm^{-1}): 1643.05 (-C=N), 1228.43 (-C-N), 1594.84 (-C=O amide), 1497.56, 1426.1 (-N-CH_2), 3247.5 (-NH-), 1699.94 (-C=O thiazolidine moiety), 760.78 (C-S-C), 497.54 (-C-Br); ^{1}H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s, 1H, CH=N-); 8.06-7.5 (s, 4H, benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH_2-C=O); 3.4-3.2 (s, 6H, 2CH_3); ^{13}C-NMR δ/ppm (300 MHz, DMSO): 165.26 (C_{12}); 154.74, 150.95 (C_2, C_6); 148.42 (C_{18}); 139.63, 131.76, 119.29 (C_{20}, C_{24}, C_{23}); 113.22 (C_{11}); 105.76 (C_5); 44.19 (C_8); 29.47, 27.5 (C_1, C_3).

EVALUATION OF ANTIOXIDANT ACTIVITY

Evaluation of antioxidant activity through phosphomolybdenic method. Different sample concentrations were obtained by dilution of stock solutions (5 mg/ml). An aliquot of sample solution (0.2 ml) was mixed with the reagent solution (2ml, 28 mM sodium phosphate; 4 mM ammonium molybdate; 0.6 M sulphuric acid). The samples were incubated at 95^0C for 90 min and then centrifuged; absorbance was measured at 695 nm. The antioxidant activity of the samples was compared with that of vitamin C (2mM) (6).

Reducing power assay. The compounds possessing electron donating groups reduce Fe^{3+} to Fe^{2+} and with iron chloride form Berlin blue with a maximum absorbance at 700 nm. To 1 ml of sample solutions obtained from stock solution (5 mg/ml), 1 ml buffer phosphate (pH 6.6) was added. The mixture was shaken vigorously and 1 ml potassium ferricyanide 1% was added. After incubation 20 min at 50^0C 1 ml trichloracetic acid 10% was added and then centrifuged at 4500 rpm for 15 minutes. To 1 ml of supernatant, 1 ml distilled water and 0.2 ml ferric chloride 0.1% were added. After 5 min the absorbance was measured at 700 nm. Vitamin C was used as reference (7).

*Scavenging effect of antioxidant activity on DPPH radical.*DPPH is a stable free radical that can accept hydrogen atoms. The reduced form of DPPH is 2,2-diphenyl-1-picrylhydrazyne of yellow color. Various concentrations of the test compounds in 200 µl DMSO were added over 2500 µl solution of DPPH radical in methanol (0.1 mM). The mixture was allowed to stand for 1 hour in the dark and then absorbance was measured at 517 nm against a blank using the UV-VIS spectrophotometer. The radical scavenging capacity was calculated according to the following equation: $I\% = (Abs_{t=o} - Abs_{t=60\ min} / Abs_{t=0})x\ 100$. $Abs_{t=60min}$ is the test compound absorbance after 1 hour. $Abs_{t=0}$ is absorbance of 0.1 mM DPPH methanol solution at 517 nm. Vitamin E was used as reference (8).

*Scavenging effect of antioxidant activity on ABTS radical.*The$ABTS^+$ radicals were produced by reacting ABTS (7mM) (2,2`-azino-bis (3-etylbenzothiazoline-6-sulphonic acid) with ammonium persulphate (2.45 mM) for 16 hours in the dark as described in literature (9). This stock solution was diluted with ethanol to obtain a solution with absorbance of 0.7 ± 0.02 at 734 nm. The tests compounds were dissolved in DMSO-ethanol (5 mg/ml) and 0.02 ml of different concentrations was added to 1.980 ml ABTS solution. After 6 min the absorbance was measured and the radical scavenging capacity was calculated according to the following equation: $I\% = 100 \times (A_{inițial}-A_{final}) / A_{inițial}$, where the $A_{inițial}$= absorbance before adding the sample, $A_{fina\ l}$= absorbance after 6 min of reaction. Vitamin E was used as referee.

RESULTS AND DISCUSSION

The conclusions referring to antioxidant activity pointed out that the chemical modulations on the structure of 2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl] hydrazono}-3-(4-brom)phenylthiazolidine-4-one (TZD-4-one) through their condensation with different aromatic aldehydes (benzaldehyde, 4-brom/4-fluor/4-dimethylamino/ 2-nitrobenzaldehyde) have a good influence on the antioxidant potential, all tested compound being more active than TZD-4-one. As to the antioxidant activity determined by phosphomolybdic reagent reduction method, it has been noticed that the

most active compounds are BT2c (EC50=29.37) and BT2e (EC50=33.13), obtained through condensation with 4-dimethylaminobenzaldehyde and 2-nitrobenzaldehyde, being more active than TZD-4-one (EC50=136.9) by up to 4.7 and 4.2 times, respectively (fig. 1). These compounds were proved to be the most active of all, reducing power included; BT2c (EC50=23.13) was 6 times and BT2e (EC50=41.6) 3.3 times more active than TZD-4-one (EC50=137.857) (fig. 2).

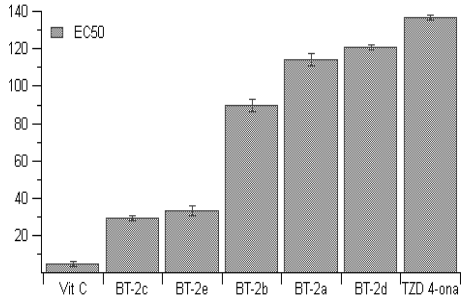


Fig. 1. Antioxidant activity

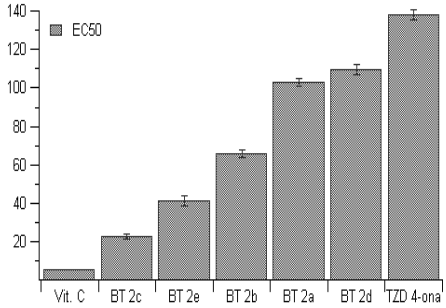


Fig. 2. Reducing power assay

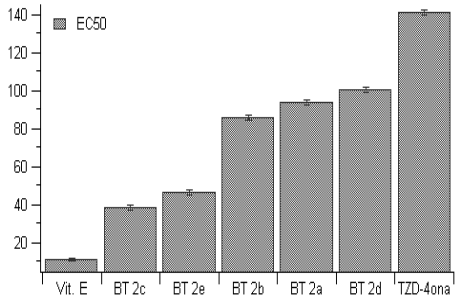


Fig. 3. Radical scavenging activity (DPPH)

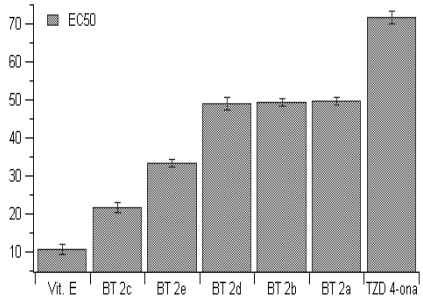


Fig. 4. Radical scavenging activity (ABTS)

The study of radical scavenging action determined by the reduction of DPPH (fig. 3) and ABTS radicals (fig. 4) showed that compound BT2c was the most active of them. Its scavenging activity was 3.7 times (EC50=38.13, DPPH method), and 3.3 times (EC50=21.17, ABTS method), respectively more intense than that of TZD-4-one. An appreciable antioxidant activity compared with TZD-4-one was also found for BT2e (EC50=46.53 and 33.2, respectively).

CONCLUSIONS

New benzylidene-thiazolidine derivatives with xanthine moiety have been synthesized, and physico-chemically characterized (melting point, yield, solubility in different organic solvents). Chemical structure was confirmed by spectral methods

(IR, ^{1}H-NMR, ^{13}C-NMR). The antioxidant potential of the synthesized compounds was evaluated, their antioxidant activity, reducing capacity and scavenging activity (by DPPH and ABTS methods) being determined. The most active proved to be the compounds resulted from condensation with 4-N-dimethylaminobenzaldehyde (BT2c) and 2-nitrobenzaldehyde (BT2e).

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DEFINING FAMILY ELEMENTS IN ADOLESCENCE

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DEFINING FAMILY ELEMENTS IN ADOLESCENCE (Abstract): The family has a permanent partner role on adolescents till adult stage. The adolescent builds up his own identity based on the affiliation to his family, but he gets away from his family for the need of leading his own life and finding his place in society. The adolescent sample was made up of 2908 college students from Timis County, aged between 15-19 years, 51.5% girls and 48.5% boys. The working **method** was the populational transversal study based on anonymous self-administration of the investigation questionnaire CORT 2004 about the risky comportment in teenagers, in a CNCSIS research project. **Results**: The study results high lighten the predominance of family organized affiliation, 78.9% living with family, 88.6%; the medium study level of 63.7% of mothers and 59.1% of fathers; the satisfaction towards family members, 71.7%; satisfaction towards family's financial level, 61.2%. In this way is underlined the role of identity development in adolescents. **Keywords:** ADOLESCENT, FAMILY, POPULATIONAL STUDY.

The adolescence is linked to professional changes that are linked to family as a hole, but also the little groups that form the family. The adolescent develop from a necessary dependent relationship to a family independence relationship. He builds up his own identity based on the family affiliation. Meanwhile he gets away from family in the need of driving his own personal life and also finding a place in society. The adolescent`s world extends out of family, and the family influence is modified (1, 2, 3).

His parents' values can be questioned and the parents became people who can make mistakes. The adolescent behavioral changes to parents start with little autonomy, setting a distance towards family, and the smallest complicity towards parents. The adolescent starts to fell the taste of independence, he wants to decide by himself, he does not like his parent`s control, he also tries to show that he is not a kid anymore, he wants immediate freedom. There also can show conflicts coming from the protection and limits imposed by the family rules, but also from the adolescent`s wish for liberty, but without responsibility.

The parental perception on physical development, emotional and sexual maturity, is that of an adult that gets control of his life. Parents` functions, future aspirations projected on the kid can become without object and can create downs towards the relation with their own kid: refuses the change, constriction, depression, and the tendency of rejection his own kid seen as a

stranger.

A permanent role of family to be the partner of their young members grow-up. The values that family transmitted to children vary from respect towards age, intern and extern roles, capacity of facing the problems, the refinement of autonomy, self-esteem, creativity and imagination, and the real development of values in perfect harmony to society.

Nowadays, the time for children education decreases in traditional family because of social responsibilities and divorces or brake-ups crash the family. It is well-known the importance of family example towards the adolescent` behavior, the presence of risky behaviors in family members with bad impact (5, 6, 7).

MATERIAL AND METHODS

The study carried out on a representative sample of 2908 high school students from Timis County, aged between 15-19 years, girls – 51.5% and boys - 48.5%. The working method was the transversal population study based on anonymous self-administration of CORT 2004 questionnaire about the investigation of risky behavior in teenagers included a research project CNCSIS (8, 9, 10, 11, 12). The answering rate was: from classes - 97.9%; from high school pupils - 76.2%; the average rate – 74.6%. The data were processed using *EpiInfo*, version 6.04, 2001.

RESULTS AND DISCUSSION

How adolescents define their family. The majority of adolescents - 78.9% (2279), indicate the membership to an organized family, with mother and father. The frequency of the unorganized (one parent, divorce, death) family is on the second place with 13.5% (381), followed by reorganized families 7.6% (220) (tab. I).

TABLE I

How adolescents define their family

Answers	Sex				Total	
	Female		Male			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
Organized	1166	78.0	1113	80.0	2279	78.9
Reorganized	125	8.4	95	6.7	220	7.6
Unorganized	196	13.6	185	13.3	381	13.5

Non answers: 28

Where and with whom the adolescents live. The adolescents from the study live with families in 2565 cases (88. 6%), at the school nursery - 177 (6.0%), most boys than girls, and 3.8% (109) of those who were asked are hosted, frequently girls. A lot of 36 (1.2%) are students who live alone (tab. II).

The instruction level of parents. The frequent school graduated by parents is high school, especially by the mother. The percentage of the fathers who graduated is 34% (964), mothers 48.6% (1382). On the second place are parents who graduated professional schools, frequently fathers: 680 (25.1%) and 420 (15.1%) mothers. Those parents who graduated university are on the third place, frequently fathers: 615 (21.6%) and 505 (17.2%) mothers, followed by professional schools graduates, 344 (12.6%) fathers and 277 (9.9%) moth-

ers. The lowest frequency is of the parents who graduated general school or less, frequently mothers: 187 (6.7%) fathers and 253 (9.0%) mothers (tab. III, IV).

TABLE II

Where and with whom the adolescents lived in the last 12 months

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
With family	1318	88.1	1247	89.1	2565	88.6
Hosted	62	4.4	47	3.2	109	3.8
In nursery	75	5.0	102	7.0	177	6.0
Alone	21	1.4	15	1.1	36	1.2
Other	50	3.3	23	1.6	73	2.4

Non answers: 49

TABLE III

The last school graduated by adolescent`s father

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
University	307	21.3	308	22.0	615	21.6
Post-highschool	179	12.9	165	12.2	344	12.6
Highschool	503	34.6	461	33.4	964	34.0
Professional school	346	23.0	334	26.2	680	25.1
General school or less	102	7.2	85	6.2	187	6.7

Non answers: 118

TABLE IV

The last school graduated by the adolescent`s mother

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
University	246	16.6	259	17.8	505	17.2
Posthighschool	160	10.6	117	9.3	277	9.9
Highschool	691	47.6	691	48.0	1182	48.6
Professional school	222	15.4	198	14.9	420	15.1
General school or less	142	9.8	111	8.2	253	9.0

Non answers: 71

The satisfaction rate of adolescents towards their relation to family members. Towards parents: 2050 (71.7%) of the adolescents are satisfied with this relation, frequent boys. The young follow, they declare that are neither satisfied nor unsatisfied, with 669 (23.4%), more frequently girls than boys. Those that are not satisfied are 141 (4.9%), more frequently girls and 25 (0.9%) of the teenagers, boys and girls, answered

that they do not have parents (tab. V).

Towards brother or sisters, the same hierarchy of the satisfaction grades: satisfied - 1749 (77.2%), more frequently, boys; not satisfied, neither satisfied - 412 (18.1%), more frequently girls; unsatisfied - 106 (4.7%), more frequently girls (tab. VI) and 590 (20.3%) of adolescents do not have brothers - 301 (21.0%) of the girls and 280 (19.7%) of the boys.

TABLE V

Satisfaction rate towards the satisfaction grade in what their relationship with their parents is concerned

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
Satisfied	989	67.1	1061	76.7	2050	71.7
Not satisfied, neither satisfied	390	26.4	279	20.1	669	23.4
Unsatisfied	96	6.5	45	3.2	141	4.9

Non answers: 23

TABLE VI

Adolescents` satisfaction rate towards their relationship to their brothers/sisters

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
Satisfied	857	73.1	892	81.5	1749	77.2
Not satisfied, neither satisfied	249	21.2	163	14.9	412	18.1
Unsatisfied	67	5.7	39	3.6	106	4.7

Non answers: 51

If we compare the satisfaction rate towards the relationship with parents with that towards brothers and sisters, it is shown a higher satisfaction rate towards brothers, at the whole lot and both sexes. Boys are frequent satisfied with their relation towards family members, in comparison to girls (fig. 1).

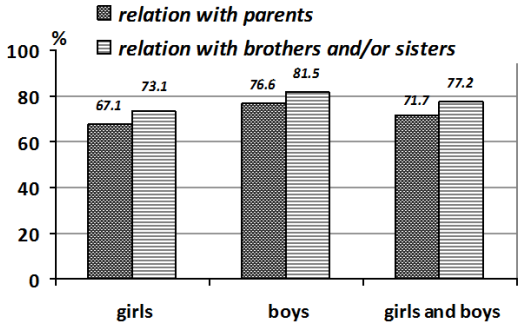

Fig. 1. Percentage distribution of satisfied adolescents towards their relationship with family members

Satisfaction rate of adolescents towards financial level of their family. Satisfaction towards the financial level of the family is predominant 1776 (61.2%), more frequently boys; 626 (21.6%) are not satisfied neither satisfied towards the financial level of their family, more frequently girls, and 471 (17.2%) are not satisfied, especially girls (tab. VII).

TABLE VII
Satisfaction rate of adolescents towards financial level of their family

Answers	Sex				Total	
	F		M			
	No.	Weighted percentage	No.	Weighted percentage	No.	Weighted percentage
Satisfied	843	56.5	933	66.2	1776	61.2
Not satisfied, neither satisfied	343	22.3	283	20.9	626	21.6
Unsatisfied	301	24.2	170	12.9	471	17.2

Non answers: 35

CONCLUSIONS

The result of the study underlines the predominance of family organized appurtenance, 78.9%; living with family, 88.6%; the medium instruction rate 63.7% of mothers and 59.1% of fathers; the satisfaction towards family members, 71.7%; the satisfaction towards the families' financial level, 61.2%. The role of family in adolescent`s life is certified through these examples.

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IMMUNOHISTOCHEMICAL PROFILE OF THE ESTROGEN AND PROGESTERONE RECEPTORS IN MAMMARY BENIGN LESIONS

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IMMUNOHISTOCHEMICAL PROFILE OF THE ESTROGEN AND PROGESTERONE RECEPTORS IN MAMMARY BENIGN LESIONS (Abstract): The immunohistochemical diagnosis for estrogen and progesterone receptors must be carried out in the tracking of every primary tumor, benign or malign, especially in the case of in situ carcinoma. **Material and method**: We have studied the expression of estrogen and progesterone receptors in benign lesions, identifying phenotypes depending on the presence of estrogen and progesterone receptors. **Results**: The result "positive" or "negative" in the report sent to the doctor is not sufficient, the inclusion of the total score in the case of a positive result, the clone used, the usage / non-usage of antigenic exposure and also the quality control being necessary. The benign mammary lesions occur in the context of a hormonal imbalance, which, in the long run runs the risk of developing a mammary carcinoma. **Conclusion**: The further study of the hormonal spectrum of those lesions, considered as pre-malignant, may lead to the identification of some groups of hyperplastic lesions, presenting a high risk for developing a mammary cancer. **Key words**: MAMMARY TUMORS, IMMUNOHISTOCHEMICAL DIAGNOSIS, HORMONAL PROFILE

The hormones control the development of the mammary carcinomas and thus the hormonal treatment is the preferred treatment for the vast majority of the ER positive metastatic breast cancers. At present, the evaluation of estrogen and progesterone expression in mammary carcinomas and their interpretation as prognostic and predictive factors, in the antiestrogenic endocrine therapy are elements of the traceability in female patients with mammary tumors (1). The immunohistochemical diagnosis for the estrogen and progesterone receptors must be carried out in the tracking of every primary benign and malignant tumor, especially in the case of in situ carcinoma. The immunohistochemical testing for the estrogen and progesterone receptors is highly indicated in the mammary tumors recurrence and metastases, due to the well known capacity of the mammary carcinomas of changing their hormonal status during their evolution. The conversion from a negative ER status to a positive ER status is beneficiary for the patients, benefiting of endocrine therapy. Conversely, the disappearance of the positive ER status is associated with a marked tumoral aggressiveness and therapy resistance. The expression of the progesterone receptor is induced by estrogen and consequently is a functional marker for ER. The normal mammary tissue presents receptors for progesterone. In mammary tumors the PR

expression is similar or slightly inferior in density, in comparison with ER, but the staining intensity is higher. PR is a weaker predictor for the endocrine therapy response and it offers instead important information regarding the clinical evolution of the disease (2). The positive mammary tumors for ER and PR present a high degree of histopathological heterogenity, do not associate ganglionic metastases, present a reduced proliferation rate and have a better prognosis (3). The ER / PR mammary tumors are associated with a favorable response to endocrine therapy and only a reduced number of cases do not respond to this therapy, due to the expression of an altered form of receptors.

OBJECTIVES

We have studied the expression of the estrogen and progesterone receptors in the benign lesions identifying phenotypes depending on the presence of estrogen and progesterone receptors.

MATERIAL AND METHODS

Out of 91 cases of benign lesion evaluated (main and adjacent ones), 16 were fibroadenomas, 23 typical moderated and fluoride ductal hyperplasia, 6 atypical ductal hyperplasia, 11 adenosis and sclerosing adenosis , 17 cystic dilatations, 15 apocrine metaplasia , 1 Phyllodes tumor and 2 cases of intraductal papilloma. The immunehistochemical protocol in the case of ER and PR receptor was similar and has been applied on twin blade slides. In order to interpret the results for ER/PR using optical microscopy we have taken into account only the nuclear coloration pattern, using Allred or Quick score, which are a semiquantitative evaluations that includes density data, the intensity of the positive reaction and also grades the positive nuclei cell percentage (percentage score), using an intensity score (tab. I).

TABLE I

Allred quantification score for estrogen and progesterone receptors

Percentage score (PS)	**Intensity score (IS)**
0 = negative	0 = negative
1 = < 1% coloured nuclei	1 = weak positive
2 = 1-10% coloured nuclei	2 = moderate positive
3 = 11-33% coloured nuclei	3 = intensely positive
4 = 34-66% coloured nuclei	
5 = 67-100% coloured nuclei	
Total score (TS) = PS + IS TS = 2 – 8	

A score of 0, 1 or 2 (less than 10% weak colored positive nuclei) has been considered negative (0), a score of 3 and 4 was considered "1+", a score of 5 and 6 was considered "2+", and a score of 7 and 8 was considered"3+".

The result "positive" or "negative" in the report sent to the doctor is not sufficient, the inclusion of the total score in the case of a positive result, the clone used, the usage / non-usage of antigenic exposure and also the quality control being necessary. The correlation between the immunehistochemical score and the response rate to hormonal therapy will be attached:

Score 0 hormonal therapy is inefficient.

Score 2-3 – 20% chance of therapy response to anti-estrogen medication.

Score 4-6 – 50% response rate.

Score 7-8 – 75% positive response to therapy.

RESULTS

In the case of negative controlled sections, where the incubation phase with the primary antibody was omitted, no coloration has been noticed. The normal adjacent mammary tissue, used as internal positive control presented a positive reaction for estrogen and progesterone receptors. The positive cellular immunoreaction has been noticed only at nuclear level. The myoepithelial, myofibroblasts and fibroblasts from the stroma of each case did not present any positive reaction. The staining for progesterone receptors has been more intense than in the case of the estrogenic receptors, but the percentage of positive nuclei was higher than in the case of the estrogen receptors.

The unmodified mammary epithelium presented a non-uniform, diffuse expression of the estrogen and progesterone receptors, with a more obvious expression at the level of ductal epithelium, compared with the lobular epithelium. The estrogen receptors have been stated in 25.3% of the benign lesion cases, and the progesterone receptors have been stated in 33% of the main benign lesions and associated lesions. The distribution according to benign lesion type of the estrogen and progesterone receptors can be seen in table 2. We have encountered 13 cases of intra and pericanalicular fibroadenomas and also 2 cases in which the fibroadenoma has been associated with the phyllodes tumor. Out of the cases of fibroadenomas studied, we have encountered a number of 5/16 (31.25%) positive cases for ER and 9/16 (56.25%) positive cases for PR. The immunoreaction of hormonal receptors was present at the level of epithelial cells nuclei. The fusiforme proliferated stromal cells (fibrocite / fibroblasts) present as “spindle cells” were negative for hormonal receptors. From table II we can note the fact that most fibroadenomas were positive for progesterone receptors.

TABLE II

Estrogen and progesterone receptor distribution in the case of benign tumors encountered (including the associated lesions)

Histological type	Total number of lesions (n=91)	ER		PR	
		+ (n=23)	- (n=68)	+ (n=30)	- (n=61)
Fibroadenomas (FA)	16	5	11	9	7
Typical ductal hyperplasia (HDT)	23	6	17	8	15
Atypical ductal hyperplasia (HDA)	6	2	4	3	3
Adenosis and typical sclerosing adenosis (Ad)	11	8	3	7	4
Intraductal florid papiloma (Pp)	2	2	0	2	0
Apocrine metaplasia (MA)	15	0	15	0	15
Cystic dilatation (DC)	17	0	17	0	17
Phyllodes Tumor (TP)	1	-	1	1	-

Regarding the quantification of estrogen and progesterone receptor expression, the vast majority of the cases we have evaluated were slightly positive (1+) and only a small amount of cases were moderately positive (2+). Out of the two fibroadenomas cases associated with the invasive ductal carcinoma, one was positive both for ER and PR, and the carcinoma was well differentiated (G1); the case associated with weak differentiated carcinoma (G3) was negative for estrogen and progesterone receptors. The fibroadenomas associated with the phyllodes tumor was positive only for the progesterone receptors.

The typical ductal hyperplasia (fig. 1) was encountered in 23 cases, out of which 6 cases (26,08%) were ER positive and 8 cases (37,7%) were PR positive. The great majority of the cases were slightly positive for ER and PR (+1). The moderate or florid ductal hyperplasia is associated with a 1.5-2 times higher risk of developing a carcinoma.

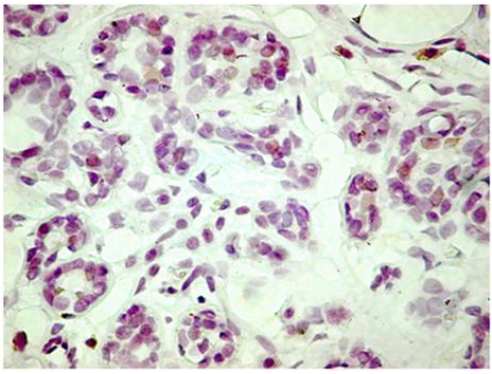

Fig. 1. The progesterone receptors expression in a typical ductal hyperplasia. One can notice heterogeneous distributed, positive nuclei, with an intensity score of 1+ and rare 2+ nuclei , with a percentage score of 3+ (around 30% positive nuclei)

Atypical ductal hyperplasia is associated with a 5 times higher risk of developing a carcinoma. We have encountered this type of lesion in 6 cases, which also presented malign lesions and DCIS (ductal carcinoma in situ), out of which 2 were ER positive (33.33%) and 3 cases were PR positive (50%).

We have encountered 12 cases of adenosis, as well as cases of sclerosing adenosis, out of which 8 cases were ER positive (72.7%) and 7 cases were PR positive (63.6%).

Intraductal florid papiloma was identified in 2 cases, positive for both ER and for PR.

In the case of apocrine metaplasia we have not noticed a positive reaction for estrogen or progesterone receptors. The ductal dilatation was negative for estrogen and progesterone receptors.

From table I one can notice that the majority of lesions (17) were quantified as being slightly positive (1+), regarding ER expression, 6 cases were moderate positive, most of them in the case of adenosis. As for the progesterone receptors expression, 22 cases were slightly positive, 7 were moderate positive and one case (adenosis) was intensely positive (fig. 2).

Depending on the estrogen and progesterone receptors, we have established the prevalence of four phenotypes ER/ PR in the case of the benign tumors (fig. 3).

Consequently, in case of fibroadenomas, we have encountered 4 cases (25%) for both estrogen and progesterone receptors (ER+/PR+), 1 case (6.25%) ER+/PR-, 5 cases (31.25%) ER-/PR+ and 6 cases (37.5%) ER-/PR-.

In the case of typical hyperplasia, 1 case (4,4%) was ER+/PR+, 5 cases (21.8%) were positive only for ER (ER+/PR-) , 7

(30,4%) were ER-/P+- and 10(43,47%) cases were negative (ER-/ PR-). Atypical hyperplasia presented the following phenotypes: 1 case (16.6%) ER+/PR+, 1 case (16.6%) ER+/PR- and 2 cases (33.3%) ER-/PR+ and ER-/PR-.

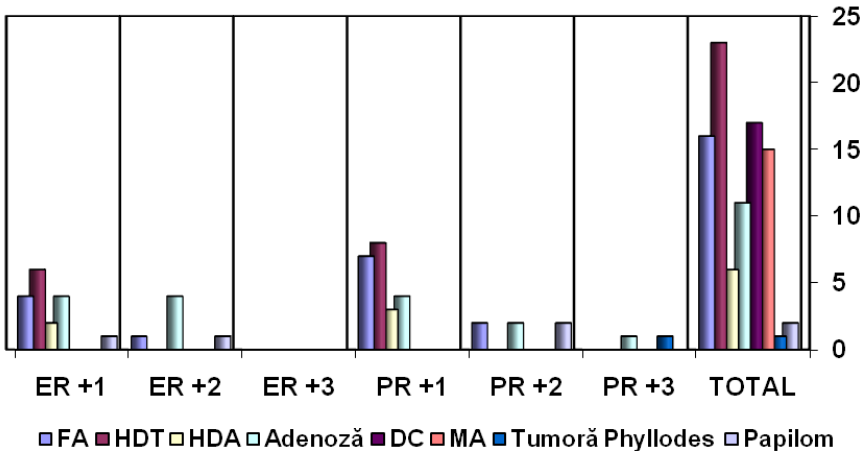

Fig. 2. ER and PR quantification of benign lesions.

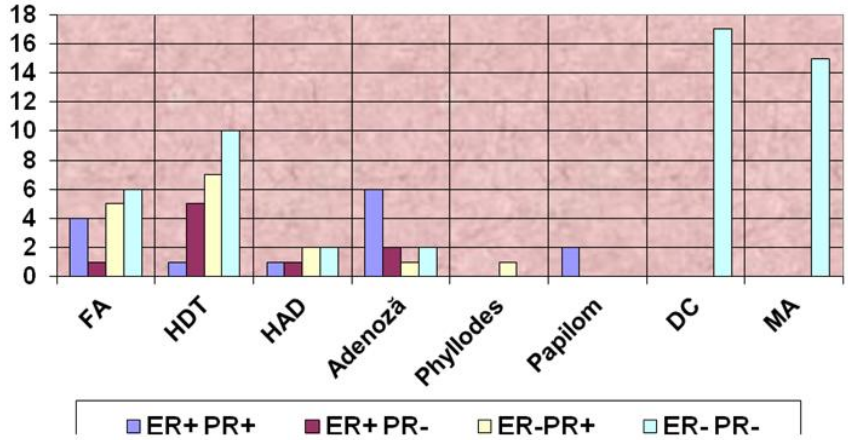

Fig. 3. ER / PR phenotype prevalence in the case of benign tumors

In the case of adenosis, 6 (54.54%) cases were ER+/PR+, 2 cases (18.18%) were ER+/PR-, 1 case (9%) was ER-/PR+ and 2 cases (18.18%) were ER-/PR-. The Phyllodes tumor encountered presented an ER-/PR+ phenotype. The florid intraductal papilomas were positive both for estrogen and progesterone receptors (ER+/PR+). The non-proliferative lesions, represented by cystic dilatations and apocrine metaplasia were not positive for ER/PR. Out of the positive cases, the most frequently encountered phenotype was ER-/PR+, followed by ER+/PR+.

DISCUSSION

The benign mammary lesions occur in the context of a hormonal imbalance, which, in the long run runs the risk of developing a mammary carcinoma. The activation of α estrogen receptor (ER-α) raises the sensitiveness of the target tissue for the action of circulatory estrogens, stimulating the DNA synthesis, cell division, active biological protein production, including pS2, TGF-α (transforming growth factor), EGF (epidermal growth factor), which influences the cellular growth and differentiation (4). The cumulative exposure to estrogens contributes to the mammary carcinogenesis by means of stimulating a premalignant cells clone or the augmentation of spontaneous mutation percentage. The estrogens may also decrease the transition time in the cellular cycle, so that the spontaneous mutations become defined before the reparatory mechanism intervention. Another mechanism could be the toxic genetic effect of estrogens. It can be explained by the loss of the suppressing gene effect, whose function is to prevent the excessive proliferation of ER positive cells in the rich estrogens environment, especially in premenopausal women (5). The allelic disequilibrium was proven in some intraductal and intralobular proliferation and is significantly increased into in situ carcinomas and atypical hyperplasia, compared with usual hyperplasia (6). Increased hormonal contents, as those observed in the case of premenopausal women or those breastfeeding are associated with a reduced number of ER+ epithelial cells, while the reduced circulatory concentrations of estrogens, as those observed in the case of postmenopausal women, are associated with an increased number of positive ER cells. In normal mammary tissue, the percentage of positive ER cells grows with the age, correlated with the diminished plasmatic concentration of the circulatory steroid hormones. In the case of premalignant proliferative lesions, as well as in the case of the in situ carcinomas, this negative association is lost, being a display of the early molecular changes that appear in the neoplastic transformation (7). The relatively elevated risk of developing a carcinoma was associated with the sclerosing adenosis lesions, intraductal florid papiloma, fibroadenoma, phyllodes tumor, fibrocystic disease with proliferative lesions adenosis-type and ductal hyperplasia. Some histopathological changes, such as apocrine metaplasia and ductal ectasia were not associated with an increased risk of developing mammary cancer. The evaluation of hormonal receptors presence in benign lesions is necessary in order to evaluate the opportunity of chemotherapy, with a positive ratio cost – benefit.

Clinical studies revealed a relationship between some histological types of benign mammary lesions and the risk of developing breast cancer, in accordance with the histological type of lesion and the hormonal status (8, 9, 10). The associated risk for the histological factors is the same for both breasts, suggesting that those markers must be regarded into a general context rather than as simple predecessor factors, depending on the histological type of lesion. There is also an interaction between the histological risk factors and the estrogen hormones in order to determine the level of associated risk. According to some studies, tamoxifen has diminished by over 86% the risk of mammary cancer in the case of a subset of female patients, diagnosed with atypical

ductal hyperplasia, suggesting the utility of the antiesteogenic therapy, especially in the case of a high risk group of female patients (11). Although there are disagreements, it is well known that small dimension tumors, in the early stage, cannot be detected using mammography (12, 13, 14).

Within the benign lesions studied, ER are expressed in 25.3% and PR in 33% of the cases. Non-malignant proliferative lesions with an increased risk of developing mammary cancer presented a positive reaction for the estrogen and progesterone receptors. Thus, atypical ductal hyperplasia, with a 5 times higher risk, presented positive ER in 33% of the cases and PR positive in 50% of the cases, with the most frequent phenotype being ER-/PR+. The sclerosing adenosis lesions, characterized by the proliferation of an epithelial and mioepithelial group of cells distorted by abundant fibrous tissue, with a 3 times higher risk of developing mammary cancer presented the highest number of positive cases for estrogen receptors (72%) and progesterone (64%), with most of the cases (45%) being ER+/PR+. In the case of fibroadenomas, estrogen receptors were present in 31% of the cases, PR in 56% of the cases, with ER-/PR+ as the most frequent phenotype (31%). The Phyllodes tumor with malign area changes, which we have encountered accompanying a fibroadenoma, preserved the progesterone receptors positive status for ER and PR and presented a reduced or moderate level of expression (2+), especially for PR. Non – proliferative lesions which were not a diagnose on their own, but were encountered in the case of fibrocystic disease , with a lower risk of developing a carcinoma , did not present a positive reaction for both estrogen and progesterone receptors (15,16,17). All cystic dilatations, either small or large, with a flattened epithelium were negative for the investigated markers (ER-/PR-). In the same manner the apocrine metaplasia lesions were ER/PR negative in all cases.

CONCLUSIONS

Regarding the benign lesions in our study, ER are expressed in 25.3% and PR in 33% of the cases. The proliferative lesions, especially sclerosing adenosis, atypical hyperplasia, florid hyperplasia and papiloma were expressed as ER and PR. The sclerosing adenosis presented most frequent ER (73%) and PR (64%). With the exception of adenosis, which expressed more frequently estrogenic receptors, in the other cases, PR presented a higher expression level. Fibroadenomas revealed PR in 56% cases and ER in 31% of the cases. The Phyllodes tumors expressed only PR. Cystic dilatations and apocrine metaplasia did not express ER/PR. The progesterone receptor prevalence in most of these cases proves a progesterone insufficiency which supports the hormonal disequilibrium theory in tumoral transformation and explains the hormonal therapy success in fibrocystic disease with proliferative lesions. The further study of the hormonal spectrum of those lesions, considered as pre-malignant, may lead to the identification of some groups of hyperplastic lesions, presenting a high risk for developing a mammary cancer and may contribute to the selection of a female patients group requiring a careful observation who might benefit from an of antiproliferative and antiestrogenic treatment.

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EXPERIMENTAL STUDY REGARDING THE CHANGES IN THE MEDIAN SAGITTAL SUTURE POST DISJUNCTION ON COMMON RABBIT BREED

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EXPERIMENTAL STUDY REGARDING THE CHANGES IN THE MEDIAN SAGITTAL SUTURE POST DISJUNCTION ON COMMON RABBIT BREED (Abstract): Widening of the upper jaw by undoing the median suture is a relatively common clinical procedure and the histological studies on sutural tissues, both immediately after disjunction and post disjunction are relatively rare. The present research aims to study cellular changes caused after undoing the median maxillary suture, on a strain of common rabbit breed, in an attempt to prove that the purpose of this therapeutic technique is obtaining neoformed osseous tissue, as a guarantee of the stability of orthodontic treatment results. Of course, changes in rabbit desmale tissues cannot be fully assimilated to the human ones. **Key words:** DISJUNCTION, MEDIAN SAGITTAL SUTURE, NEOFORMED OSSEOUS TISSUE

Intermaxillary disjunction is defined as the undoing of the median palatine suture by means of orthopedic forces. Thus, we obtain dimensional expansion of the upper jaw in transverse direction. According to Boboc (1) "intermaxillary disjunction provides the opportunity to obtain significant widening of the upper arch, the palate, the nasal cavities and even the face in a short period of time".

MATERIAL AND METHODS

The disjunction technique involves two essential features, namely: firm anchorage on the side areas, as well as using a disjunction appliance able to support the effort of "fracture" of the upper jaw.

To this end, two metal trays were made by the size of the rodent's side areas, on a dry skull, which allowed, on one hand, to obtain a working model easy to use, as well as imprinting in order to fabricate the disjunctor.

Given that the experimental rabbits were part of the same strain, individual changes in the side areas proved minimal (common breed rabbits have had the average weight of 2700 g and a dental formula represented by four incisors, six premolars and 6 molars, fully erupted). In order to individualize the contact between the metal trays and the dental area, there were applied retentions in

trays, which were secured on the side areas by means of an autopolimerisable composite material. Thus, the appliances made could be used throughout the batch of rabbits studied, but also in some cases, reused in experimental animals.

For disjunction, a Dentaurum screw was used, with parallel expansion, whose arms were welded to the metal trays (fig. 1).

Fig. 1. The disjunctor – a Dentaurum screw was used, whose arms were welded to the metallic trays

The appliance was tested on a rabbit jaw euthanized beforehand, to observe its effectiveness and to correct the position of the screw from the arch and from the direction of disjunction.

The disjunctors were applied on a number of 10 rabbits (using batches of three rabbits each and a witness rabbit). General anesthesia was administered, using neuroleptanalgesia, namely *Acepromazine* 0.4 mg/kg body weight (BW) and *Ketamine* 0.5mg/kg body weight (BW). The rabbit used as witness was euthanized without cementing its disjunctor. In nine rabbits, the disjunctors were cemented on the premolars and the molars of the upper arch, on one side and the other of the median line, with an autopolimerisable composite material (*Structur 2, Voco,* Germany) (fig.2).

Then, the disjunctors were activated during a single session (ultra-rapid disjunction). The first batch of rabbits was euthanized immediately after cementing and activating the appliances, the second batch after 7 days and the third batch after 14 days.

For histological analysis, the median suture was dissected and sections from all 10 rabbits were prepared, after euthanasia (the witness, batch 1 during activation, batch 2 after 7 days and also batch 3 after 14 days). 10% buffered formalin was used for each part, as mounting medium. Decalcification solutions based on formic acid were used. The material was processed by classical technique of paraffin embedding, with sections cut in 4 microns. Staining used was Haematoxylin-eosin. The microscope used was Olympus BX50, Ocular x10, x20 and x40 (the histopathologic examination was achieved at Clinical Hospital of Orthopedics-Trauma and Osteoarticular TBC).

Fig. 2. The disjunctor was anchored on the side areas by means of the autopolimerisable composite material

RESULTS AND DISCUSSION

It was noticed that orthopedic forces developed by the disjunction appliance have resulted in undoing the median sagittal suture, leading to the beginning of a process of tisular reparation (2), which is completed by forming a new bone. Stadialization of these structural changes, depending on the moment when the batches of animals are sacrificed, is well defined in terms of tisular reorganization (3, 4).

Thus, in the witness rabbit (without disjunctor applied), the median suture presents lamellar osseous tissue (mature), Haversian type and trabecular with adipose marrow, the edges of the palatine bones in the suture area being in direct contact with a thin layer of fibrous tissue (fig. 3).

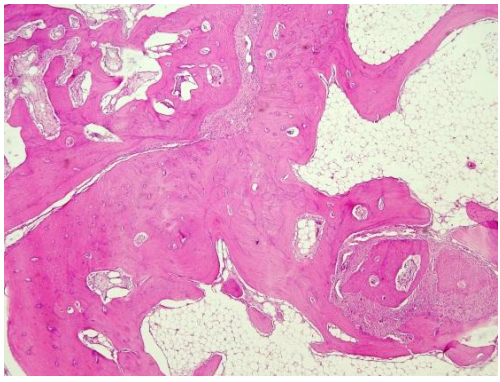

Fig. 3. Median suture in the witness rabbit with lamellar osseous tissue, Haversian type and trabecular

Sections from different levels of the osseous parts removed from the first batch of rabbits, immediately after disjunction, presenting the characteristic aspect of fragmented osseous tissue and hemorrhagic suffusions, as well as polymorphous inflammatory infiltrate (fig. 4).

In sections resulted from the osseous parts removed from the rabbits in the second batch of study (at an interval of 7 days after disjunction), we can observe a stromal cell reaction, with an increased number of fibroblasts (fig. 5), as well as an appositional ossification, through a process of osseous metaplasia in the presence of active osteoblasts increased in size which mark the limits of the osseous matrix (fig. 6). This phenomenon can also be observed in the vicinity of the periodontal ligament (fig. 7).

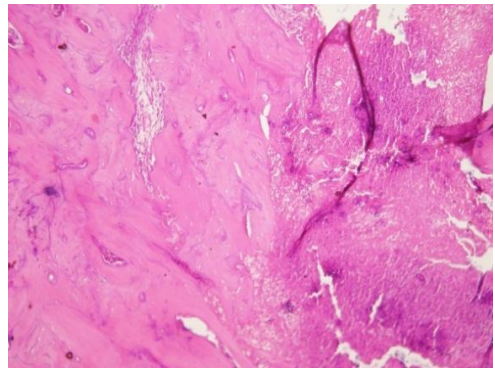

Fig. 4. Cortical bone with area where the osseous tissue is fragmented Hemorrhagic suffusions and polymorphous inflammatory infiltrate are being associated

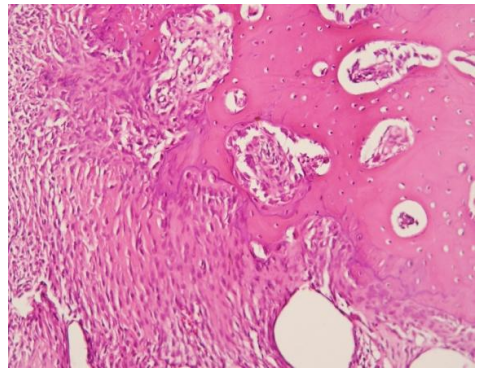

Fig. 5. Mesenchymal tissue with a tendency to densify itself in contact with the osseous tissue

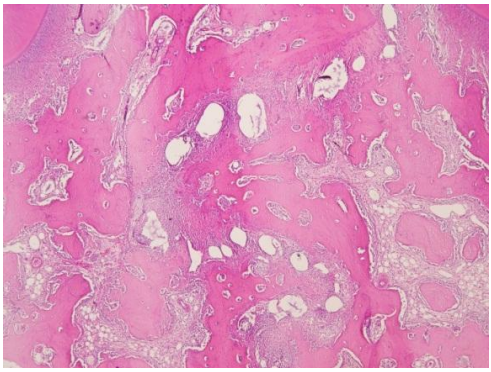

Fig. 6. Appositional ossification – process of osseous metaplasia in the presence of active osteoblasts

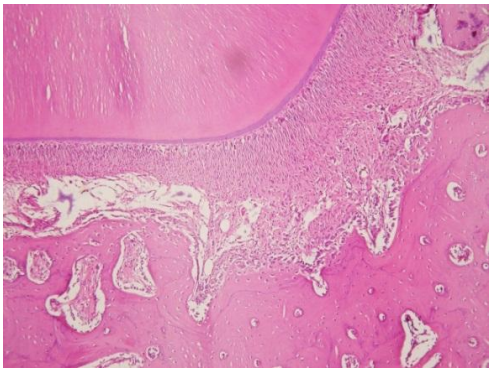

Fig. 7. Appositional ossification is also found in the vicinity of the periodontal ligament

In contrast to the tendency of ossification, in figure 8, are captured osteoclasts responsible for the resorptive osseous processes. The aspect of ischemic necrosis with cracks in the compact bone is associated with centers of osteoclastic activity, which resorb the necrotic bone, associated with elements of simultaneous appositional ossification.

The predominant osteoformative activity following the osteoclastic wave, with cellular dense mesenchymal tissue and in the presence of highly active osteoblasts in contact with the lamellar bone where the primitive bone is formed, as a result of the microfractures that occurred after traction (fig. 9).

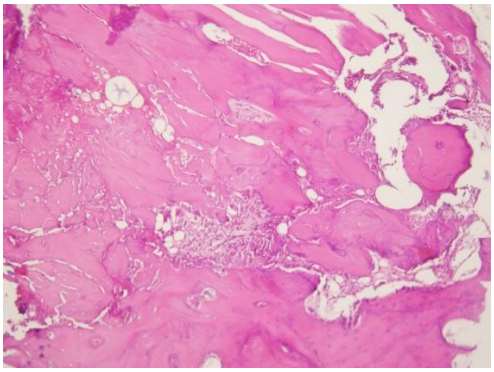

Fig. 8. Ossification is counterbalanced by a process of osseous resorption with the involvement of the osteoclasts

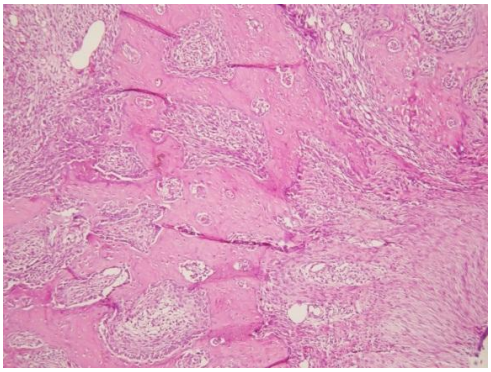

Fig. 9. Predominant osteoformative activity in the presence of the mesenchymal tissue, as well as the osteoblasts found in the vicinity of the lamellar bone

In the third batch of rabbits, sacrificed at 14 days after the disjunction, we detect fragments of cortical bone with appositional ossification (fig. 10).

In the next image, we observe that the suture clearly tends to close itself by forming neoformed osseous tissue (fig. 11). The presence of the immature non-lamellar bone is indicated on the side slopes of the

crack, as well as the presence of the osteocytes in the mature lamellar bone.

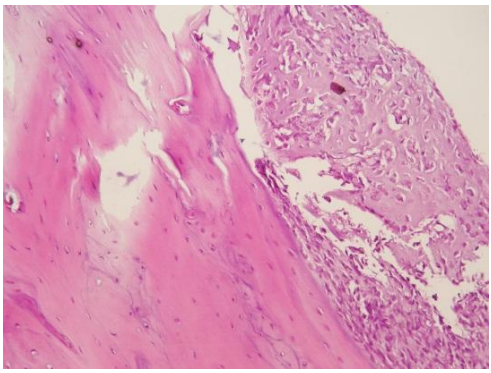

Fig. 10. The area of appositional ossification with uncalcified osteoid matrix and young cortical bone

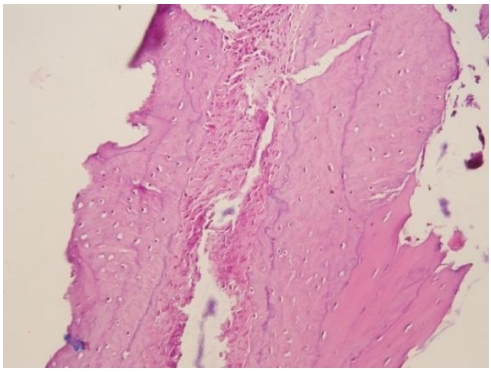

Fig. 11. The tendency of the crack in the area of the median suture to close itself by the process of ossification

CONCLUSIONS

The neoformed bone is made of a substrate particularly favorable for dental movement, allowing the development of the processes of resorption and apposition, the cornerstone of any orthodontic treatment using fixed techniques.

According to the succession of the levels studied in the experiment presented on laboratory animals, we notice that the process of ossification clearly tends to cover the dehiscent palatal median suture area; we must put on hold the fact that the results of our study are not directly transferable to the human tissue.

Rapid and ultra-rapid activation remains an accessible technique and, most of the time, after increasing the diameters, the treatment is suitable to be continued with fixed appliance.

As a consequence, osseous structural changes obtained after disjunction demonstrate mainly that this therapeutic method is useful in situations where the lack of space in the maxillary is important, the reparatory processes taking place and forming new osseous tissue, thus getting a permanent character. In this way, the premises of a much more judicious distribution of teeth are created, in terms of a lower biological sacrifice, offering an additional guarantee for maintaining the stability of the result of the orthodontic treatment.

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REFLECTIONS ON FERTILITY DYNAMICS IN ROMANIA

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REFLECTIONS ON FERTILITY DYNAMICS IN ROMANIA (Abstract): **Aims** to highlight the changes occurred in the evolution of the fertility phenomenon in Romania, focusing on developments in the general fertility rate, total fertility rate, number of live births, and on the construction of specific indicators to reveal the source of demographic change. Several theories on the factors that may cause fertility decline it were outlined, underlining the presence of these factors during fertility dynamics in our country. After 1990, population decline may be explained by a close inter-relationship between economic theories (worsening economic conditions lead to decreased fertility) and the second demographic transition (postponement of births, fertility change model). **Key words:** DEMOGRAPHIC CHANGE, FERTILITY, ECONOMIC FACTORS, THE SECOND DEMOGRAPHIC TRANSITION.

Demographic changes have profound implications on future economic and social development of a population (1). While the rise of the life expectancy is considered to be a major achievement for the European Community, decline of birth rates, fertility (tab. I) and its consequences - an aging population - are challenges which the European Union must deal with (2).

TABLE I
Total cohort fertility rate, 1935-1965
(Demography Monitor 2005 (4))

EU/RO	1935	1945	1955	1965
EU 25	2,37	2,11	1,94	1,77
EU 15	2,36	2,10	1,90	1,73
EU 10	2,40	1,96	1,77	1,96
RO	-	4,48	3,97	

It is considered that all European Union countries (EU) will face in the coming years an increasing proportion of the total elderly population and a declining proportion of people from active age groups and capable of working (3). Thus, according to data from the European Commission's 2008 demographic report, since 2008 the number of people aged over 60 will increase by about 2 million people every year for a period of 25 years. (4).

In the context of a rising interest about the demographic development of the EU, discussions on the implications of demographic change have increased in number and seriousness in Europe.

Even if there are common issues and common demographic conditions (5) - reducing the number of live births, fewer marriages, rise of the average age of marriage (6) - throughout all Europe, what it is primarily needed is to understand the circumstances and specific national circum-

stances and then to identify the possible solutions to be applied in other countries where there are different contexts. Romania, as a member of the European Union, it is not an exception in terms of population trends.

Given the European context, it is required in Romania to be paid attention to reflect accurate demographic changes. This paper aims to assess the phenomenon of fertility trends in the population of Romania from 1965 until 2007. Through this research it is desired to be observed the specific conditions of our country in terms of demographic change.

In 2007, the large generation of 1967 (so-called generation of "decrees") has reached 40 years, women of this generation's contribution to the phenomenon of fertility is decreasing as magnitude and consequently, in importance. In this paper, wanting to surprise the moment when the influence of large generations born in the communist period has begins to recede, we stopped with the analysis in 2007.

As the objectives of the present research, we aimed to document changes in the number of live births over the period 1965-2007 and to determine the influence that the alteration of specific group-age fertility rates had it on the population.

MATERIAL AND METHODS

The present research is a descriptive transversal study on the phenomenon of fertility in the female population of childbearing age in Romania (7, 8).

To describe the evolution of the fertility phenomenon in Romania it was set up a database containing the following data as follows: number of live births in calendar years, the number of live births by mother's age group by calendar year, the number of women of childbearing age groups quinquennial age, in calendar years. Subsequently, usual indicators were constructed, as the general fertility rate, total fertility rate, but, also, less used indicators such as ratio of the live births born in two different calendar years (years used for comparison), the difference between the number of live births born in two different calendar years (years used for comparison) and the difference between number of live births born in year 0 and number of live births expected to be born in year 0 (based on the assumption that the fertility in year 1 is applied to population of childbearing age of year 0 - year 0 being the year of comparison, and year 1, the reference).

The symbols used to describe how to construct the indicators used in the study are as following:

- $f_x^1 = Age - specific\ fertility\ rate\ for\ age\ x\ , for\ year\ 1$
- $f_x^0 = Age - specific\ fertility\ rate\ for\ age\ x, for\ year\ 0$
- $F_x^1 = number\ of\ women\ of\ childbearing\ age\ for\ age\ group\ x, for\ year\ 1$
- $F_x^0 = number\ of\ women\ of\ childbearing\ age\ for\ age\ group\ x, for\ year\ 0$
- $g_x^1 = number\ of\ live\ births, for\ year\ 1$
- $g_x^0 = number\ of\ live\ births, for\ year\ 0$

The indicators build and used in the research are as following:

1. The ratio between the numbers of live births during the two years of reference

$$I_{N1/0} = \frac{\sum_{x=15}^{49} f_x^1 F_x^1}{\sum_{x=15}^{49} f_x^0 F_x^0}$$

2. The difference in absolute number of live births between the two years of refer-

ence $\Delta_{N1/0} = \sum_{x=15}^{49} f_x^1 F_x^1 - \sum_{x=15}^{49} f_x^0 F_x^0$

3. The ratio of fertility between the two years of reference

$$I_{N_{1/0}}^{fg} = \frac{f_g^1}{f_g^0} = \frac{\sum_{x=15}^{49} f_x^1 F_x^1}{\sum_{x=15}^{49} F_x^1} \div \frac{\sum_{x=15}^{49} f_x^0 F_x^0}{\sum_{x=15}^{49} F_x^0} = \frac{\sum_{x=15}^{49} f_x^1 g_x^1}{\sum_{x=15}^{49} g_x^0}$$

4. The difference between live births in year 0 and expected live births in year 0, if it is applied fertility of the female childbearing age population from year 1 to the female childbearing age population from year 0

$$\Delta_{N_{1/0}}^{fg} = \left(f_g^1 - f_g^0\right) \sum_{x=15}^{49} F_x^1$$

RESULTS

Romania is experiencing today an unprecedented number of demographic changes in the demographic history of the last century, changes that lead to an aging population (8). The most important factors leading rapidly to an aging population are: persistently maintaining the fertility rates at a very low level, increased life expectancy and the transition of generations born in the communist period to the retirement period. Below are captured, in a succinct picture, the trends of fertility phenomenon during 1965-2007 and their demographic consequences at the population level.

1. Evolution of General Fertility Rate in Romania

General Fertility Rate (number of live births per 1,000 women of childbearing age) had a downward trend since 1991. The downward trend in the General Fertility Rate between years 1956 - 1965, led to the political decision to take action in order to rectify the situation. In the period 1956 - 1965 in terms of legislation, it was permissible to make abortion on demand, in the context of the communist ideology of the time when it was encouraged female labor force participation, and women were regarded as an important work force for the economy. In 1966, the government at that moment decided to ban abortion on demand by Decree 770/1966, decree which came into force in October the same year (9). The consequence was that General fertility rate reached a value of 105.5 live births per 1,000 women of childbearing age in 1967; starting next year, it began to decline more or less sharply depending on the coercive measures applied in different moments by the communist authorities of the time. Thus, the lowest values of the General fertility rate in the communist period were recorded in years 1966 (55.7 $‰_0$), in 1973 (70.4$‰_0$) and 1983 (59.5$‰_0$), followed by consecutive years of increases in values. From the sociological perspective, the effects of Decree 770/1996 decreased gradually during time, even if the authorities have introduced ever more repressive measure. In addition to legal provisions, the period 1980-1989 was characterized by the lack of availability of contraceptive measures, but also by the deterioration of living conditions of the population. The downward trend in the General Fertility Rate has widened since 1990. On 26 December 1989, the National Salvation Front Council -the revolutionary power at that time- issued the Decree Law No. 1, through which were repealed several laws and, decrees. One of the acts that were repealed it was the Decree 770/1966 on the prohibition of abortion (10), (11). From an economic perspective, the first period after 1990 is characterized by strong fluctuations in the rate of inflation (in 1991, the average monthly rate of inflation - the average monthly price increases - was 10.3%, for

1993 to reach 12.1%) (11).

2. Model of Age-Specific Fertility Rate: Evolution

In addition to the downward evolution of the General Fertility Rate, fertility rates specific by age groups followed the same trend. The new model of fertility suggests the change of the fertility pattern from an early one, where the highest fertility rate is recorded at specific age group 20-24 years, to a late fertility model, a model where the highest rate of fertility is recorded at the age groups of 25-29 years.

3. Evolution of Total Fertility Rate

During the period of observation, total fertility rate went from a level of 2.9 children per woman in 1956. Registration for two consecutive years a total fertility rate values below the "replacement" level- that is 1.9 children per woman- led to the Government intervention to take the coercive measures mentioned above.

Following the implementation of pronatalist policies, in 1967 it was recorded a level of 3.7 children per woman. Peak level of 1967 has not been recorded in subsequent years; in 1968 the value registered being 3.6 children per woman, the evolution being still downward. After 1990, total fertility rate has continued to decrease, however, the trend becoming very sharp: the value of 1.8 children per woman in 1990 will reach a level of 1.3 children per woman in 1995 (fig. 1).

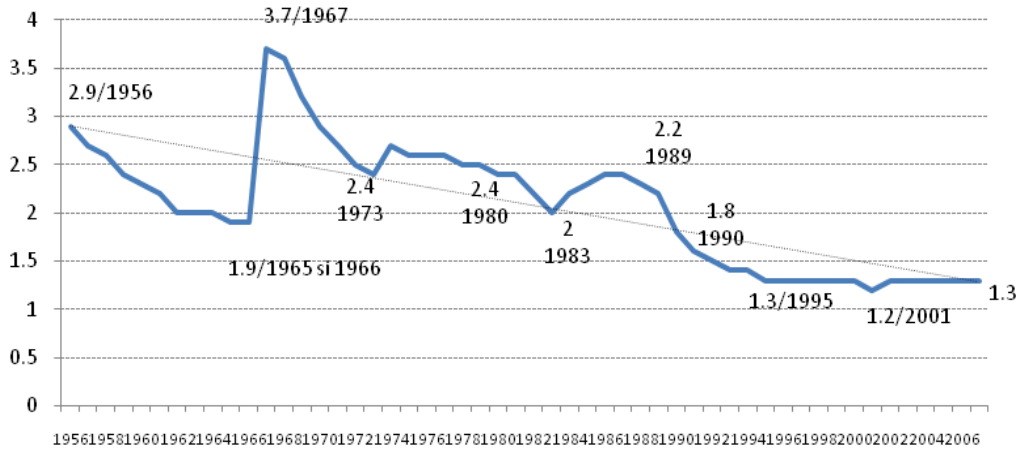

Fig. 1. The evolution of total fertility rate, Romania, during 1956-2007

Highlighting the demographic losses

Number of live births

Evolution of live births had a downward trend since 1967. If this year in Romania the number of live births for the period reached a record level with a value of 527 764 live births, in subsequent years, the downward trend fluctuated; the decrease of the level of the number of live births in 1966 (273,678 live births) 1973 (378696 live births) and 1983 (321 498 live births) have lead to coercive measures that had therefore increased the number of live births in consecutive years. Cancellation of legal restrictions regarding abortions on demand in 1990, led in 1991 to recording a

number of live births under the level registered in the year 1965. Downward trend continued until 2007 (fig. 2).

Demographic losses due to lower birth rates and fertility rates are dramatic. Since 1990 is registered a shortage in number of live births from year to year. Comparing the number of live births in 1990 and subsequent years show an annual population loss taking into account as the reference the year 1990. In 2007, the deficit of live births compared to 1990 was 100 018 live births (fig. 3).

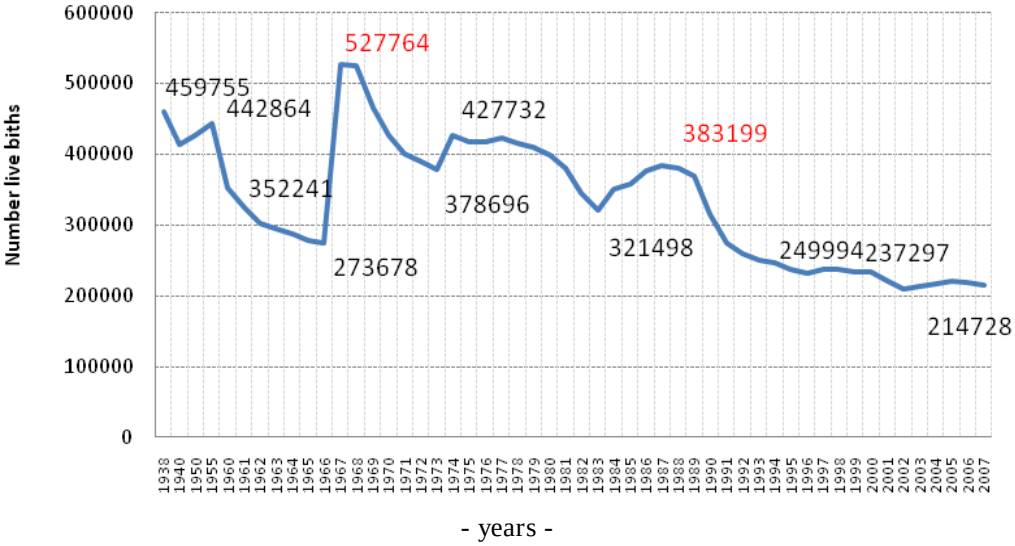

Fig. 2. The evolution of live births in Romania, during 1938-2007

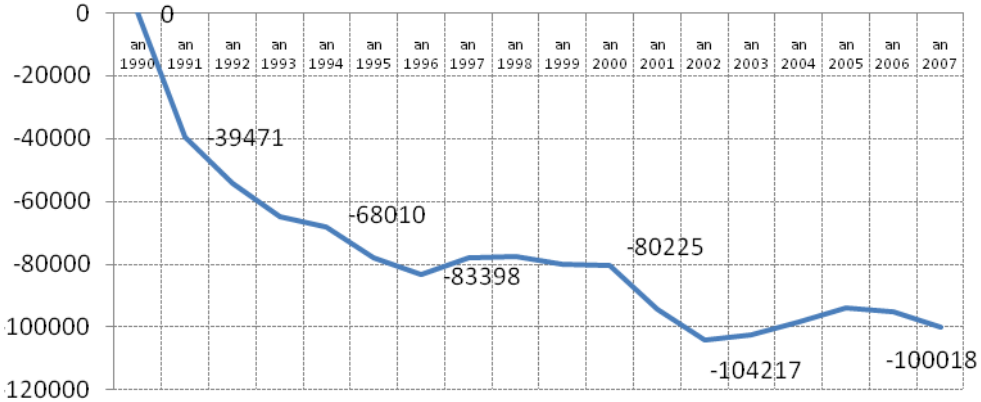

Fig. 3. The evolution of the difference between the number of live births from thee years after 1990 and the live births in 1990, Romania, 1990-2007

Comparison of the number of live births recorded in the consecutive years of 1990 and the number of live births registered in 1990 has as result a deficit every year since 1990. This deficit reached a record value of 30% in 2007 compared to 1990 (fig. 4).

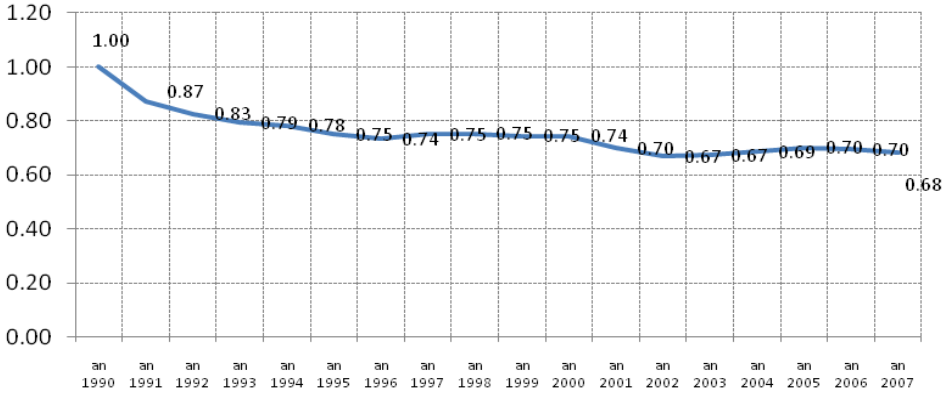

Fig. 4. The evolution of the ratio of live births of the years after 1990 and the live births in 1990, Romania, 1990-2007

5. Quantifying the influence of decreasing General Fertility Rate on the number of live births

If the General Fertility Rate in 1990 would be retained for each subsequent year, the number of live births expected in these circumstances would have been higher than the level actually recorded. Applying the General Fertility Rate from 1990 to the real number of female population of childbearing age recorded for each subsequent year of 1990 lead to a highlighting of the live births' deficit due to the general rate of fertility decline. The number of live births in 2007, while maintaining the General Fertility Rate in 1990, would have been 309 291 live births from 214 158 live births actually registered, which means that while preserving the general rate of fertility 1990, in 2007 a number of 95033 live births it would be born in addition to the actual observed number. This difference of 95 033 live births between the number of actual (observed) live births in 2007 and the expected level while maintaining General Fertility Rate from 1990, is quantifying the decrease of the number of live births due to influence of General Fertility Rate on the number of live births (fig. 5).

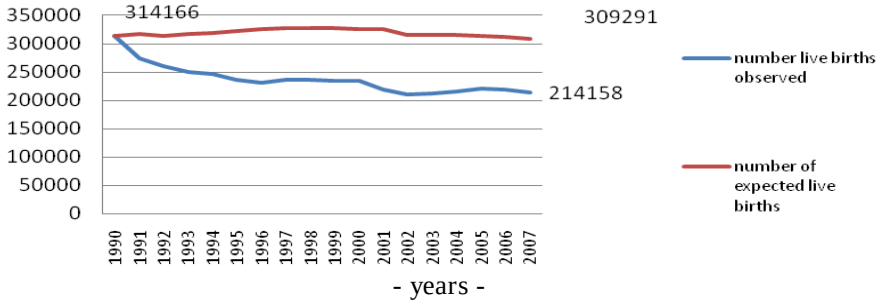

Fig. 5. The evolution of the number of live births observed and expected number of live births if the general rate of fertility would be preserved at the level of year 1990, Romania, 1990-2007

DISCUSSION AND CONCLUSIONS

Fertility decline is a phenomenon that has affected and affects not only our country but is known at European level (12). The decline of fertility is due to a networking and an interaction of many factors, but the evidence suggests that the main factors incriminated are still different from country to country. Thus, changes in fertility trends can be given by the second demographic transition, by delaying the moment of birth or may be the population reaction to the economic crisis (13, 14). Data from the literature suggests that worsening economic conditions contribute to decreased fertility (15), however, is difficult to prove directly, the population decline is due to which factor. For Romania it is possible that each of them to be contributed, albeit at different moments of time. Thus, immediately after the 1989 revolution, it is possible that the main factors that led to the decline in fertility to be economic ones (16), but in close relation to contraceptive policies. Immediately after the change of political regime in 1989, the laws that prohibited abortion on demand have been eliminated and economic conditions have become precarious. Not only macro-economic climate has become unstable, uncertain, but poverty has affected millions of people around the Eastern European region (17).

In Romania, during 1990-2007, the deficit of live births increased, reaching in 2007 to register 100,018 fewer live births from 1990, a decline of 30%. In addition, if the General Fertility Rate from 1990 it would being kept, the number of live births that it would have been registered in 2007 it was 309,291 as against 214,158 who had been actually registered. Registration of this deficit cannot be apart to the worsening economic conditions, as previously mentioned. The relationship between fertility phenomenon and economic conditions, family services and related policies has been supported by other researchers, which stated that the loss of resources in Eastern European countries where the political system was changed in 1980 drew to an increased decline of fertility (18).

For Romania, to the records of the influence of the phenomenon of economic elements, elements of the second demographic transition are added, probably from the 2000s. Specific sides of the second demographic transition would be the postponing of the timing for family formation, delaying their first birth, the growth of consensual unions and cohabitation instead of marriage and increasing the number of children born outside marriage. All these explanations have been events that led to the decline of fertility in Romania during the 2000s. The couple's fertility history from our country is changing (level of education of those who are starting a family, the trajectories of mental and physical health of family members, other aspects of the life course), all having their impact and their influence on fertility trends.

In this work we wanted to capture the moment when the quota of contributions of the numerous fertile generation of year 1967 begins to diminish. The female fertile population is still numerous, at the moment, being composed of numerous large generations of women born in the communist period, but changes are foreseen to begin on age structure of this population. For the future, these changes suffered by the female population of childbearing age and their influence on the fertility trend and on the actual number of live births must be taken into consideration.

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EVALUAREA UNOR PARAMETRI BIOFIZICI AI ERITROCITULUI UMAN PRIN SPECTROSCOPIE FT-IR – STUDIU PRELIMINAR

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ASSESSMENT OF SOME BYOPHYSICAL PARAMETERS OF HUMAN ERYTHROCYTE BY FT-IR SPECTROSCOPY – PRELIMINARY STUDY (Abstract): Based on the biochemical composition of erythrocyte membrane and asymmetric distribution of phospholipids, proteins, and cholesterol, FT-IR spectroscopy can monitor the distribution and interaction pattern of membrane constituents. **Material and methods:** The study series included 21 clinically healthy subjects aged between 20 and 60 years. Complete blood counts were performed and the serum biochemical compounds (cholesterol, triglycerides, total protein, glucose) were determined. **Results and discussions:** The parameters that can be assessed by erythrocyte FT-IR in relation with the biochemical factors that may influence membrane fluidity are: degree of fatty acids unsaturation, saturated fatty acids/unsaturated fatty acids ratio, cholesterol/phospholipids ratio, and phospholipids/protein ratio. Based on the obtained results, in the assessment of membrane status the following vibration modes were selected as spectral parameters: vibration associated valence bond (=CH), asymmetric valence CH_2 groups, modes associated with P=O bond and amine bands I and II specific for proteins. Other parameters, such as ν(C-O) specific to glucose, may provide additional information on glucose metabolic status. **Conclusions:** By correlating biochemical markers with these spectral parameters information on red cell membrane status, status that may reflect different pathological processes, can be obtained. **Key words:** ERYTHROCYTE, BLOOD BIOCHEMICAL PARAMETERS, FT-IR SPECTROSCOPY, MEMBRANE FLUIDITY

Studiul membranei celulare folosind spectroscopia FT-IR este o metodă relativ recent introdusă în studiul sistemelor biologice. Pentru studiul statusului membranar plasmatic se poate alege ca model membrana eritrocitară din mai multe considerente: disponibilitate în număr mare a globulelor roşii, obţinerea probelor de studiu este relativ simplă, este un model membranar recunoscut şi se pot face corelaţii cu alţi parametri hematologici în contextul fiziologic sau patologic dat (1).

Plecând de la compoziţia biochimică a membranei eritrocitului şi distribuţia asimetrică a fosfolipidelor, proteinelor şi colesterolului, se poate urmări prin spectroscopie FT-IR distribuţia şi modul de interacţiune a componentelor membranare, putându-se pune, indirect, în evidenţă aspectul de mozaic fluid membranar. În funcţie de compoziţia biochimică, membranele biologice, inclusiv cea eritrocitară, sunt caracte-

rizate de diferiţi parametri biochimici sau biofizici. Astfel, studii recente prin spectroscopie FT-IR, au subliniat modificările induse de stresul mecanic sau biochimic ca fiind un inductor patologic (2). Stresul oxidativ, prin intermediul radicalilor liberi, atât la nivel celular cât şi extracelular induce modificări ale membranei eritrocitare (3).

Aspectul spectral al eritrocitului urmăreşte în domeniul 4000-400 cm^{-1} frecvenţele de absorbţie ale grupărilor caracteristice fosfolipidelor membranare (4, 5), colesterolului şi proteinelor eritrocitare (5).

TABELUL I

Grupele funcţionale majore ale membranei eritrocitare şi absorbţiile IR caracteristice (5)

Numărul de unda (cm^{-1})	**Atribuirea benzii de absorbţie**
3020-3000	ν(=CH): acizi graşi nesaturaţi, esteri colesterolici
2990-2950	$\nu_{as}(CH_3)$: fosfofolipide,esteri colesterolici, acizi graşi
2950-2880	$\nu_{as}(CH_2)$: fosfolipide, lanţuri lungi ale acizilor graşi
2880-2860	$\nu_{s}(CH_3)$: acizi graşi, fosfolipide
2870-2830	$\nu_{s}(CH_2)$: fosfolipide, lanţ lung acizi graşi
2996-2795	$\nu_{s}(CH_3)$, $\nu_{as}(CH_3)$, $\nu_{as}(CH_2)$, $\nu_{s}(CH_2)$: acizi graşi, fosfolipide
1739-1713	ν(C=O): fosfolipide, esteri colesterolici, gliceride
1713-1589	ν(C=O): (amida I) lanţ proteină β
1589-1474	δ(N-H): (amida II)lanţ proteină α
1480-1430	$\delta_{s}(CH_3)$, $\delta_{as}(CH_3)$, $\delta_{as}(CH_2)$, $\delta_{s}(CH_2)$: fosfolipide, acizi graşi, gliceride
1420-1370	$\nu(COO^-)$: ion carboxil aminoacizi
1257-1201	ν(P=O): fosfolipide
1138-1061	ν(C-O):carbohidraţi, lactat
ν_s valenţă simetrică; ν_{as} valenta asimetrică; δ_s forfecare simetrică; δ_{as} forfecare asimetrică;	

MATERIAL ŞI METODĂ

Ne-am propus căutarea unor corelaţii între parametrii biochimici sangvini, vârstă şi parametrii spectrali FT-IR ai membranei eritrocitare. Scopul studiului este de a stabili în ce măsură informaţiile obţinute prin analiză spectrală FT-IR a membranei eritrocitare pot fi puse în relaţie cu statusul fiziologic sau patologic al pacientului.

Subiecţi

Studiul prezentat este unul preliminar, în vederea identificării parametrilor spectrali membranari eritrocitari care pot fi corelaţi cu diferiţi parametri biochimici sanguini. Lotul este format din 21 subiecţi cu vârsta cuprinsă între 20-60 ani (6 bărbaţi, 15 femei), clinic sănătoşi, pentru care s-a efectuat hemoleucograma analitică (număr hematii, număr leucocite, hematocrit (Hct) hemoglobină (Hb), volum mediu eritrocitar (MCV), hemglobina medie eritrocitară (HEM), număr trombocite) şi la care s-au dozat compuşi biochimici serici: colesterolul plasmatic, trigliceridele plasmatice, proteine totale, glicemia.

Material

Pentru hemoleucogramă s-a utilizat Analizatorul automat ABXP60-RO, iar

pentru biochimia serică Analizatorul automat SLIM-RO.

Pentru analiza prin spectroscopie FT-IR s-au utilizat aceleaşi probe de sânge din care s-a realizat hemoleucograma (sânge venos recoltat dimineaţa, *à jeun* în vacutainere de 5 ml cu 10mM Na_4EDTA).

Metodă

Probele au fost pregătite pentru a fi studiate prin spectroscopie FT-IR la un interval de 6 ore de la recoltare, timp în care au stat la frigider la 4˚C.

Probele s-au centrifugat 5 minute la 1000g, după care s-au separat de plasmă prin aspirarea surpernatantului.

Eritrocitele rămase s-au spălat pentru a elimina resturile de plasmă, prin centrifugare de 3 ori la 1500 g cu soluţie izotonă de soluţie NaCl 0.9 % în raport de 1:1, după care s-au realizat diluţii de eritrocite spălate: soluţie NaCl 0.9 % în raport de 1:19 (5). Probele eritrocitare au fost apoi analizate prin spectroscopie FT-IR prin realizarea unor pelicule plasmatice, respectiv eritrocitare, realizate din 5 µl probă, pe suport de KRS-5, prin uscare în vid (5 mm Hg la 20˚C) (5, 6). Fiecare probă a fost analizată in triplicat, pentru a urmări reproductibilitatea rezultatelor, iar pentru analiza statistică s-a utilizat spectrul probelor cel mai bine uscate (în funcţie de picurile de absorbţie caracteristice). Probele s-au înregistrat în transmisie, cu un spectrometru FT-IR Bruker Vertex 70. Domeniul de lungimi de undă: 4000 – 400 cm^{-1}. Fiecare probă a fost înregistrată prin acumularea a 32 de spectre, cu rezoluţie de 2,0 cm^{-1}.

REZULTATE

Analiza membranei eritrocitare prin FT-IR, în funcţie de factorii biochimici care pot influenţa fluiditatea membranei, permite urmărirea următorilor parametri eritrocitari: gradul de nesaturare al acizilor graşi, raportul acizi graşi saturaţi/acizi graşi nesaturaţi, raportul colesterol/fosfolipide, raport fosfolipide/proteine (7). Se mai pot urmări nivelul glucozei şi al lactatului la nivel eritrocitar, marker al activităţii metabolice eritrocitare.

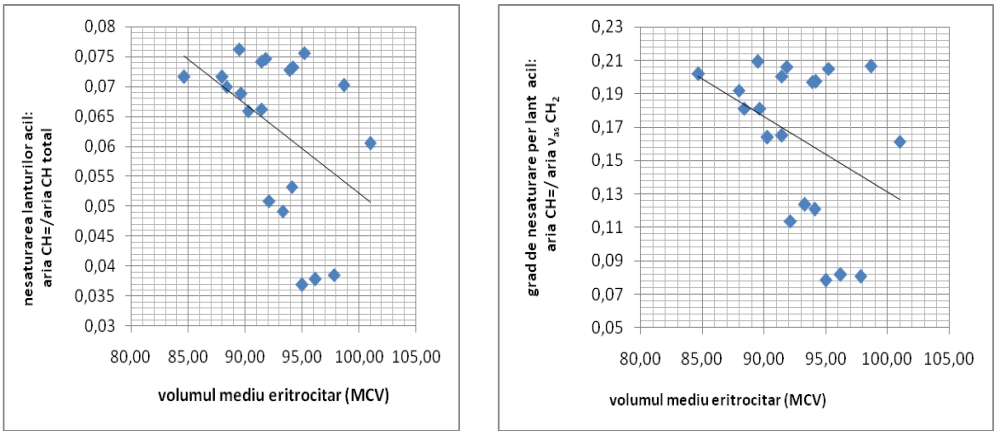
S-a observat o corelare invers proporţională între volumul mediu eritrocitar (MCV) şi gradul de nesaturare al eritrocitelor. Pentru estimarea numărului de legături olefinice raportate la lanţurile acil ale acizilor graşi eritrocitari am raportat aria specifică ν(=CH) la aria$\nu_{as}(CH_2)$ (fig. 1) după un model din literatură (8, 9).

Vibraţiile de valenţă simetrică specifice legăturii $\nu_s(CH_2)$, precum si cele ale grupei υ(P=O) sunt specifice fosfolipidelor. Scăderea ariei pentru $\nu_s(CH_2)$ şi ν(P=O) este corelată cu creşterea gradului de rigiditate a membranei (10). Creşterea rigidităţii membranei se corelează cu concentraţia de colesterol plasmatic în acord cu datele din literatură (7) (fig. 2).

Măsurătorile noastre au fost efectuate la temperatura constantă de 22°C, valoarea medie pentru $\nu_s(CH_2)$ fiind de 2871,277 cm^{-1} şi o valoare pentru deviaţia standard σ = 0,306. Se poate stabili o corelare între abaterea faţă de medie pentru $\nu_s(CH_2)$ şi valorile concentraţiei pentru colesterolul plasmatic, respectiv trigliceridele plasmatice (fig. 4**).**

Proteinele au ca parametri specifici spectrali banda amida I (determinată, în principal, de vibraţia de valenţă a grupei C=O), aflată în domeniul 1695-1574 cm^{-1}, şi banda amidă II (combinaţie de deformaţie N-H şi valenţă C-N), care se observă în domeniul 1574-1471 cm^{-1}.

Un aspect interesant este modul în care se corelează aria specifică glucozei eritrocitare ν(C-O) în domeniul 1062-997 cm^{-1}, cu aria specifică $\nu_s(CH_2)$, ca indicator al mobilităţii moleculare membranare (fig. 6).



a. b.

Fig. 1. a. Relaţia dintre volumul mediu eritrocitar şi gradul de nesaturare în ansamblu pentru lanţurile acil al acizilor graşi din membrana eritrocitară. **b.** Relaţia dintre volumul mediu eritrocitar şi gradul de nesaturare per lanţ de acil al acizilor graşi din membrana eritrocitară

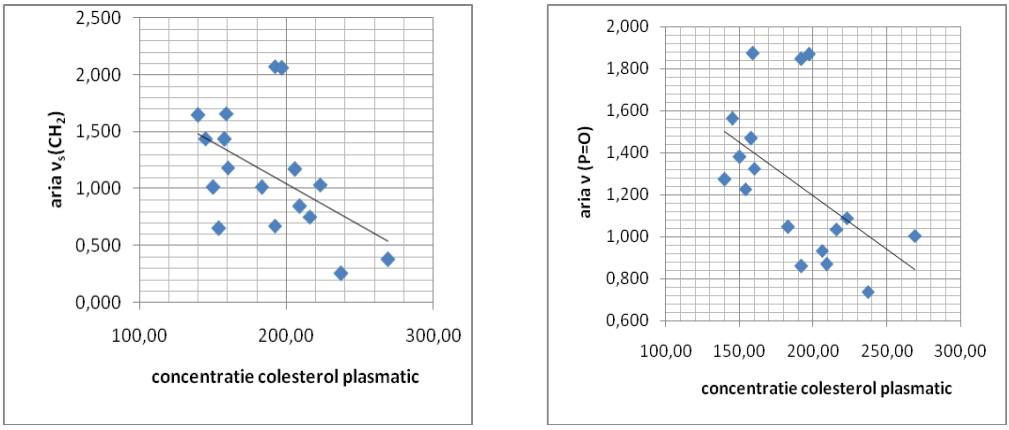


Fig. 2. Relaţia ariei pentru νs(CH2) , respectiv νs(P=O) şi concentraţia colesterolului plasmatic

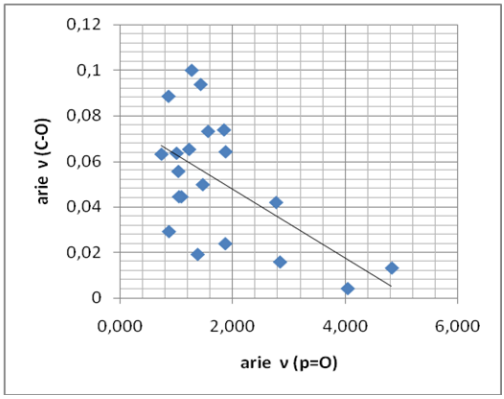


Fig. 3. Relaţia ariei pentru ν(C-O) specifică colesterolului membranar şi ν_s(P=O)

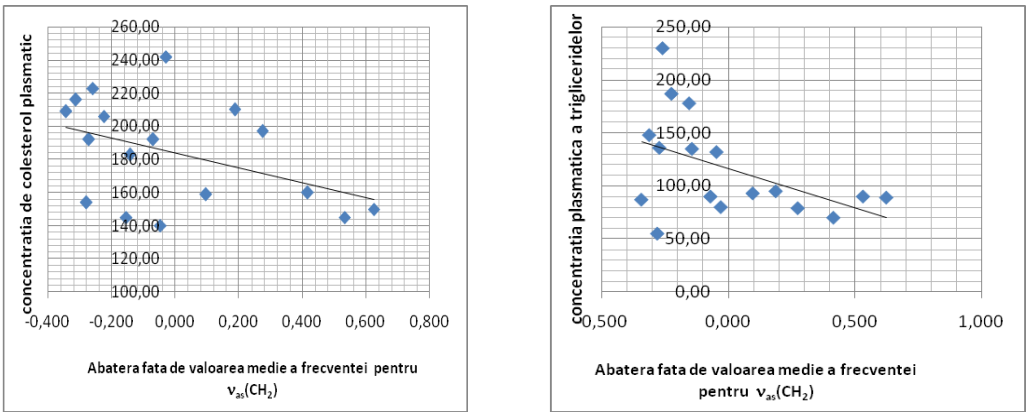


Fig. 4. Dependenţa benzii de absorbţie $\nu_s(CH_2)$ de colesterol şi TG plasmatice

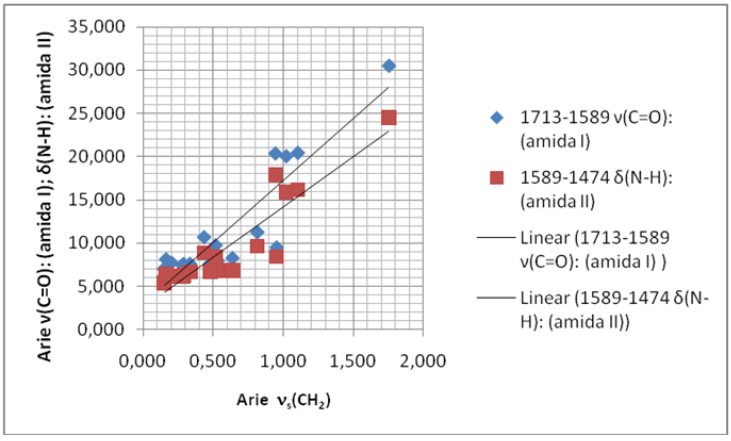


Fig. 5. Corelare între vibraţiile specificeproteinelor şi cele corespunzătoare fosfolipidelor

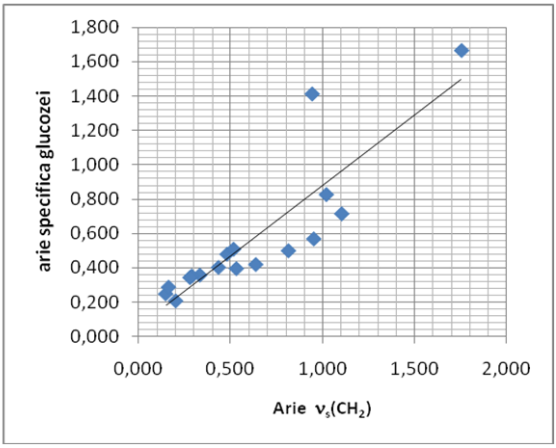


Fig. 6. Corelare între aria legăturii ν(C-O), specifică glucozei, şi aria specifică benzii $\nu_s(CH_2)$

DISCUŢII

Acizii graşi nesaturaţi sunt evidenţiaţi spectral prin absorbţiile specifice grupărilor olefinice ν(=CH) în domeniul 3030 – 3004 cm^{-1}. Raportul dintre ariile benzilor din domeniile 2800-3030 caracteristic vibraţiilor grupării CH şi 3030 – 3004 cm^{-1} (8) oferă informaţii referitoare la gradul de nesaturare al lanţurilor de tip acil al acizilor graşi membranari (8, 11). Creşterea ariilor benzii de valenţă olefinică se traduce prin creşterea gradului de nesaturare membranar (8, 9).

Faptul că ariile specifice legăturilor olefinice se corelează cu MCV poate fi expresia statusului fiziologic eritrocitar (fig.1), eliminarea eritrocitului din circulaţie fiind iniţiată de rigidizarea membranei datorită printre altele şi proceselor de oxidare membranară (12, 13). Rigidizarea cât şi creşterea în volum a eritrocitului face ca durata de viaţă a acestuia sa fie una redusă ca urmare a deformabilităţii reduse şi expresiei unor markeri specifici (14).

Modificarea fluidităţii membranare evidenţiată prin absorbţiile asociate CH_2, sau CH_3 simetrice sau asimetrice este în strânsă legătură cu compoziţia mediului extern plasmatic (fig.2) (colesterol, trigliceride, glucoză) (7).

Nucleele sterolice interacţionează cu regiunea lanţurilor hidrocarbonate, fosfolipidice în principal, cea mai apropiată de grupările polare ale capului lipidic, lăsând restul lanţului flexibil. Rezultă astfel o imobilizare parţială a fosfolipidelor pusă în evidenţă prin modul în care se modifică aria specifică $\nu_s(CH_2)$ şi cea a ν(C=O), asociată colesterolului, în domeniul 1739-1702 cm^{-1} (fig. 3). Astfel, putem întări ideea că prezenţa unui anumit procent de colesterol membranar diminuează procentul de fosfolipide care participă la tranziţiile conformaţionale (7), (15).

Asher şi *Levin* (15) au studiat influenţa temperaturii şi a unei cantităţi de colesterol asupra modului de împachetare a lanţurilor alchil din dipalmitoilfosfatidilcolină, urmărind vibraţiile de valenţă simetrică şi asimetrică ale grupelor CH_2. S-a constatat că tranziţia de fază produce o deplasare cu 4 cm^{-1} a $\nu_{as}(CH_2)$, când creşte brusc numărul conformerilor *gauche*. Adăugarea unei cantităţi >20 mol% de colesterol a dus la o tranziţie de fază fără salt de frecvenţă, datorită scăderii mobilităţii lanţului alchilic. Autorii au considerat că modul de împachetare s-a modificat de la unul hexagonal la unul quasi-hexagonal. O deplasare mai mică de frecvenţă a $\nu_{as}(CH_2)$, asociată rigidizării lanţului alchilic, a fost observată de *Rooney şi colab*. (16) în cazul membranelor eritrocitare îmbogăţite în colesterol. Scăderea concentraţiei trigliceridelor plasmatice, cât şi a colesterolului duce la scăderea pantei spectrului, pus în evidenţă prin abaterea faţa de valoare medie în intervalul M±σ (fig.4) Aceste modificări ar putea fi explicate prin prisma adaptării homeovâscoase a membranelor (7).

Raportul fosfolipide/proteine, în favoarea proteinelor**,** are un efect de reducere a difuziunii laterale atât a lipidelor cât şi a proteinelor. Proteinele suferă anumite schimbări de formă pentru a realiza funcţiile specifice. Studiile experimentale asupra vâscozităţii membranare, în funcţie de activitatea proteinelor membranare, sunt dificil de realizat deoarece vâscozitatea membranară poate fi schimbată la temperatură constantă prin schimbarea compoziţiei membranare, schimbări care pot afecta proprietăţile proteinelor prin afectarea interacţiunii lipid-proteină. A apărut astfel o idee conform căreia modificările vâscozităţii membranare ar trebui să afecteze activi-

tatea proteinelor membranare. Pentru susţinerea acestei idei a fost introdusă noţiunea de „adaptare homeovâscoasă", care menţine vâscozitatea membranară la o valoare optimă pentru exprimarea activităţii proteice. Aceasta este probabilă, deoarece studii referitoare la compoziţia fosfolipidică sugerează că modificările de fluiditate constituie un factor semnificativ în privinţa activităţii proteice (1).

Rezultatele obţinute de noi arată o dependenţă liniară a ariilor de vibraţie specifice pentru banda amidă I, respectiv amidă II, faţă de ariile specifice fosfolipidelor $\nu_s(CH_2)$ (fig.5), ceea ce sugerează o scădere a mobilităţii proteinelor în relaţie cu scăderea mobilităţii fosfolipidelor.

CONCLUZII

Studiul FT-IR al membranei eritrocitare a permis o evaluare preliminară a statusului membranar prin aprecierea mişcărilor moleculelor în funcţie de benzile de absorbţie caracteristice componentelor majoritare membranare: fosfolipide, colesterol şi proteine. din cauza eşantionului relativ mic de pacienţi, validarea statistică a rezultatelor obţinute până în prezent nu poate fi realizată. În schimb, rezultatele preliminare au permis alegerea ca parametri spectrali în evaluarea statusului membranar a următoarelor moduri de vibraţie: vibraţia de valenţă asociate legăturii (=CH), cea de valenţă asimetrică a grupelor CH_2; modurile asociate legăturii P=O, precum şi benzile amidă I şi II, specifice proteinelor. De asemenea, urmărirea altor parametri, cum ar fi ν(C-O) specifică glucozei, poate aduce informaţii despre statusul metabolic al eritrocitului.

Corelarea acestor parametri spectrali cu diferiţi markeri biochimici poate oferi informaţii despre statusul membranar eritrocitar, status care poate fi pus în relaţie cu diferite procese patologice.

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NOUTĂȚI

NOI MODALITĂȚI DE DIAGNOSTIC ÎN INFECȚIILE CU *KINGELLA KINGAE*

Kingella kingae este frecvent implicată în infecţiile ostearticulare la copil. Deoarece metodele de izolare clasice sunt dificile, s-a încercat standardizarea metodelor de diagnostic prin tehnici de biologie moleculară. În acest scop s-a folosit ca secventă de interes toxina RTX, genomul bacterian nefiind secvenţiat până în prezent. Scopul studiului a fost dezvoltarea unei metode rapide, sensibile şi specifice utilă pentru a stabili diagnosticul etiologic al acestor infecţii. Au fost aplicate două metode Real Time PCR (în sistem Taq Man şi SYBR green), ambele având ca scop indentificarea secvenţelor nulceotidice rtxA. Sensibilitatea şi specificitatea metodelor a fost evaluată folosind 67 tulpini bacteriene (31 au fost *Kingella kingae).* Apoi, 52 probe pozitive şi/sau negative în cultură şi/sau PCR au fost testate folosind aceste două tehnici. Ulterior s-a folosit tehnica PCR "în cuib" şi secvenţierea tulpinilor izolate pentru a confirma prezenţa *Kingella kingae* în probele luate în lucru. Rezultatele obținute au demonstrat acurateţea metodelor mai sus prezentate în stabilirea diagnosticului etiologic. (Lehours P, Freydière A, Richer O et al, The rtxA Toxin Gene of Kingella kingae: a Pertinent Target for Molecular Diagnosis of Osteoarticular Infections. *J Clin Microbiol* 2011; 49: 1245-1250).

Teodora Vremeră

EMERGENȚA REZISTENȚEI LA CARBAPENEME LA TULPINI DE *KLEBSIELLA PNEUMONIAE* IZOLATE DIN INFECȚII ALE TRACTUSULUI URINAR

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CARBAPENEM RESISTANCE IN *KLEBSIELLA PNEUMONIAE* CLINICAL ISOLATES FROM URINARY TRACT INFECTIONS (Abstract): Resistance to carbapenems by KPC (*Klebsiella pneumoniae* carbapenemase) production in *Klebsiella pneumoniae* clinical isolates was first described ten years ago in the USA and recently reported in other countries. This enzyme inactivates all beta-lactam antibiotics and is associated with fluoroquinolone and aminoglycoside resistance. **Material and methods**: We investigated the carbapenem resistance in 498 *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from urinary tract infections during 2009 in the Iasi "Dr. C. I. Parhon" Clinical Hospital. Antimicrobial susceptibility testing was performed according to CLSI guidelines. To detect ESBL (e*xtended spectrum beta-lactamase*) and KPC production we used phenotypic tests and molecular biology methods (PCR). **Results**: From all tested strains, only two *K. pneumoniae* strains showed modified susceptibility to carbapenems. The modified Hodge test was positive for the strain resistant to ertapenem, meropenem, imipenem (K_A) and negative for the strain resistant only to ertapenem (K_B). Both K_A and K_B isolates were negative for bla_{KPC} and bla_{TEM} genes, but harbored bla_{SHV} and $bla_{CTX\text{-}M}$ genes, respectively. **Conclusions**: We report the first carbapenem resistant *K. pneumoniae* strains from Northeast Romania. The resistance is not mediated by KPC-carbapenemase; the possibility of dual mechanisms through cefalosporinases production and porins loss is suggested. **Key words:** *KLEBSIELLA PNEUMONIAE*, CARBAPENEMS, RESISTANCE MECHANISMS

Carbapenemele sunt folosite în special în tratamentul de primă intenție a infecțiilor severe determinate de bacili gram-negativi. Instalarea rezistenței la aceste antibiotice a condus la limitarea opțiunilor terapeutice, asociată cu o rată înaltă de mortalitate. Fenomenul este relativ frecvent întâlnit la bacili gram-negativi nefermentativi (*Pseudomonas aeruginosa, Acinetobacter baumannii*), mai recent fiind raportat în unele zone geografice şi la reprezentanți ai familiei *Enterobacteriaceae*, în special la *Klebsiella pneumoniae* (1).

Acest fenotip de rezistență poate fi explicat prin producerea de carbapenemaze (aparținând claselor moleculare Ambler A, B, D), care inactivează toate β-lactaminele, sau printr-un mecanism dual în care sunt impli-

cate cefalosporinaze (beta-lactamaze cu spectru extins, AmpC) asociate cu reducerea permeabilității membranei externe (pierderea unor porine membranare OmpK35, OmpK36) (2). Raportarea în diferite țări de pe glob a tulpinilor rezistente la carbapeneme reprezintă o preocupare relativ recentă, motiv pentru care am demarat acest studiu.

MATERIAL ŞI METODE

Lotul de studiu a inclus 498 tulpini de *E.coli* şi *K.pneumoniae* izolate din uroculturi în anul 2009, la Spitalul Clinic "Dr. C. I. Parhon" Iaşi. Testarea sensibilității la antibiotice am efectuat-o prin antibiograma difuzimetrică conform standardelor CLSI 2009 (3). Tulpinile cu sensibilitate modificată la cefalosporine cu spectru larg au fost testate față de trei carbapeneme: ertapenem (ETP), imipenem (IPM) şi meropenem (MEM). Pentru izolatele cu diametrul zonei de inhibiție ≤ 21 mm față de ETP sau/şi MEM am determinat concentrația minimă inhibitorie (CMI) utilizând sistemul automat Phoenix BDExpert; am efectuat screening-ul pentru β-lactamaze cu spectru extins (BLSE) şi pentru carbapenemaze prin testul dublului disc, respectiv testul Hodge modificat (THM) (3). Activitatea beta-lactamazică a extractului sonicat din cultura tulpinii cu THM pozitiv a fost evaluată calitativ prin hidroliza nitrocefinului.

La tulpinile rezistente la carbapeneme am urmărit detectarea genelor ce codifică beta-lactamaze cu spectru extins (tip CTX-M, SHV, TEM) şi carbapenemaze tip KPC prin trei reacții PCR, într-un termociclor Mastercycler personal (Eppendorf, Germania), conform unor metode descrise anterior (1,4,5). Primerii utilizați, dimensiunea ampliconilor şi temperaturile de atașare sunt prezentate în tabelul I.

TABELUL I

Primerii utilizați pentru amplificare genică

Gena țintă	Primer	Secvență	Dimensiune amplicon	Temp de atașare	Bibliografie
*bla*CTX-M	CTXM-1 CTXM-2	5'-ATGTGCAGYACCAGTAARGT-3' 5'-TGGGTRAARTARGTSACCAGA-3'	593 bp	50˚C	5
*bla*SHV	SHVfw SHVrev	5'-GATGAACGATTTCCCATGATG-3' 5'-CGCTGTTATCGCTCATGGTAA-3'	214 bp	51˚C	4
*bla*TEM	TEMfw TEMrev	5'-AGTGCTGCCATAACCATGAGTG-3' 5'-CTGACTCCCCGTCGTGTAGATA-3'	431 bp	51˚C	4
*bla*KPC	KPCfwd KPCrev	5'ACTTCCTGAGGCCGTCCGTA-3' 5'-GCCATCGTCAGTGCTCTACAG-3'	1185 bp	55˚C	1

REZULTATE ŞI DISCUȚII

În anul 2009, 552 (88,6%) din uroculturi au fost pozitive cu bacili gram-negativi din familia *Enterobacteriaceae*, *E. coli* şi *K. pneumoniae* reprezentând cei mai frecvenți agenti etiologici (429, respectiv 69 tulpini). Un procent de 21,9% tulpini *E.coli* şi 30,4% tulpini *K. pneumoniae* au prezentat sensibilitate modificată la cefalosporine cu spectru extins; dintre acestea, numai la două tulpini *K. pneumoniae* diametrul zonelor de sensibilitate la ETP a fost redus. Ambele tulpini au fost rezistente la peniciline cu şi fără inhibitori de beta-

lactamază, cefalosporine, monobactam precum și la antibiotice din alte clase (tab. II). Una dintre tulpini, K_A a dovedit rezistență la ETP, MEM și IPM (CMI > 8 µg/ml); cealaltă tulpină, K_B a fost rezistentă la ETP, dar sensibilă la MEM și IPM. *Screening*-ul pentru BLSE a fost negativ în ambele cazuri. Testul Hodge modificat a fost pozitiv pentru K_A și negativ pentru K_B (fig. 1).

TABELUL II

Sensibilitatea la antibiotice a tulpinilor de *Klebsiella pneumoniae* K_A și K_B

Antibiotic[a]	**CMI**[b] **(µg/ml)/ categorie de sensibilitate (S, R)**[c]			
	KA		KB	
AM	> 16	R	> 16	R
AMC	> 16/8	R	> 16/8	R
PIP	> 64	R	> 64	R
TZP	> 64/4	R	> 64/4	R
CZ	> 16	R	> 16	R
FOX	> 16	R	> 16	R
CAZ	> 16	R	> 16	R
CTX	> 32	R	> 32	R
CES	> 16/8	R	> 16/8	R
FEP	> 16	R	> 16	R
ATM	> 16	R	> 16	R
IPM	> 8	R	≤ 1	S
MEM	> 8	R	2	S
AK	≤ 8	S	≤ 8	S
GM	≤ 2	S	> 8	R
SXT	1/19	S	> 2/38	R
TE	> 8	R	> 8	R
CIP	≤ 0,5	S	> 2	R
LEV	≤ 1	S	> 4	R
COL	≤ 0,5	S	≤ 0,5	S

[a]AM, ampicilină;
AMC, amoxicilină-clavulanat;
PIP, piperacilină;
TZP, piperacilină-tazobactam;
CZ, cefazolin;
FOX, cefoxitin;
CAZ, ceftazidim;
CTX, cefotaxim;
CES, cefoperazonă-sulbactam;
FEP, cefepim;
ATM, aztreonam;
IPM, imipenem;
MEM, meropenem;
AK, amikacină;
GM, gentamicină;
SXT, sulfametoxazol-trimetoprim;
TE, tetraciclină;
CIP, ciprofloxacin;
LEV, levofloxacin;
COL, colistin.
[b] Concentrația minimă inhibitorie.
[c] S, sensibil; R, rezistent.

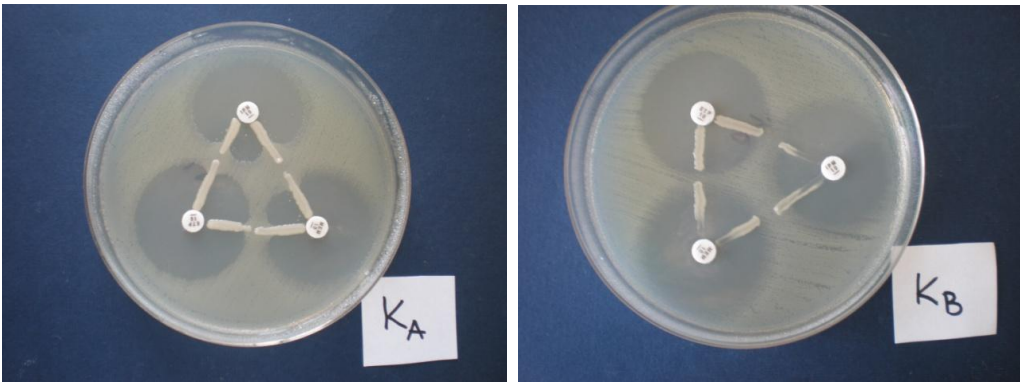

Fig. 1. Testul Hodge modificat: pozitiv pentru tulpina KA (tulpina E.coli ATCC 25922 cultivă spre discul cu carbapenem la locul de intersecție cu tulpina testată – aspect de frunză de trifoi); negativ pentru tulpina KB (conturul zonei de inhibiție circular, nemodificat).

Rezultatele acestor teste fenotipice neau determinat să suspectăm intervenția unor mecanisme diferite de rezistență la carbapeneme: prin producere de carbapanemaze (K_A) sau prin producere de β-lactamaze cu spectru extins în asociere cu pierderea unor porine (K_B). Extractul enzimatic obținut prin sonicarea culturii bacteriene K_A a hidrolizat nitrocefinul, acțiune inhibată de cloxacilină, fapt care sugerează prezența unei cefalosporinaze de tip AmpC (6). Analiza genetică prin PCR cu primeri specifici pentru genele *bla*KPC, *bla*TEM, *bla*CTX-M, *bla*SHV a fost negativă pentru KPC şi TEM; tulpina K_A posedă o genă SHV, iar K_B una CTX-M (tab. III). Lipsa secvențierii face imposibilă precizarea tipului de enzimă SHV (clasic sau BLSE).

TABELUL III
Rezultatele analizei prin PCR

	*bla*CTX-M	*bla*SHV	*bla*TEM	*bla*KPC
Tulpina K_A	-	+	-	-
Tulpina K_B	+	-	-	-

Rezistența *K. pneumoniae* prin producere de carbapenemaze KPC a fost semnalată în urmă cu un deceniu în SUA iar ulterior în alte țări (Canada, Columbia, Brazilia, China, Israel, Marea Britanie, Grecia, Franța, Italia, Polonia, Elveția, Spania) (1, 7, 8, 9). Fenomenul este alarmant întrucât acest tip de enzime inactivează toate antibioticele β-lactamice, se asociază cu rezistență la antibiotice din alte clase (aminoglicozide, fluorochinolone) şi sunt codificate de gene plasate pe elemente mobile, ceea ce face posibil transferul acestui mecanism de rezistență la alți bacili Gram-negativi (*Enterobacteriaceae, Pseudomonas aeruginosa*). Aceste tulpini au de asemenea un potențial ridicat de răspândire pacient-pacient, necesitând măsuri severe de supraveghere epidemiologică. În România acest fenotip nu a fost încă raportat.

În plus, literatura cuprinde multiple semnalări ale diverselor combinații de determinanți de rezistență: alterarea permeabilității membranei externe asociată cu cefalosporinază AmpC (2) sau cu betalactamaze cu spectru extins (CTX-M, SHV-2), însoțite de o creşterea variabilă a CMI la carbapeneme, cel mai constant fiind afectat ertapenem (10, 11). Totodată, s-au înregistrat până la 25% rezultate fals pozitive la testul Hodge modificat, mai ales pentru izolate producătoare de CTX-M sau hiperproducătoare de AmpC (11, 12).

În acest context putem explica rezistența la ertapenem a tulpinii K_B prin asocierea dintre CTX-M şi pierderea unor porine. Tulpina K_A nu s-a dovedit producătoare de carbapenemază KPC, rezistența la cele trei carbapeneme implicând sinteza de beta-lactamaze multiple (AmpC, SHV) însoțită de o scădere a permeabilității membranei externe.

CONCLUZII

Au fost izolate din infecții urinare primele două tulpini *K. pneumoniae* din Nord-Estul României cu sensibilitate modificată la carbapeneme. Mecanismul de rezistență nu apare mediat de carbapenemaze, fenotip relativ recent descris în literatură, care limitează posibilitățile actuale de terapie antibacteriană şi necesită măsuri drastice de supraveghere epidemiologică. Testele fenotipice şi moleculare care ne-au fost accesibile pledează pentru un mecanism dual de rezistență, prin producere de cefalosporinaze asociată cu pier-

derea de porine.

Interpretarea testului Hodge modificat, recomandat de standardul testării sensibilității la antibiotice pentru detectarea fenotipică a carbapenemazelor la *Enterobacteriaceae*, trebuie făcută cu prudență, datorită unor rezultate fals pozitive, mai ales în zonele cu prevalență crescută a tulpinilor producătoare de beta-lactamaze cu spectru extins, cum este țara noastră.

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VALOAREA MODELULUI TRIDIMENSIONAL ÎN RECONSTRUCŢIA PIERDERILOR DE SUBSTANŢĂ SEGMENTARE ALE MANDIBULEI. OBSERVAŢII PE MARGINEA UNUI CAZ CLINIC

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THE VALUE OF 3-DIMENSIONAL MODEL IN MANDIBULAR SEGMENTARY DEFECT RECONSTRUCTION. A CASE REPORT (Abstract) : The use of autogenous bone graft in the reconstruction of mandibular defects following tumor extirpation is a reliable method for further functional rehabilitation. The exact amount of bone needed for harvesting is usually achieved by estimation of the gap and direct measuring. We present the case of a 51-years old patient referred to our clinic for a recurrent mandibular keratocyst. Besides clinical examination, the diagnosis work-up consisted of ortopantomography and computertomography (CT). Based on CT images, a graphic reconstruction of the mandible was realized and a 3D negative template of the scheduled mandibular defect was printed. The tumor was removed by a segmental resection of the mandible, while a fragment of bone tissue similar to the 3D model was harvested from the iliac crest. This bone fragment replaced the mandible defect and was fixed by means of four plates and titanium screws. No postoperative complications occurred. The pathology result confirmed the diagnosis of keratocyst. In conclusion, this method proved to be useful for precise planning of the shape and size of the graft in addition to exact placement of the graft in an acceptable prosthodontically position for future dental implant rehabilitation. **Key words** : 3-DIMENSIONAL MODEL, MANDIBULAR DEFECTS, FREE BONE GRAFT

Reconstrucţia pierderilor de substanţă osoasă segmentare ale mandibulei este o problemă complexă şi mereu actuală în chirurgia reparatorie maxilo-facială. Restaurarea arhitecturii mandibulare este esenţială pentru echilibrul şi simetria feţei ca şi pentru o masticaţie, deglutiţie şi fonaţie normală (1).

Întocmirea planului reconstructiv este întotdeauna condiţionată de sediul şi întinderea pierderii de substanţă, de natura acestor pierderi, de starea părţilor moi de vecinătate şi a dentiţiei precum şi de vârsta şi starea generală a bolnavului.

De asemenea, un rol deosebit de important în reconstrucţia definitivă a defectelor osoase în continuitate ale mandibulei o are materialul reconstructiv alături de reconstrucţia tridimensională cât mai exactă a segmentului osos îndepărtat (2). Studiile experimentale şi clinice au demonstrat superioritatea incontestabilă a transplantelor autogene proaspete comparativ cu oricare alt material de

Fig. 1. Aspect preoperator extraoral

Fig. 2. Aspect preoperator intraoral

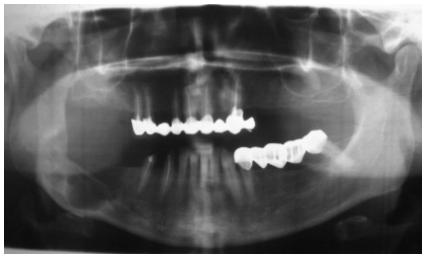

Fig. 3. Aspect ortopantomografic

Fig. 4. Aspect septat al leziunii

Fig. 5. Întreruperea ambelor corticale osoase

reconstrucţie (3,4,5,6). Pentru aprecierea cât mai exactă a dimensiunilor pierderii de substanţă de un real folos sunt modelele reproductibile tridimensionale confecţionate pe baza tomografiei computerizate (CT) (7).

PREZENTAREA CAZULUI

Pacienta G.R., în vârstă de 51 ani, s-a internat în martie 2010 pentru un keratochist recidivat de ram orizontal şi unghi drept al mandibulei. Prima intervenţie chirurgicală a fost efectuată în urmă cu 5 ani şi a constat în chistectomie cu meşare. La această ultimă internare pacienta prezenta o discretă deformare a regiunii genio-maseterine drepte în 1/3 inferioară, determinată de prezenţa formaţiunii tumorale care evolua din grosimea osului erodând şi desfiinţând în totalitate ambele corticale osoase (fig. 1). Mucoasa acoperitoare era integră dar aderentă de leziune, iar la palparea bimanuală se percepea fluctuenţă (fig. 2).

Ortopantomografia a evidenţiat o radiotransparenţă multiloculară la nivelul ramului orizontal drept mandibular în 1/3 distală : 4.6 (spaţiu edentat) – unghi drept (fig. 3).

Examenul CT a relevat prezenţa unei leziuni expansive radiotransparente, septate, cu contur policiclic, având aceeaşi localizare ca şi pe ortopantomografie, care întrerupea ambele corticale osoase (fig. 4, 5).

Pe baza examenului CT s-a efectuat reconstrucţia grafică a mandibulei afectate (fig. 6a şi 6b).

În scopul recoltării unui fragment osos cu aceeaşi dimensiune ca a osului rezecat, necesar reconstrucţiei osoase imediate, am confecţionat preoperator un model tridimensional (3D) pe baza examenului CT. În acest sens datele obţinute au fost procesate şi transformate în format 3D, ulterior fiind convertite în format stereolitografic (STL) utilizându-se 3 tipuri de software (8). După această operaţiune dificilă, cu ajutorul unei imprimante 3D s-a realizat modelul anatomic ce include şi leziunea (fig. 7).

Fişierul STL de la modelul anterior a fost introdus într-un alt program de software şi s-a creat astfel un model nou folosindu-se o funcţie "imagine în oglindă" luându-se ca

Fig. 6a. Reconstructia grafică a mandibulei vedere frontală

Fig. 6.b. Reconstructia grafică a mandibulei vedere inferioară

Fig. 7. Model anatomic

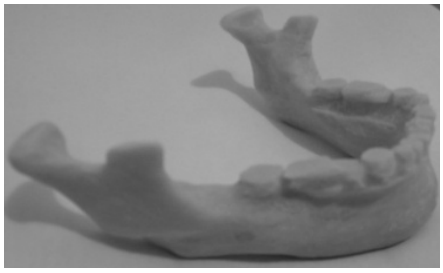

Fig. 8. Model final

Fig. 9. Aspect intraoperator al tumorii

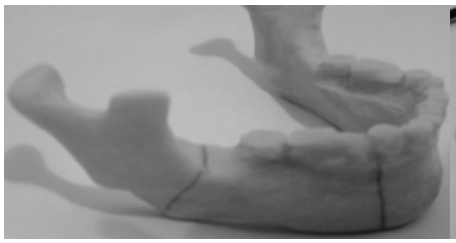

Fig. 10. Liniile de osteotomie

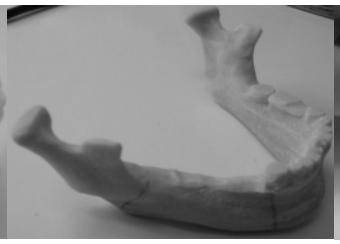

Fig. 11. Radierea dinţilor

Fig. 12. Modelul "ideal" secţionat

reper ramul orizontal controlateral (sănătos) al mandibulei. Acest model a fost ulterior creat prin imprimare 3D şi sterilizat pentru a fi utilizat în sala de operaţie (fig. 8).

Intervenţia chirurgicală a constat în extirparea tumorii prin rezecţie segmentară de mandibulă 45-unghi drept, cu abord cutanat (fig. 9). Plastia pierderii de substanţă s-a realizat utilizând modelul tridimensional "ideal". Acesta a fost marcat în dreptul liniilor de osteotomie (fig. 10) şi după radierea dinţilor (fig. 11) a fost secţionat izolându-se astfel un fragment identic cu pierderea de substanţă osoasă (fig. 12). Apoi s-a recoltat din creasta iliacă dreaptă un fragment similar cu cel al modelului tridimensional şi s-a

Fig. 13. Fixarea transplantului în sediul receptor

Fig. 14. Aspect postoperator

fixat în locul pierderii de substanţă mandibulară cu ajutorul a 4 plăcuţe şi şuruburi din titan (fig. 13).

Evoluţia postoperatorie a fost favorabilă.

La 10 luni de la intervenţia chirurgicală nu s-a înregistrat nici o complicaţie (fig. 14).

Diagnosticul histopatologic al piesei de rezecţie a fost de keratochist.

DISCUŢII

Pierderile de substanţă osoasă segmentare ale mandibulei au ca etiologie traumatismele, infecţiile, tumorile şi radionecroza (9). Cel mai frecvent, defectele mandibulare sunt rezultatul extirpării chirurgicale a tumorilor.

În funcţie de localizare, Peri şi col. au clasificat defectele osoase în continuitate ale mandibulei astfel :

- tip I segment simfizar ;
- tip II segment lateral ;
- tip III segment fronto-latero-terminal ;
- tip IV segment condilian + apofiza coronoidă (10).

Cazul prezentat se încadrează în tipul II al clasificării.

Defectele osoase segmentare mandibulare generează prejudicii clinice, morfologice şi funcţionale în strânsă dependenţă cu mărimea defectului şi localizarea sa. Astfel necesitatea reconstrucţiei ramului orizontal mandibular rezidă din următoarele caracteristici :

- constituie un element esenţial al pârghiei mandibulare suportând cele mai mari forţe masticatorii ;
- conţine nervul alveolar inferior responsabil de inervaţia osului mandibular, a dinţilor şi a buzei inferioare ;
- reprezintă în zona distală locul de inserţie a celor mai importanţi muşchi ridicători ai mandibulei (11).

Obligativitatea evaluării cât mai fidele a dimensiunilor pierderilor de substanţă osoasă a determinat crearea modelelor tridimensionale pornind de la examenul CT. Acest lucru a fost menţionat pentru prima dată în 1980 de Alberti (12), iar construcţia primului model cranio-facial a fost raportată în 1987 de către Brix şi Lambrecht (13). Până la realizarea şi utilizarea în practică a acestor modele tridimensionale, aprecierea dimensiunilor pierderilor de substanţă segmentare mandibulare s-a efectuat şi încă se mai efectuează pe baza piesei rezecate şi a plăcii martor de conturare (piesă componentă a truselor de reconstrucţie).

În ceea ce priveşte refacerea continuităţii mandibulare, în prezent există metode chirurgicale variate, fiecare având indicaţiile, limitele şi complicaţiile proprii. Studiile experimentale şi clinice au demonstrat valoarea de necontestat a grefelor de os autolog, acestea reprezentând "standardul de aur" în reconstrucţiile pierderilor osoase în continuitate ale mandibulei (14,15). Dacă dimensiunea pierderii osoase este mai mică de 5 cm se poate utiliza grefa de os autolog nevascularizat (11,16,17). În acest sens, o sursă importantă de os cortico-spongios autogen proaspăt o reprezintă creasta iliacă care poate livra orice formă şi mărime de transplant în cantitatea dorită (18). Pentru a se asigura succesul intervenţiei chirurgicale un rol deosebit de important îl are pregătirea patului de transplantare, modul de recoltare şi modelare a grefonului, fixarea acestuia în *situs*-ul receptor precum şi îngrijirea postoperatorie. Dezavantajele majore ale acestui tip de transplant osos decurg din lipsa aportului vascular propriu cu riscuri legate de resorbţia osoasă sau de apariţia mai frecventă a complicaţiilor infecţioase ce pot conduce la rejetul grefei, comparativ cu grefa osoasă vascularizată.

În cazul prezentat, cantitatea necesară de os s-a recoltat în funcţie de modelul tridimensional confecţionat preoperator. Acest lucru a permis recoltarea unui fragment osos iliac de dimensiuni ideale şi a favorizat scurtarea duratei intervenţiei chirurgicale. În plus reconstrucţia osoasă va permite protezarea ulterioară a zonei edentate, restabilind funcţiile aparatului dento-maxilar.

CONCLUZII

Reconstrucţia imediată a pierderilor de substanţă segmentare ale mandibulei mai mici de 5 cm, utilizând grefele nevascularizate din osul iliac, reprezintă soluţia ideală. Mo-

delul tridimensional permite o planificare precisă şi mai uşoară a plastiei osoase cât şi o recoltare a osului mult mai exactă prin folosirea tiparului negativ a spaţiului ce urmează a fi reconstruit. Deşi laborioasă şi costisitoare, beneficiile aduse de folosirea modelului îl recomandă ca un element valoros şi deosebit de util în protocolul reconstructiv.

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IN VITRO EVALUATION OF KETOPROFEN CONTROLLED RELEASE FROM VARIOUS FORMULATIONS

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IN VITRO EVALUATION OF KETOPROFEN CONTROLLED RELEASE FROM VARIOUS FORMULATIONS (Abstract): The aim of this study is to investigate the potential utility of some hydrogels, based on chitosan, chitosan modified with phthalic anhydride and 75/25 poly(N-isopropyl acrylamide)/alginate, for preparing drug release systems containing ketoprofen, as model drug. **Material and method**: The *in vitro* release profiles and swelling studies were done in ethanol medium, where the studied drug presents high solubility, at 25^0C (room temperature). The ketoprofen release was observed by monitoring the absorbance at λ_{max} = 254 nm as a function of time. **Results**: The experimental results indicated that the smallest amount of drug was released from chitosan matrices, appreciatively 31%. **Conclusions**: The composition of hydrogels had an important effect on ketoprofen release. **Key words**: ALGINATE, CHITOSAN, SWELLING, CONTROLLED RELEASE, KETOPROFEN

Among the temperature-sensitive hydrogels reported to date, poly (N-isopropyl acrylamide) (PNIPAAm) and its copolymers have been widely used for cell separation as well as for pharmaceutical and tissue engineering applications because of their unique thermo-responsive properties (1,2, 3). PNIPAAm undergoes a reversible temperature-sensitive coil-to-globule transition in aqueous solutions at approximately 32^0C (its lower critical solution temperature, LCST), which lies between room temperature and physiological temperature (37^0C). The LCST of PNIPAAm can be increased by copolymerization with hydrophilic monomers or decreased by using hydrophobic monomers (4,5,6).

Alginate, a polysaccharide extracted from brown algae has been used as a hydrophilic component of hydrogels for many biomedical applications. The ability of alginate sodium salt to pharmaceutical industry, being applied as a carrier (hydrophilic matrix) for controlled release of oral dosage forms. Matrices incorporating alginate salts or a combination of alginate with other polymers have been employed to successfully prolong release of many drugs (7,8).

Since chitosan itself is non-toxic (9), biodegradable (10) and biocompatible (11), several biological applications have been reported for chitosan, including chelation processes (12), a cholesterol trap (13) and a drug carrier (14,15). Recently, there has been a growing interest in chemical modification of chitosan to improve its solubility and widen its applications (16,17). The chemical modification is a powerful tool to control the interaction of the polymer with drugs, to enhance the drug loading efficiency as well as tailor the drug release period. Chemically modified chitosans have great utility in controlled release and targeting studies of almost all class of bioactive molecules.

Chitosan has been modified by ionic crosslinking (e.g. with tripolyphosphate, TPP) to prepare intelligent drug delivery systems

(18), and by carboxymethylation for repair and regeneration of bone tissues (19) or use as antioxidant agent (20).

The aim of the present investigation to compare the kinetics of swelling and ketoprofen release from different matrices as N-isopropyl acrylamide (NIP)/alginate (Alg) hydrogel (75/25), chitosan (C) and chitosan modified with phthalic anhydride (CF) matrices.

EXPERIMENTAL

Materials

The monomer N-isopropyl acrylamide (Aldrich) 97% (NIPAAm), the polysaccharide alginic acid extracted from brown algae (Fluka), M_w = 48 000 - 186 000 with reduced viscosity at 25^0C of 2.41 mL·g^{-1}, c = 0.2 g/dL (Alg) and the crosslinking agent N,N'-methylene bis-acrylamide (Fluka) were all used as received.

Chitosan – (Aldrich) with weight average molecular weight M_w = 400 000 and acetylating degree of 68% was used.

Phthalic anhydride-modified chitosan was synthesized as follows: the N- substituted chitosan derivative was obtained by treating chitosan with phthalic anhydride, an Aldrich product, in dimethylsulfoxide (DMSO) under nitrogen flow and mixing for 6 hours at 80°C according to Hirano's method (21). The obtained modified chitosan was further purified by reprecipitation in ice-distilled water and sodium carbonate (Na_2CO_3); then the precipitate was collected, centrifuged and washed with ethyl ether and then dyalized against acetone for 3 days in order to remove the traces of solvent. The structure of obtained product was confirmed by of FT-IR and H-NMR spectra (22).

Ketoprofen is a classical nonsteroidal anti-inflammatory (first generation), analgesic and antipyretic drug efficient in the treatment of the rheumatoid arthritis and osteoarthritis, spondylitis, as well as soft tissue injuries and also to reduce fever. It is

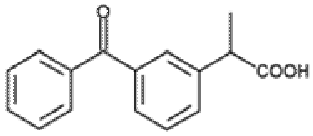

Fig. 1. Structure of ketoprofen (23)

a water insoluble substance but it dissolves in solvents like ethanol, methylene chloride, dimethylsulphoxide (DMSO) and dimethylformamide (DMF). The structure of ketoprofen is presented in fig. 1.

METHODS

Swelling kinetics

The kinetics of the swelling was carried out by weight measurements performed at room temperature in ethanol. The equilibrium swelling degree was calculated according to the Eq. (1).

$$Q_{max}\,(\%) = (W_t - W_d)/W_d \bullet 100 \qquad (1)$$

where W_t is the weight of the swell samples at time t and W_d is the dry weight of the sample.

To determine the kinetics of solvent diffusion into the matrices (swelling) the following equation was used (24):

$$F_t = \frac{W_t}{W_{eq}} = k_{sw} t^{n_{sw}} \qquad (2)$$

where W_t and W_{eq} represent the amount of ethanol solution, absorbed by the matrices at time t and at equilibrium, respectively, k_{sw} is the swelling constant characteristic of the system and n_{sw} is the power law diffusion exponent which takes into account the type of solvent transport. Eq. (2) applies to initial states of swelling (swelling degree less than 60%) and linearity is observed when log F_t as a function of log t is represented.

Drug loading and *in vitro* release studies

The drug loading of the hydrogel matrices was carried out by mixing ketoprofen with dried hydrogel in powder form. During the drug release study, at predetermined time intervals, 1 mL sample was withdrawn

from the release medium and concentration of ketoprofen at 254 nm in the release medium were determined using a UV-VIS spectrophotometer HP 8450A.

The concentrations of the drugs were calculated based on calibration curves determined for drug at specific maximum absorption wavelengths.

A simple, semi-empirical equation using Korsmeyer and Peppas model was used to kinetically analyze the data regarding the drug release from studied matrices system which is applied at the initial stages (approximately 60 % fractional release) (25, 26,27,28,29,30,31) :

$$M_t / M_\infty = k_r t^{n_r} \quad (3)$$

where $M_t/M_á$ represents the fraction of the drug released at time t, M_t and M_8 are the absolute cumulative amount of drug released at time t and at infinite time (in this case maximum release amount in the experimental conditions used, at the plateau of the release curves), respectively, k_r is a constant incorporating characteristics of the macromolecular matrix and the drug n_r is the diffusion exponent, which is indicative of the release mechanism. In the equation above a value of $n_r = 0.5$ indicates a Fickian diffusion mechanism of the drug from matrix, while a value $0.5 < n_r < 1$ indicates an anomalous or non-Fickian behaviour. When $n_r = 1$ a case II transport mechanism is involved while $n_r > 1$ indicates a special case II transport mechanism (32,33,34,35).

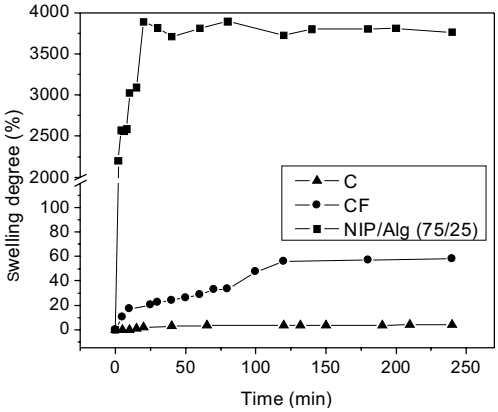

Fig. 2. Swelling profiles of studied hydrogels in ethanol medium, at 25 ^{0}C

The corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release versus time.

FT-IR spectroscopy

The hydrogels loaded with ketoprofen were analyzed by FT-IR spectroscopy, using the KBr pellet technique. The spectra were scanned on a Bruker VERTEX 70 (USA) device, over a 4000-500 cm^{-1} range, at a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSIONS

Swelling kinetic studies

The swelling behaviour of the tested matrices in ethanol medium at 25^{0}C is shown in fig. 2.

To determine the swelling behavior, the chitosan-based matrices were tested in saturated ethanol vapors atmosphere, at 25^{0}C. The swelling of chitosan in ethanol is very low of only 4%. It is evident that the character of swelling curves for both chitosan matrices is different, because of modifying process with phthalic anhydride, the swelling ratio in this case increased being of 58%.

For 75/25 NIP/Alg hydrogels, the swelling studies were done by immersing the samples in ethanol solution at 25^{0}C, reaching a stable equilibrium after 30 minutes with a swelling degree of 3800 %.

In tab. I, the kinetic parameters of swelling for studied hydrogel are given.

The values obtained for swelling parameter (n_{sw}) in ethanol medium at 25^{0}C varies in range between 0.01-0.15 indicating an anomalous mechanism of swelling.

In vitro release studies

In case of chitosan-based matrices, the modification process with phthalic anhydride, determines a difference between amounts of drug released, the highest percent, about 43%, being obtained for CF-

TABLE I
Kinetic parameters of swelling for studied hydrogels

Hydrogels	n_{sw}	k_{sw} (min^{-n})
C	0.01	0.94
CF	0.05	0.65
75/25 NIP/Alg	0.15	0.53

-based matrices and the lowest for chitosan matrix. Release profiles are similar with the swelling ones. To explain this behaviour FT-IR spectroscopy is used. Appreciatively 96% amount of drug released characterizes the 75/25 NIP/Alg hydrogels.

Infrared spectroscopy was used to study the interactions between the drug and the polymers. The spectra of the studied systems are given in fig. 4.

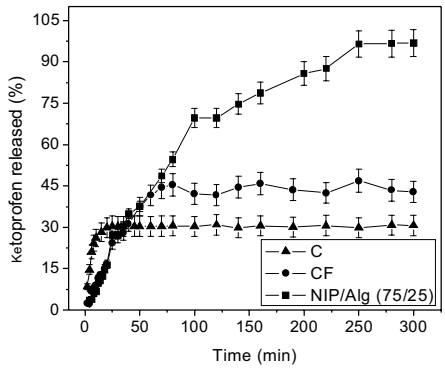

Fig. 3. The release profiles of ketoprofen in ethanol from studied hydrogels, at 25^{0}C

Ketoprofen has a carboxylic acid group, which can interact with the functional groups of the polymers. For ketoprofen, the cha-

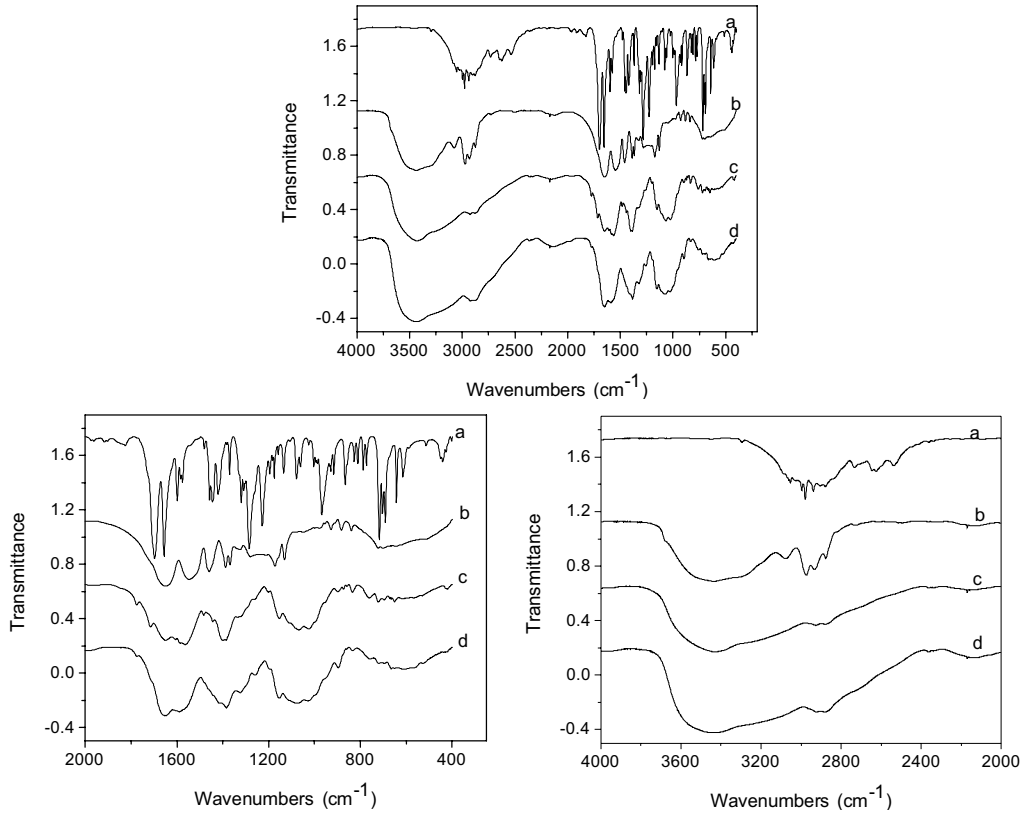

Fig. 4. FT-IR spectra in two representative spectral region of (a) ketoprofen, and studied matrices : (b) 75/25 NIP/Alg, (c) chitosan modified with phthalic anhydride, (d) chitosan, loaded with ketoprofen

TABLE II
The kinetic parameters of ketoprofen released from studied matrices

Hydrogels	Korsmeyer-Peppas equation			First order kinetic model	
	n_r	R	$k_r 10^3$ (min^{-n})	$k_r 10^3$ (min^{-1})	R
C	0.72	0.99	55.25	33.04	0.98
CF	0.83	0.99	14.14	8.16	0.99
75/25 NIP/Alg	1.18	0.99	4.43	7.52	0.98

racteristic bands appeared at 2876 cm^{-1} due to CH stretching of CH3 group masked by symmetric OH, 1697 cm^{-1} due to the C=O stretching vibration of the carboxylic group, a band observed at 1655 cm^{-1} represented by the carbonyl C==O stretching vibrations, and a wide band between 811 cm^{-1}-642 cm^{-1}, characteristic to C-H deformation of aromatic ring (fig. 4a).

In case of NIP/Alg (75/25) ketoprofen loaded hydrogels, the IR spectra showed characteristic peaks of ketoprofen at 2974 cm^{-1}-2876 cm^{-1}, 2169 cm^{-1}-2125 cm^{-1}, 1650 cm^{-1} regions and 839 cm^{-1}-1094 cm^{-1} band (fig. 4b).

For chitosan-phthalic anhydride ketoprofen loaded matrices, the IR spectra showed characteristic peaks of ketoprofen at 2978 cm^{-1}-2877 cm^{-1} band, 1384 cm^{-1}, 1651 cm^{-1}, and the region 834 cm^{-1}-615 cm^{-1}, respectively (fig. 4c).

The spectra of ketoprofen loaded hydrogels prepared with chitosan showed that the peaks at 1697 cm^{-1} and 1655 cm^{-1} were found at 1652 cm^{-1} and 1646 cm^{-1} respectively and the band 811 cm^{-1}- 642 cm^{-1} is replaced by 758 cm^{-1}-502 cm^{-1} bands, respectively (fig. 4d) which indicate major modifications in the structure after loading.

These bands correspond to amide groups indicating formation of a new compounds and possible drug-matrix interactions through covalent bonding and/or hydrogen bonding. This explains the slower release rate and smaller release amount of ketoprofen from the chitosan matrix.

Kinetic mechanism

The kinetic parameters for ketoprofen release in ethanol solution from tested hydrogels are presented in table II.

The values of the diffusion exponent (n_r) obtained for ketoprofen release from tested matrices indicates an anomalous transport mechanism for chitosan and chitosan modified with phthalic anhydride hydrogels, namely 0.72 and 0.83, respectively, which appeared by coupling Fickian diffusion with the relaxation of the hydrogel network. For 75/25 NIP/Alg hydrogel the n_r value is 1.18 sugesting a case II transport mechanism.

CONCLUSIONS

Potential applications of chitosan-based matrices and 75/25 NIP/Alg hydrogels in controlled drug delivery were tested. The composition of hydrogels had an important effect on ketoprofen release.

The swelling exponent values $n_{sw} < 0.5$ indicated a non-Fickian character of ethanol diffusion into the hydrogels.

The values of the diffusion exponent (n_r) obtained for ketoprofen release from chitosan-based matrices indicates an anomalous transport mechanism and a case II transport mechanism for 75/25 NIP/Alg.

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NOUTĂŢI

TERAPIE CU ^{131}I ÎN TUMORI MALIGNE NON-TIROIDIENE ?

Sistemul de transport Na/I (Na/I symporter, NIS) este o proteină existentă în mod normal în membrana celulelor tiroidiene, care mediază influxul de iod, atât stabil, utilizat, ulterior, fiziologic în sinteza hormonilor tiroidieni, cât şi radioactiv (^{131}I, ^{123}I, ^{124}I), permiţând vizualizarea scintigrafică tiroidiană şi mediind radioiodoterapia cancerului tiroidian. Captarea scintigrafică tiroidiană de $^{99m}TcO_4^-$ este, de asemenea, mediată de proteina NIS. Studii experimentale recente aduc în discuţie posibilitatea utilizării NIS pentru imagistica neinvazivă a expresiei genice şi, posibil, radioiodoterapia unor tumori maligne non-tiroidiene cărora li s-a indus genetic expresia membranară a acestei proteine (Baril P, Martin-Duque P, Vassaux G. Visualization of gene expression in the live subject using the Na/I symporter as a reporter gene : applications in biotherapy. *Br J Pharmacol* 2010 ; 159 (4) : 761-771) .

Cipriana Ştefănescu

ACTIVITATEA UNEI NOI FLUOROCHINOLONE IN CERCETARE, FINAFLOXACIN, ASUPRA UNOR IZOLATE DE *ACINETOBACTER BAUMANII*

Autorii studiului compară activitatea unei noi fluorochinolone, finafloxacin, cu cea a ciprofloxacinului, asupra unor izolate de *Acinetobacter baumanii*, în condiţii de pH diferite : 7,2 *versus* 5,8. Finafloxacinul a demonstrat o activitate superioară ciprofloxacinului, în condiţii de aciditate. Activitatea celor două flourochinolone a fost comparabilă, în condiţii de pH 7,2. Finafloxcin promite a fi un nou agent antimicrobian pentru tratamentul infecţiilor cu *Acinetobacter baumanii* în compartimentele acide ale organismului uman (Higgins PG, Stubbings W, Wisplinghoff H, Seifert H. Activity of the investigational fluoroquinolone Finafloxacin against Ciprofloxacin-sensitive and –resistant *Acinetobacter baumannii* isolates. *Antimicrob Agents Chemother* 2010 ; 54 (4) : 1613-1615).

Cristina Tuchiluş

STUDIU CLINICO-MICROBIOLOGIC PRIVIND PROPRIETĂŢILE ANTIBACTERIENE ALE UNOR MATERIALE DE RESTAURARE CU CARACTER BIOACTIV

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CLINICAL AND MICROBIOLOGICAL STUDY REGARDING SURFACE ANTIBACTERIAL PROPERTIES OF BIOACTIVE DENTAL MATERIALS (Abstract): In the new era of dentistry the coronal restoration materials must possess „bio-active" features represented by fluor ions release, chemical adhesion and antibacterial agents. **Aim**: Our study aims to determine the surface antibacterial properties of glassionomer cements and compomers. **Materials and method**: The study group included 64 patients with high cariogenic risk with 80 teeth with acute and chronic dental caries affecting proximal and occlusal dental surfaces. The teeth with cariogenic lesions were restored with zinc-oxide-eugenol (n=20), glassionomer cement GC Fuji Triage (n=20), glassionomer cement modified with resins Fuji II LC (n=20), compomer Dyract (n=20). DENTOCULT SM test (Orion Diagnostica, Finland) was used for bacterial analyses. The samples from bacterial biofilm were collected from the restorated dental surfaces (study group) and intact enamel surfaces (control group). The recorded data were processed using non-parametrical statistical tests. **Results**: The lowest mean value of bacterial indices was recorded for glassionomer cement Fuji Triage (0.4), and Fuji IILC (1.2), material with highest surface antibacterial properties. The highest value (1.5) was recorded for compomer Dyract. The Kruskal-Wallis test proves the significant statistical differences between the three bioactive materials. **Conclusion**: The materials with bioactive features have the ability to inhibate the growth of *Streptococcus mutans* in bacterial biofilm to the surfaces of coronal restoration. **Key words**: BIOACTIVE MATERIALS, SURFACE ANTIBACTERIAL PROPERTIES, DENTOCULT SM, GLASSIONOMER CEMENTS, COMPOMERS

ESTHETIC REHABILITATION THROUGH CAD/CAM TECHNOLOGY - CASE REPORT

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ESTHETIC REHABILITATION THROUGH CAD/CAM TECHNOLOGY-CASE REPORT (Abstract): With the progress of dental materials and techniques, CAD/CAM system currently allows the manufacture of high precision prosthetic restorations. Zirconia is an ideal base for porcelain because the bonding process between the zirconia and the porcelain is stronger than it is with porcelain and metal. This means that the porcelain is less likely to fracture away from a zirconia base. Zirconia can also be colored to match teeth and it is translucent, mimicking the natural look of teeth far better than any crown with a metal base could. The aim of this article is to describe a case of oral rehabilitation, involving CAD/ CAM technology for full dental crowns, using zirconia as framework. **Keywords:** DENTAL CROWNS, ZIRCONIA FRAMEWORK, CAD/CAM TECHNOLOGY.

The popularity of metal-ceramic restorations is largely due to its qualities- high mechanical resistance and reasonable esthetics. Among others, such restorations have some color disadvantages, the metal infrastructure being difficult to be completely masked by the opaque layer (1, 2). Since the esthetic expectations in dentistry are growing, research in the field of materials for all-ceramic restoration and dental implants was intensified (3). In the last years, zirconia based ceramics became an optimal alternative in the prosthetic rehabilitation due its mechanical and optical properties (4, 5, 6). With the progress of dental materials and techniques, Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) system currently allows the manufacture on fixed prosthesis, implants (7,8) and became a reality in dental practice. Zirconia framework is esthetically and biologically better accepted than the metal framework, and is very well tolerated by patients. It is a fact that systems based on zirconia present beyond the esthetic factors, high strength and radio-opacity, plaque accumulation reduction and low thermal conductivity (9, 10). The aim of this article is to describe a case of oral rehabilitation, involving CAD/CAM technology for dental full crowns, using zirconia as framework material.

CASE REPORT

A 38-year-old female patient came to the dental clinic with esthetic problems, determined by an inappropriate aspect of the metal-ceramic crown on the tooth 2.1. The prosthetic device was poorly cervical fitted and had an improper color appearance. After

clinical and Rx examination, we decided to replace the restoration with a metal-free full crown based on a zirconia framework. The old crown was removed and the patient was provisionally protected with a preformed acrylic temporary crown. The impression was registered, using the wash-technique, then, the temporary crown was cemented with Temp Bond. Based on the impression, the dental technician created two dental casts: one for scanning and the other for the final adaptation and tray-in of the prosthetic device. For the zirconia infrastructure realization, the Schütz Dental Tizian CAD-CAM system was used. The first step consists in a highly accuracy optical impression of the model, by scanning the plaster model inside the Tizian Smart-Scan chamber (fig. 1).

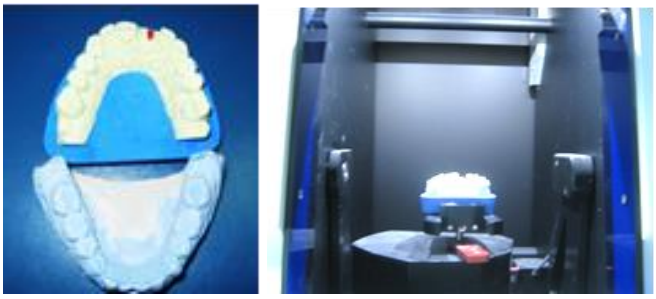

Fig. 1. Scanning the model in the Tizian Smart-Scan chamber

The results of the scanning process were viewed on the monitor, in profile and in section, to check the accuracy of each detail. After scanning, the information was stored in the database, in order to create a virtual model (fig. 2). For designing the framework through software, the opposite arch and occlusal relationship should also be scanned, allowing the mounting of the 3D models in the program.

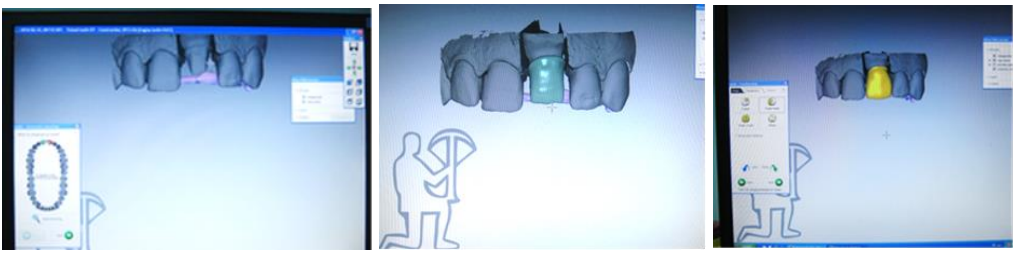

Fig. 2 Building the virtual prosthetic restoration

The design of prosthetic piece was obtained virtually (the CAD phase) and sent to the milling machine, in order to manufacture the framework by milling the ceramic blocks (CAM phase). The IPS e.Max system used was zirconia infiltrated with lithium-disilicate glass ceramic. The framework includes esthetics, mechanical performance, economic feasibility and utilization. The zirconia framework must be veneered with another ceramic system, the Tizian ceramic (Schutz Dental) being used because it offers a very dense surface, the hardness values are very similar to those of natural enamel, is very gentle on the antagonists and is color-stable during time (fig. 3).

Fig. 3 Milling the zirconia framework

The external polished surfaces were first cleaned in deionized water, and subsequently by ultrasound in butanone and ethanol. The final try-in of the restauration was realized on the model and in the oral cavity, as well (fig. 4). The final setting of the crown was done with self-curing resin cement for zirconia, RelyX™ Unicem (3M Espe Dental).

Fig. 4 The dental crown control

DISCUSSION

The current popularity of metal-ceramic restorations declined as a result of certain disadvantages: structural weakness, risk of errors due to the complexity of the fabrication procedure, esthetic inconveniences. The emergence of new materials and technologies open the way to achieving dental restorations with longevity over time and well accepted by patients. All-ceramic crowns based on zirconia frameworks have advantages of their mechanical and optical properties; in addition, zirconia crowns are manufactured using CAD/CAM technology, 10, 11), which allows shortening of working time, after the elimination of many laboratory steps. Zirconia is an ideal base for porcelain because the bonding process between the zirconia and the porcelain is stronger than it is with porcelain and metal. This means that the porcelain is less likely to fracture away from a zirconia base. Zirconia can also be colored to match teeth and it is translucent, mimicking the natural look of teeth far better than any crown with a metal base could. The metal free crown is long lasting, easier to wear and more durable than other crowns (12, 13).

CONCLUSIONS

Our clinical experience demonstrates that metal ceramic prosthesis is not always very well accepted by the patients because of some color disadvantages. Zirconia is a modern possibility to replace the metal frame in porcelain crowns and bridges and fulfill the purpose of restoring function and simultaneously ensure fundamental esthetics requirements on oral rehabilitation.

The CAD-CAM technology allowed our

prosthesis realization in a very short time, as result of eliminating a lot of laboratory steps, because zirconium dental restorations are made by cutting a solid block of zirconia with very high accuracy. The clinical exam demonstrated very good esthetic and functional results and after six months there are no biological or mechanical complications. Because of the absence of internal metal structure, light passes through similar to the natural tooth and it is reflected in the same way, which is very important, especially for frontal restorations. Being a biocompatible material, it is very well accepted by the body and there is no risk of allergic reaction and because is thermo-insulating, the temperature variations are not transmitted to the dental pulp, thereby decreasing the risk of a possible future pulp inflammation.

So, we concluded that zirconia crown technology is an exceptional therapeutic solution, aesthetical and mechanically speaking. Also, in the future, zirconium will be a convenient solution for the dental labs because the technology is fast and accurate.

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EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME IMINES DERIVATIVES OF L-ARGININE

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EVALUATION OF THE ANTIOXIDANT ACTIVITY OF SOME IMINES DERIVATIVES OF L-ARGININE (Abstract): L-Arginine is an α-amino acid which plays important roles in different diseases or processes, such as Alzheimer disease, inflammatory process, healing and tissue regeneration and it also could be useful as an anti-atherosclerotic agent. **Aim**: Considering the large amount of studies on the beneficial effects of different antioxidants, this paper is focused on the evaluation of the antioxidant potential of some imine derivatives, synthesized by the authors and described in a previous article. **Material and Methods**: The evaluation of the antioxidant power was performed using phosphomolydenum-reducing antioxidant power (PRAP) and ferric reducing antioxidant power (FRAP) assays, tests described in the literature and which are used with some minor modifications. **Results**: It was found that most of the imine derivatives are more active than the L-Arginine in the PPAP and FRAP assays. The most active derivative was the compound obtained by condensation of L-arginine with 2,3-dihydroxybenzaldehyde (2k) and 2-nitrobenzaldehyde (2g). **Conclusions**: Following the described protocol, some imine derivatives of L-arginine were evaluated in terms of antioxidant potential using *in vitro* methods. The most favorable influence was obtained by the aromatic substitution with *nitro* and *hydroxyl*, the corresponding derivatives being the most active derivatives compared to L-arginine. **Keywords**: L-ARGININE, IMINE, ANTIOXIDANT ACTIVITY.

L-Arginine is one of the most metabolically versatile amino acids. It is classified as a semi-essential or conditionally essential amino acid, depending on the stage of development and on the individual state of health (1). L-Arginine serves as a precursor for the synthesis of polyamines, proline, glutamate, creatine, agmatine and urea (2). It also serves as a precursor for the synthesis of proteins and is a substrate for different enzymes. For example, the nitric oxide synthase (NOS) converts arginine to nitric oxide (NO) and citrulline. NO is a signal molecule involved in many processes such as: vasodilatation, angiogenesis (3, 4), immune responses, epithelialization and formation of granulation tissue and inhibition of platelet activation/aggregation (5, 6). L-Arginine is also reported to act as an free radical scavenger, because it inhibits the activity of pro-oxidant enzymes, thus acting as an antioxidant, all these effects being mediated by NO

(7, 8). The imine derivatives are cited the in literature for their wide range of biological activities such as: antifungal, antibacterial, antimalarial (9), ant proliferative (10), anti-inflammatory (11), antiviral and antipyretic (12) and antioxidant (13) effects. The increasing interest in imines is due to their easy synthesis and wide applicability, antioxidant effects included.

Based on these considerations, new compounds derived from L-arginine were synthetized (14) and evaluated for their antioxidant properties.

MATERIAL AND METHODS

The organic solvents (*p.a.* quality) and the standard reagents used for the antioxidant tests were purchased from Sigma Aldrich Company and Fluka Company. All solvents and reagents were used without prior purification.

Chemistry

The imine derivative synthesis. The synthesis of the imine derivatives consisted in the condensation of L-Arginine in methanol with various aromatic aldehydes (4-chloro/4-fluoro/4-bromo/2-nitro/3-nitro/4-nitro/2-hydroxy/3-hydroxy/2-methoxy/2,3-dihydroxy/2,4dihydroxy/ 2,4, 6-trihydroxy-benzaldehyde) which led to the corresponding derivatives (2a-m) (Fig. 1).

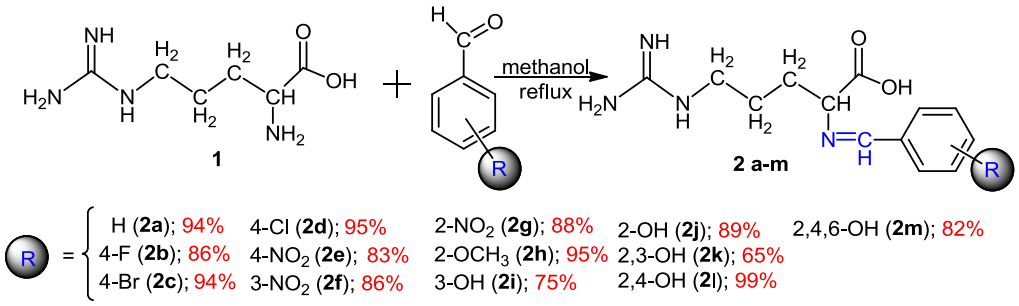

Fig. 1. Synthesis of L-arginine derivatives

The synthesis and spectral characterization of the synthesized compounds were reported in our previous article (14).

Antioxidant assays

Phosphomolydenum-reducing antioxidant power (PRAP) assay. The total antioxidant capacity of the synthesized compounds was evaluated using phosphomolybdenum method (15) with some minor modifications. The method is based on the formation of phosphomolybdenum blue complex by the reduction of Mo^{6+} to Mo^{5+} under the action of electron donating compounds. The maximum absorption of the complex was recorded at 695 nm and the reducing antioxidant effectiveness is correlated with high absorbance values.

To obtain a stock solution with the concentration of 5 mg/mL, the imine derivatives were dissolved in DMSO (dimethyl sulfoxide), and L-arginine in double distilled water. From the stock solution different volumes were taken (10 µL, 20 µL, 40 µL, 60 µL) and then completed to by adding DMSO up to 200 µL. To the mixture 2000 µL of reagent (28 mM phosphate, 4 mM ammonium heptamolybdate,

0.6 M sulfuric acid) was added. After preliminary stirring, the solutions were incubated at 95 ^{0}C for 90 min, cooled in cold water and centrifuged at 4600 rpm for 15 min. The supernatant was separated and the absorbance was read at 695 nm against a blank consisting of 2000 µL reagent and 200 µL DMSO. For each compound the effective concentration 50 (EC_{50}) was calculated by linear regression which is the sample concentration at which 50% of the substrate is reduced. Ascorbic acid was used as a positive control and L-arginine, prepared in a similar manner as the samples, as a reference. All determinations were performed in triplicate and the results were expressed as arithmetic mean ± standard deviation (SD).

Ferric reducing antioxidant power (FRAP) assay. The reducing power of the synthesized compounds was measured by the method described in the literature (16, 17) with some modifications. The method is based on the reduction of ferricyanide to ferrocyanide, which is quantified as Prussian Blue, complex which has a maximum absorption band at 700 nm.

To obtain a stock solution with the concentration of 10 mg/mL, the imine derivatives were dissolved in DMSO (dimethyl sulfoxide), and L-arginine in double distilled water. From the stock solutions serial dilution were made in order to obtain the following concentrations: 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL. To 0.5 mL of sample with the above mentioned concentrations, 0.5 mL of 0.2 M phosphate buffer at pH 6.6 was added. The reaction was then initiated by the addition of 0.5 mL of potassium ferricyanide 1% w/v, after which the samples were incubated at 50°C (oven) for 20 min and the reaction was stopped by the addition of 05. mL of 10% (w/v) trichloroacetic acid. After vigorous shaking the samples were centrifuged at 4500 rpm for 15 min. To 1 mL of the supernatant 1 mL of doubly distilled water and 0.2 mL of 0.1% ferric chloride were added. After 5 min the absorbance was read at 700 nm against a blank (the reaction mixture without the sample).

A high absorbance indicates a high reducing power. For each compound EC_{50} was calculated by linear regression, which is the sample concentration at which 50% of the substrate is reduced. Ascorbic acid was used as a positive control and L-arginine, prepared in a similar manner as the samples, as a reference. All determinations were performed in triplicate and the results were expressed as arithmetic mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phosphomolydenum-reducing antioxidant power (PRAP) assay. The total antioxidant capacity of the imine derivatives of L-arginine (2a-m) was evaluated at four different concentrations: 0.0272 mg/mL, 0.045 mg/mL, 0.090 mg/mL and 0.135 mg/mL. The absorbance values of the phosphomolydenum complex recorded at 695 nm are plotted in Fig. 2. It is noted that the ability of the tested compounds to reduce Mo^{+6} to Mo^{+5} increases with the concentration. The highest increase in absorbance from the concentration of 0.0272 mg/mL to the concentration of 0.135 mg/mL was recorded for the compounds 2e (R = 4-NO_2 (from 0.023 ± 0.013 to 0.322 ± 0.021), 2g (R = 2-NO_2, from 0.109 ± 0.001 to 0.571 ± 0.002) and 2k (R = 2,3-OH, from 0.333 ± 0.006 to 1.233 ± 0.019), the increase was 4 to10 times. For the tested compounds there are not reference data in the literature.

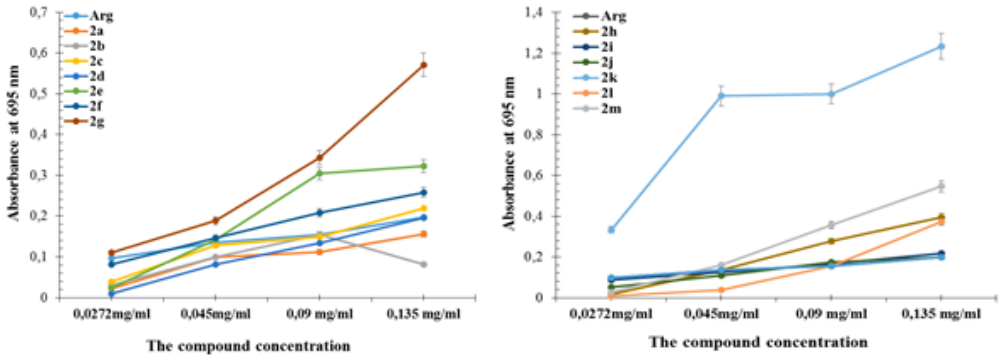

Fig. 2. Absorbance of the imine derivatives (**2a-m**) recorded at 695 nm

The analysis of EC_{50} values (Table I) showed that the most active compounds are 2k (R=2,3-OH, EC_{50}=0.033±0.001) and 2g (R=2-NO_2, EC_{50}=0.087±0.004), for which the total antioxidant capacity is about 38 times and respectively 15 times greater than L-arginine (EC_{50}=1.271±0.042). The compounds 2m (R=2,4,6-OH, EC_{50}=0.196± 0.063), 2f (R=3-NO_2, EC_{50}=0.228±0.008) and 2e (R=4-NO_2, EC_{50}=0.265±0.009) also showed an appreciable total antioxidant activity, being 6.5 times (2m), 5.5 times (2f) and 4.8 times (2e) more active than L-arginine.

TABLE I

EC_{50} values (mg/mL) of the phosphomolybdenum reducing antioxidant power of derivatives 2a-m

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
L-Arg	1.271 ± 0.042	2e	0.265 ± 0.009	2j	0.962 ± 0.023
2a	1.383 ± 0.063	2f	0.228 ± 0.008	2k	0.033 ± 0.001
2b	1.454 ± 0.069	2g	0.087 ± 0.004	2l	1.321 ± 0.045
2c	0.916 ± 0.033	2h	0.814 ± 0.046	2m	0.196 ± 0.063
2d	1.420 ± 0.094	2i	0.975 ± 0.026	ascorbic acid	0.006 ± 0.001

* EC_{50} values are the average of three determinations ± standard deviation

Ferric reducing antioxidant power (FRAP) assay. The absorbance values of Prussian Blue complex formed in the presence of imine derivatives at different concentrations (0.189 mg/mL, 0.374 mg/mL, 0.757 mg/mL, 1.515 mg/mL) and recorded at 700 nm are plotted in Fig. 3.

From the analysis of the results it can be noticed that the absorbance of the samples (**2a-m**) and consequently the reducing power increases with the increase in concentration. Except for compound 2a, all tested derivatives are more active than L-arginine, at all concentrations. This highlights the favorable influence of the aromatic ring substitution on the antioxidant activity. The most active compounds were 2e (R = 4-NO_2), 2g (R = 2-NO_2) 2k (R = 2,3-OH) and 2m (R = 2,4,6-OH), for which the absorbance value at 1.515 mg/mL was

approximately 11 times (2e), 14 times (2g), 15 times (2k) and 10 times (2m) higher than that of L-arginine, determined under similar experimental conditions.

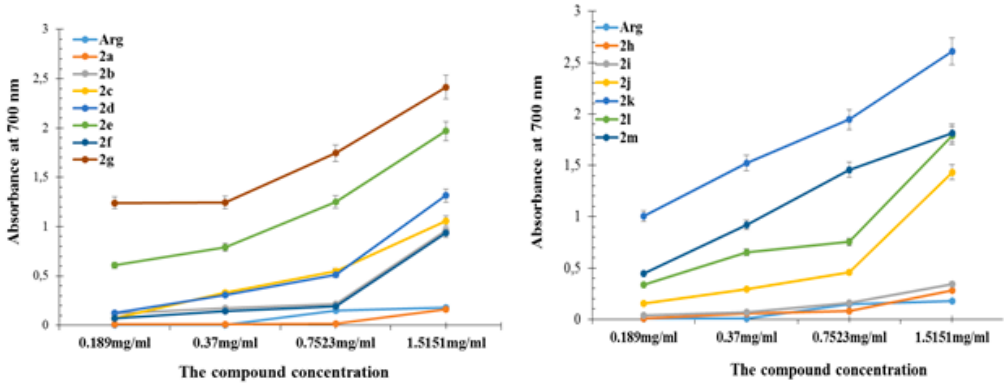

Fig. 3. Absorbance of the imine derivatives (**2a-m**) recorded at 700 nm

The EC_{50}values calculated based on the absorbance values recorded at 700 nm are presented in Table II; low EC_{50}values indicate a high reducing power. The most active compound was found to be compound 2g (R=2-NO_2, EC_{50} = 0.044 ± 0.001) which is about 58 times more active than L-arginine (EC_{50} =2.571±0.024) and 70 times more active than the unsubstituted derivative 2a (EC_{50}=3.08±0.016). Compound 2k (R=2,3-OH, EC_{50}=0.067± 0.001) also had a significant reducing power, being about 38 times more active than L-arginine. Also, compounds 2e (R=4-NO_2, EC_{50}=0.174±0.023) 2m (R=2,4,6-OH, EC_{50}=0.238 ± 0.006) and 2l (R=2,4-OH, EC_{50} =0.372 ± 0.015) were found to be about 15 times, 11 times and 7 times, respectively more active than L-arginine. For the tested compounds there are not reference data in the literature.

TABLE II

Reducing power (EC_{50}, mg/mL) of derivatives 2a-m in comparison with ascorbic acid and the parent compound

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
L-Arg	1.471 ± 0.024	2e	0.121 ± 0.023	2j	0.974 ± 0.002
2a	0.732 ± 0.016	2f	0.145 ± 0.005	2k	0.112 ± 0.038
2b	0.805 ± 0.031	2g	0.086 ± 0.001	2l	0.091 ± 0.001
2c	0.564 ± 0.023	2h	1.157 ± 0.084	2m	0.859 ± 0.006
2d	1.099 ± 0.009	2i	1.238 ± 0.022	ascorbic acid	0.006 ± 0.001

* EC_{50} values are the average of three determinations ± standard deviation

CONCLUSIONS

In this study the authors assessed the antioxidant potential of 13 imine derivatives of L-arginine using *in vitro* methods: phosphomolydenum-reducing antioxidant power (PRAP) and ferric reducing antioxidant power (FRAP) assays. Most of the studied compounds showed an improved antioxidant

activity compared to L-arginine, fact that proves a favorable influence of structural modulation on the antioxidant effects of L-arginine. For both methods used for the evaluation of the antioxidant potential it was shown that the aromatic substitution with *nitro* and *hydroxyl* has the most favorable impact on the antioxidant activity.

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MULTIDISCIPLINARY APPROACH TO PALLIATIVE CARE WITHIN THE REGIONAL INSTITUTE OF ONCOLOGY IAŞI – PHYSICIANS' PERCEPTION

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MULTIDISCIPLINARY APPROACH TO PALLIATIVE CARE (Abstract). **Aim:** The purpose is to present an analysis regarding the information, training, need and barriers arising in the provision of palliative care among physicians of different specializations within the Regional Institute of Oncology Iaşi. **Materials and methods:** The data was collected through the questionnaire method, which was filled in by a number of 38 physicians. **Results:** All the staff surveyed was confronted in their professional activity with patients who required palliative care. Only 21.1% of the staff surveyed had training in palliative care certified through courses, internships or volunteering. 39.5% of the physicians surveyed believed that their patients did not benefit from all the palliative care they would have required. All the staff surveyed (with one exception - 2.6%), considered that additional training in palliative care would bring benefits in terms of quality of life of the patients who require palliative care. All the medical staff in oncology/radiology and 95.7% in the other sections considered training in the field of palliative care to be necessary as regards the professional curriculum ($p = 0.827$). **Conclusions:** The respondents stated that better training in the field of palliative care could contribute to enhancing the quality of life of their patients. The physicians surveyed shared the need to follow training courses in palliative care, claiming at the same time that a training module with a duration of approximately 3 months within residency would be beneficial. **Keywords:** PALLIATIVE CARE, TRAINING, DEVELOPMENT, MULTIDISCIPLINARY.

In Romania, palliative care (PC) is currently undergoing a dynamic development and the number of specialized services (mostly bed units) is increasing (1). At the same time, there is a need not only to cover the norms for the staff in the field, but especially to ensure specialized human resources (2) in order to provide quality palliative care services.

According to a sociological study conducted at national level (3, 4), 20.7% of the population has been or is currently confronted with having to provide care for an incurable patient with an advanced or terminal illness, of whom only 1.2% benefited from specialized care services. It is a low percentage which demonstrates the insufficient level of specialized services covered

in terms of the need of palliative care (5).

The education and training of the medical staff in palliative care therefore becomes extremely important. In Romania, the most complex form of specialization in palliative care is currently the achievement of the certification in complementary studies (lasting approximately 2 years), however, there are also shorter courses (both for physicians and nurses), which have a leading role in the initiation in the field or in deepening specific themes.

Palliative care requires a coordinated team approach. No one individual and no one discipline can address the complexity of issues that arise throughout the provision of palliative care (6). Although care is generally provided by a nucleus-team consisting of physician, nurses, psychologist and social workers (7), most often a broader contribution of professionals in the medical field is necessary, in order to provide care for other related specialties as well.

The palliative care in patients with cancer is an important part of the field, at the level of Iaşi county palliative care being provided both in public and private hospital units.

MATERIALS AND METHODS

The purpose of this paper is to present an analysis regarding the information, training, need and barriers arising in the provision of palliative care among physicians of different specializations within the Regional Institute of Oncology Iaşi. The data was collected through the questionnaire method, which was filled in by a number of 38 physicians, and the results were processed by means of *SPSS 16.0* program.

The criteria of inclusion in the study were represented by: the professional degree of physicians regardless of specialty and the informed consent of the participants. The criteria of exclusion from the study were represented by: other professional degrees apart from that of physician and the refusal to process the data after filling in the questionnaire.

RESULTS AND DISCUSSION

The results show that most of the physicians participating in the survey specialized in surgery (31.6%), followed by those from the specialties of medical oncology (28.9%) and hematology (26.3%). A percentage of 10.5% is represented by the physicians specialized in radiotherapy and 2.6% belonging to other specialties (tab. I).

TABLE I
Medical specialties

Specialty	n	%
Medical oncology	11	28.9%
Radiotherapy	4	10.5%
Hematology	10	26.3%
Surgery	12	31.6%
Other (gynecology)	1	2.6%

The distribution on professional titles shows that most of the surveyed physicians are primary care physicians and medical specialists, representing a percentage of 57.8% compared to resident physicians, who account for 42.2% (tab. II).

TABLE II
Professional titles

Professional title	n	%
Primary health care physicians	11	28.9%
Medical specialists	11	28.9%
Resident physicians	16	42.2%

The majority of the surveyed physicians (36.8%) stated a work experience between 1

and 3 years in the provision of care for patients with cancer. A significant percentage (28.9%) declared a seniority of more than 10 years in the provision of care for patients with cancer, and a low percentage (7.9%) less than 1 year experience (tab. III).

TABLE III
Work experience in the provision of care for patients with cancer

Work experience	n	%
< 1 year	3	7.9%
1-3 years	14	36.8%
3-5 years	6	15.8%
5-10 years	4	10.5%
> 10 years	11	28.9%

The results showed that all the staff surveyed was confronted in their professional activity with patients who required palliative care (fig. 1). In the cases studied, the physicians surveyed were most frequently (78.9%) confronted with fewer than 10 cases of palliative care per month, patients who needed hospitalization or ambulatory consultation (fig. 2).

The data analysis revealed that only 21.1% of the staff surveyed had training in palliative care certified through courses, internships or volunteering (fig. 3).

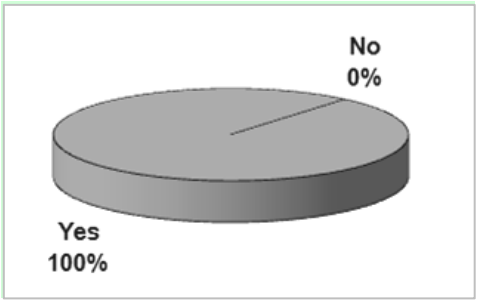

Fig. 1. Confrontation with need of palliative care

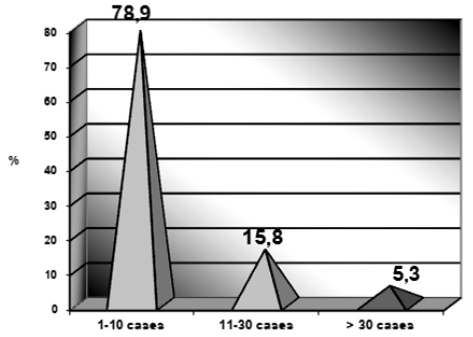

Fig. 2. Number of cases/month who needed palliative care

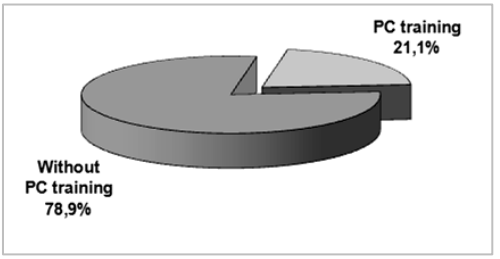

Fig. 3. Training in palliative care

A percentage of 34.2% of the participants considered that the patients whom they treated in their capacity of attending physicians received all the palliative care they required, while 26.3% believed that further knowledge or other material resources would have been necessary in order to provide their patients with full palliative care. 39.5% of the physicians surveyed believed that their patients did not benefit from all the palliative care they would have required, but could not say whether this was due to the absence of knowledge in the field or to some specific material resources (fig. 4).

All the staff surveyed (with one exception - 2.6%), considered that additional training in palliative care would bring benefits in terms of quality of life of the patients who require palliative care (fig. 5).

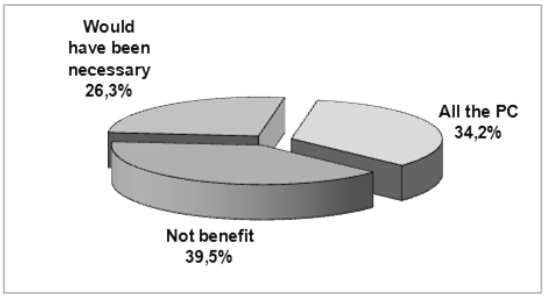

Fig. 4. Benefiting of palliative care

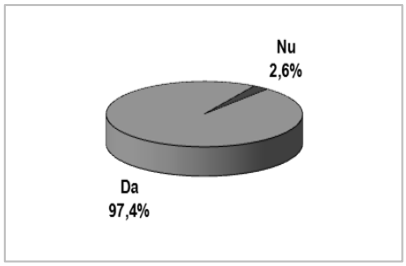

Fig. 5. The necessity of additional training in palliative care

The main barriers to providing palliative care for the patients, according to the respondents, were represented by: insufficient time allotted to the patient, due to the large number of patients that require care/consultation (65.8%); insufficient communication skills in interacting with patients or their family (52.6%); insufficient knowledge regarding pain control (47.4%); lack of guidelines/protocols of palliative care (47.4%); administrative issues (47.4%) (fig. 6).

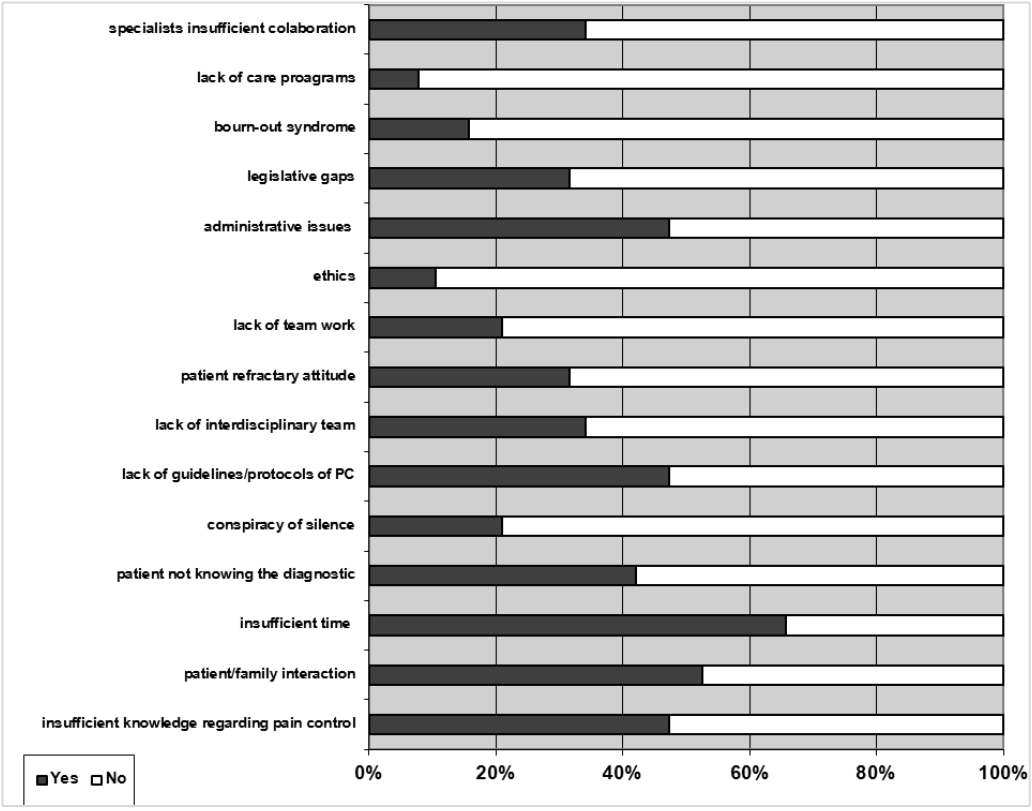

Fig. 6. Barriers to providing palliative care

Due to the fact that the physicians in medical oncology and radiotherapy specialties work with a large number of cancer cases, the 38 physicians surveyed were divided into two groups, as follows: an oncology/radiotherapy group and a group comprising all other specialties. The comparative responses of the people specialized in oncology / radiology *vs.* other specialties revealed that the distribution of respondents according to professional title was uniform (p=0.676), in the oncology / radiology services predominating physicians with seniority under 3 years in cancer patient care (p = 0.041), which is due to the higher number of medical residents and young specialists. Moreover, more than 10 patients per month requiring palliative care/ambulatory consultation was noted in 40% of the physicians in the oncology/radiology group, while the ratio in the other services was of 8.7% (p = 0.049). Another finding showed that a percentage of 46.7% of the physicians in oncology/radiology services had training in the field of palliative care, while the lack of such training was detected in the other specialties (p = 0.007).

In oncology/radiology services, 46.7% of the physicians considered that they provided all the palliative care that the patients required, and 40% claimed that other resources (material or knowledge) would have been necessary in order to provide all the palliative care necessary to their patients. In contrast, as regards the other services, 56.5% of the respondents said that they did not provide this type of care (p = 0.028). The comparative analysis of the results of the two groups indicate that all the medical staff in oncology / radiology sections and 91.3% in the other sections considered training in the field of palliative care as being necessary in order to ensure good services (p = 0.667) and additional training in the field, according to the respondents, would bring an improvement in the quality of life of the patients (p = 0.827) (tab. IV).

As regards the main barriers to providing palliative care, approximately 47% of the staff who took part in the survey said that they have insufficient knowledge about pain control (p = 0.793) and 60% of the physicians in oncology / radiology sections and 47.8% from the other sections considered as the main barrier in the field of palliative care the reduced communication skills in interacting with the patient or his/her family (p = 0.687). In the oncology/radiology services, 86.7% of the physicians considered that they have insufficient time to provide all the care their palliative patients would require, while from the other services only 52.2% of the respondents said that they do not have enough time for palliative consultations (p = 0.031).

Great differences were noted in the percentage between physicians in oncology/radiology and the other services (73.3% *vs.* 21.7%) as regards the opinion on the patient's ignorance of his/her diagnosis as a barrier to providing palliative care (p = 0.005). No significant differences in percentage were found between the physicians' appreciation of hiding the diagnosis from the patient in providing palliative care as a barrier, although 1/3 of oncologists/ radiologists and only 13% of those belonging to other specialties considered the "conspiracy of silence" to be such a barrier. (p = 0.275). The lack of guidelines and protocols on palliative care was specified by 56.5% of the physicians in the group of other specialties and 33.3% of those in oncology/radiology, the percentage differences being statistically insignificant (p = 0.286).

TABLE IV

Statistical analysis of studied groups

Parameters	Oncology/ radiology group (n=15)	Other specializations group (n=23)	p values of Chi-square
Professional title			
Primary care physicians	5 (33.3%)	6 (26.1%)	0.676
Medical specialists	5 (33.3%)	6 (26.1%)	
Resident physicians	5 (33.3%)	11 (47.8%)	
Work experience in PC			
< 1 year	-	3 (13.0%)	**0.041**
1-3 years	9 (60.0%)	5 (21.7%)	
3-5 years	-	6 (26.1%)	
5-10 years	1 (6.7%)	3 (13.0%)	
> 10 years	5 (33.3%)	6 (26.1%)	
Number of PC cases/month			
None	-	-	**0.049**
1-10 cases	9 (60.0%)	21 (91.3%)	
11-30 cases	4 (26.7%)	2 (8.7%)	
> 30 cases	2 (13.3%)	-	
Training in PC	7 (46.7%)	1 (4.3%)	**0.007**
Benefiting of PC			
All the PC	7 (46.7%)	6 (26.1%)	**0.028**
Would have been necessary	6 (40.0%)	4 (17.4%)	
Not benefit	2 (13.3%)	13 (56.5%)	
Additional training in PC	15 (100%)	21 (91.3%)	0.667
Improvement in the quality of life of the PC patients	15 (100%)	22 (95.7%)	0.827

The frequency of physicians in oncology/radiology who remarked upon the lack of an interdisciplinary team for a holistic approach to address the cases was slightly higher compared to the other specialties (46.7% *vs.* 26.1%) (p = 0.338). It was noted that a percentage of 60% of the physicians in oncology/radiology declared that they are faced with a reluctant attitude of the patient or his/her family in the confrontation with the oncological disease, an aspect which was only found in 13% (p = 0.007) of the cases in the other specialties. The lack of knowledge or the lack thereof in relation to teamwork is considered to be a barrier to providing palliative care by 20-22% of the physicians surveyed (p = 0.781).

In the cases studied, approximately 47% of the physicians in both study groups considered administrative issues related to space, equipment, medication, etc., as barriers to ensuring palliative care (p = 0.793). The legislative gaps or poor legislation in the field of palliative care was remarked upon by 40% of the physicians in the group of oncology / radiology sections and by 26.1% of those from other specializations, the differences in percentage being statisti-

cally insignificant (p = 0.586). The burnout syndrome was mentioned only by 40% of the physicians in oncology/radiology (p = 0.004). Another aspect that resulted from the data studied showed that the lack of programs / initiatives dedicated to palliative care, in the cases studied, was mentioned rarely, with a slightly higher frequency in the group of physicians in the oncology / radiology sections (13.3% *vs.* 4.3%) (p = 0.698). Approximately 1/3 of the subjects in both study groups showed lack of and insufficient collaboration with other professionals in the hospital (p = 0.797) (tab. V).

TABLE V

Barriers to the provision of palliative care

Parameters	Oncology/ radiology group (n=15)	Other specializations group (n=23)	p values of Chi-square
Insufficient knowledge on pain control	7 (46.7%)	11 (47.8%)	0.793
Interaction patient / family	9 (60.0%)	11 (47.8%)	0.687
Insufficient time	13 (86.7%)	12 (52.2%)	**0.031**
Patient's ignorance of diagnostic	13 (73.3%)	5 (21.7%)	**0.005**
"Conspiracy of silence"	5 (33.3%)	3 (13.0%)	0.275
Lack of guidelines / IP protocol	5 (33.3%)	13 (56.5%)	0.286
Lack of an interdisciplinary team	7 (46.7%)	6 (26.1%)	0.338
Refractory patient attitude	9 (60.0%)	3 (13.0%)	**0.007**
Lack of teamwork	3 (20.0%)	5 (21.7%)	0.781
Ignorance of ethical issues	2 (13.3%)	2 (8.7%)	0.932
Administrative issues	7 (46.7%)	11 (47.8%)	0.793
Legislative gaps	6 (40.0%)	6 (26.1%)	0.586
Burnout syndrome	6 (40.0%)	-	**0.004**
Lack of programs providing care	2 (13.3%)	1 (4.3%)	0.698
Insufficient collaboration between specialists	5 (33.3%)	8 (34.8%)	0.797

As regards the usefulness of completing training in the field of palliative care, approximately 47% of the participants considered additional training to be useful for the development of communication skills with the patient or his/her family (p = 0.793), while the completion of professional training was considered useful for the development of teamwork according to 73.3% of the oncologists/radiologists and 87% of the physicians from the other specialties; however, the differences in percentage are statistically insignificant (p = 0.528). Approximately 1/2 of the subjects in both study groups considered such training to be useful in deepening their knowledge of medical ethics (p = 0.793). Moreover, approximately 1/3 of the subjects in both study groups believed that the therapy and control of other symptoms could be improved by completing professional training (p = 0.866). With one exception (an oncologist) the subjects wished to attend palliative care courses in the coming months (p = 0.827), while

13.3% of the physicians in the oncology/radiology group requested an advanced level of these courses.

It should be noted that all the medical staff in oncology/radiology and 95.7% in the other sections considered training in the field of palliative care to be necessary as regards the professional curriculum (p = 0.827), the duration of courses ranging between the study groups: 80% of the group of oncologists/radiologists preferred a duration of 3 months for the course, while the physicians in the other specialties mostly (60.9%) preferred a duration of 1 month (p = 0.007).

According to all the subjects surveyed, the extension of palliative care services was regarded as useful (tab. VI).

TABLE VI

The usefulness of completing training in the field of palliative care

Parameters	Oncology / radiology group (n=15)	Other specializations group (n=23)	P values of Chi-square
The usefulness of completing training in the field of PC			
development of communication skills	7 (46.7%)	11 (47.8%)	0.793
teamwork	11 (73.3%)	20 (87.0%)	0.528
medical ethics	8 (53.3%)	12 (52.2%)	0.793
therapy/ control of symptoms	5 (33.3%)	7 (30.4%)	0.866
The wish to attend PC courses	14 (93.3%)	23 (100%)	0.827
The utility to complete professional curriculum	15 (100%)	22 (95.7%)	0.827
The duration of courses 1 month 3 months 6 months	 2 (13.3%) 12 (80.0%) 1 (6.7%)	 14 (60.9%) 8 (34.4%) -	**0.007**
The utility of extension of PC services	15 (100%)	23 (100%)	**-**

CONCLUSIONS

In our study results showed that all the respondents have been confronted in their professional activity with cases of palliative care, which, in a hospital specialized in oncology, have an increased frequency. Throughout their professional activity, the respondents stated that they have provided palliative care to their patients, in their capacity of attending physicians, regardless of their level of training in the field. Moreover, the respondents admitted that their patients could often have benefited from a better palliative care, for which both deeper knowledge in the field, as well as additional material resources, would have been necessary.

The respondents stated that better training in the field of palliative care could contribute to enhancing the quality of life of their patients. For this purpose, the physicians surveyed shared the need to follow training courses in palliative care, claiming at the same time that a training module with duration of approximately 3 months within residency would be beneficial.

Finally, all the respondents considered the development and the expansion respectively of palliative care services to be useful.

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NEWS

PERICYTES IN HEALTH AND DISEASE

Pericytes also known as perivascular cells are contractile cells located on the external side of the endothelium in micro vessels. They communicate with endothelial cells via direct physical contact or through paracrine signaling pathways. These interactions are important for blood vessel maturation, remodelling, and maintenance. Pericytes are also known as Rouget cells after their discoverer, Charles Rouget, but the more suitable term pericyte (peri; around, cyte; cell) was coined by Zimmerman in the early 1920s, as the cells were found to wrap themselves around and along the vessel. They share many characteristics with smooth muscle cells that surround larger blood vessels as well as fibroblasts and myofibroblasts. Pericytes are of mesenchymal origin and have been shown to have mesenchymal stem cell properties capable of differentiating into other types of mesenchymal cells, including fibroblasts, osteoblasts, chondrocytes, and adipocytes. This article covers the areas of origin, structure, pericyte's interaction with endothelial cell, markers, functions; and role of pericytes in health and disease. It provides the current state of knowledge and thoughts for future investigations in these areas. Of interest is pericyte differentiation to other types of mesenchymal cells. Damage to a tissue initiates proliferation of fibroblasts, activation of fibroblasts to myofibroblasts, and the formation of new blood vessels. Endothelial cells and pericytes are interdependent, so failure of proper communication between the two cells can lead to numerous human pathologies. With time, a number of other functions have been attributed to pericytes, and it seems like we are now only starting to understand the complexity of this versatile, controversial and highly intriguing cell type (Munde PB, Khandekar SP, Dive AM et al. Pericytes in Health and Disease. International Journal of Oral & Maxillofacial Pathology. 2014; 5 (1): 02-07).

Doina Butcovan

DENTAL STUDENTS' PSYCHOLOGICAL WELL-BEING DURING EXAMINATION PERIOD AND HOLIDAY

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DENTAL STUDENTS' PSYCHOLOGICAL WELL-BEING DURING EXAMINATION PERIOD AND HOLIDAY (Abstract). **Introduction**: Psychological well-being is recognized as an important health component, which influences the behavior, ability to cope with stressful events, work performance, and generally the ability to achieve one's full potential. **Aim:** To comparatively assess the psychological well-being of dental students during the summer semester examination period and summer holiday. **Material and methods:** A single-arm, prospective study was conducted in second year dental students from the Faculty of Dental Medicine, Bucharest. The psychological well-being was assessed using the WHO-Five Well-being Index. **Results:** Students' psychological well-being was statistically significantly better during the summer holiday (median=19) than during the summer semester examination period (median=11.5), Z=3.69, p <.001. The perceived satisfaction regarding financial means, faculty colleagues, home, family and overall life satisfaction was the same during summer semester examination period and summer holiday, but it was significantly correlated with the WHO-Five Well-being Index score corresponding to the summer holiday, and no association was observed with the WHO-Five Well-being Index score corresponding to the summer semester examination period. **Conclusions:** Within the limits of this study, psychological well-being is likely to be negatively influenced, on a fairly large scale, by the semester examination period. Therefore, it is recommended to identify the most appropriate methods of examination with regards to the psychological load that might be a threat to the validity of students' evaluation. Additionally, training students about adequate coping strategies, designed as interventions at individual or group level, may be required. **Keywords:** DENTAL STUDENTS, MENTAL WELL-BEING, STRESS, PERSONAL SATISFACTION, EDUCATIONAL PROCESS, EDUCATION

Psychological well-being is as an important health component, rightly recognized by its inclusion in the definition of health by the World Health Organization. Well-being may influence the behavior, the ability to cope with stressful events, the work performance and generally the ability to achieve one's full potential (1-3).

In direct relation to the academic activities, the most stressful period for the students is the examination period, with high psychological demands related to taking multiple exams in a limited time-frame, these scores usually having the greatest

impact on their academic achievements for the respective semester. The marked stress during this period can adversely affect student's health and performance, with possible implications on the correctness of his/her evaluation (4, 5).

The aim of the study was to comparatively assess the psychological well-being of dental students during the summer semester examination period and summer holiday.

MATERIAL AND METHODS

Study design, settings and participants. A single-arm, prospective study was designed and implemented on a convenience sample formed by second year dental students from the Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. The psychological well-being was assessed through a self-administered email questionnaire, filled in during summer semester examination period and during summer holiday. Enrolment was done in the 2014 summer semester examination period, on a voluntary basis. Repeater students were excluded. Informed consent was given by the participants when providing the email address to be sent the link to the questionnaire.

Variables. The main study outcome was the psychological well-being, that was assessed using the WHO-Five Well-being Index (WHO-5; WBI), developed by the Psychiatric Research Unit, Mental Health Centre North Zealand, Hillerod, Denmark, a World Health Organization Collaborating Centre. The Romanian language version was used, available on the website of Psychiatric Research Unit at the Mental Health Centre North Zealand. WHO-Five Well-being Index is a depression screening tool with good psychometric validity and sensitivity (6-8).It consists of a 5-item questionnaire, with a time frame of previous two weeks. Its interpretation is in accordance to the score obtained, that ranges from 0 to 25, with corresponding percentage score ranging from 0 to 100. A score below 13, corresponding to a percentage score below 50, or choosing answers with the minimum scores (0 or 1), indicates the need to perform a more detailed examination for the diagnosis of depression, the MDI questionnaire (Major Depression Inventory, World Health Organization) being recommended. For monitoring change, a difference of percentage score of 10 indicates a significant change in the psychological well-being (1, 6).

Other study variables were demographics (age, sex) and satisfaction with factors that may relate to psychological well-being (i.e. satisfaction with financial means, academic activities at faculty, faculty colleagues, home, general health, friends, family and overall life satisfaction).The latter were registered as self-rated satisfaction, using a 10 point numerical rating scale, with whole numbers (1 to 10 integers), that was defined by the extremes, from 1 (very unsatisfied) to 10 (very satisfied). The respondent was asked to select the number that best reflects their satisfaction level regarding the issues mentioned.

Data collection. Students were prospectively followed-up, the data being collected at baseline, during the summer semester examination period (T1) and during the summer holiday (T2). The summer semester examination period, according to the structure of the academic year of the Faculty of Dental Medicine of the University of Medicine and Pharmacy Carol Davila in Bucharest, had a 4 weeks length, from

07.06.2014 to 06.07.2014. Data was collected at T1 after two weeks from the beginning of the summer semester examination period, until it ended. Data was collected at T2 after three weeks from the completion of the summer semester examination period, after 28.07.2014, until the summer holiday ended.T2 moment was planned considering the need for a recovery period after the studied stress factor (semester examination period), considering the time-frame of previous two weeks of the WHO-Five Well-being Index, and also considering the possibility of student delay in responding the questionnaire.

Data collection was conducted online, by creating a questionnaire in Google Docs Form. This questionnaire was administered using the email address provided by the students. For the questionnaire completion on summer holiday (T2), two email reminders were sent to those who originally completed it in the summer semester examination period (T1). At T2, the respondents were no longer able to consult the previous responses, given at T1.

Data analysis. Statistical analysis was performed with SPSS software, version 13.The Wilcoxon signed-ranks test was used to evaluate differences in the scores of WHO-Five Well-being Index, at T1 and T2. Spearman's test was used for nonparametric correlation analysis between two factors. The significance level was set at $p<.05$.

RESULTS

Participants. Initially, a number of 39 second year dental students from the Faculty of Dental Medicine agreed to participate in this study and provided the email addresses. From these, 34 dental students responded to the email-delivered questionnaire during summer semester examination period (T1), corresponding to a response rate of 87%. They were aged between 19 and 24 years (mean and median - 20 years), of which more than half (n=22) were female. From the 34 dental students that responded to the questionnaire at T1, 20 students responded also to the same questionnaire in the summer holiday (T2),the response rate being 59%.They were aged between 20 and 24 years (mean=21 years; median=20 years), of which more than half (n=11) were female.

Psychological well-being. During the summer semester examination period (T1),the 34 dental students that responded to the questionnaire, obtained scores of WHO-Five Well-being Index varying from 4 (percentage score 16) to 20 (percentage score 80), with a mean of 12 (percentage score 49) and median of 12.5 (percentage score 50). According to the methodology of interpreting WHO-Five Well-being Index results, it was recommended to perform a more detailed examination for the diagnosis of depression in more than half of the analyzed students (n=20; 59%).

In the summer holiday (T2), the 20 students who completed the questionnaire again obtained scores of WHO-Five Well-being Index between 10 (percentage score 40) and 24 (percentage score 96), with a mean of 18 (percentage score 72) and median of 19 (percentage score 76). According to the interpretation methodology of WHO-Five Well-being Index results, it was recommended to perform a more detailed examination for the diagnosis of depression in only 3 (15%) of these students.

Using Wilcoxon signed-ranks test it was observed that psychological well-being is significantly better in the summer holiday (T2) than in the summer semester

examination period (T1)- in T2 median=19 (percentage score 76), in T1 median=11.5 (percentage score 46), Z = 3.69, p <. 001. A significant change of psychological well-being from T1 to T2, with differences of the value of the percentage score above 10, was observed in the majority of students (n=18; 90%).Seventeen students registered a psychological well-being improvement in T2, with positive changes of the percentage score of WHO-Five Well-being Index that ranged from 12 to 52 (mean, respectively median of percentage score change of 30, respectively 28) and one student registered psychological well-being decrease, with a negative change of the percentage score of WHO-Five Well-being Index of 12. The remained two students didn't register a significant change of their psychological well-being, one of them scoring the same at T1 and T2, and the other one scoring higher at T2, but the score difference from T1 was below the cut-off value of 10 for the percentage score of WHO-Five Well-being Index (Fig. 1).

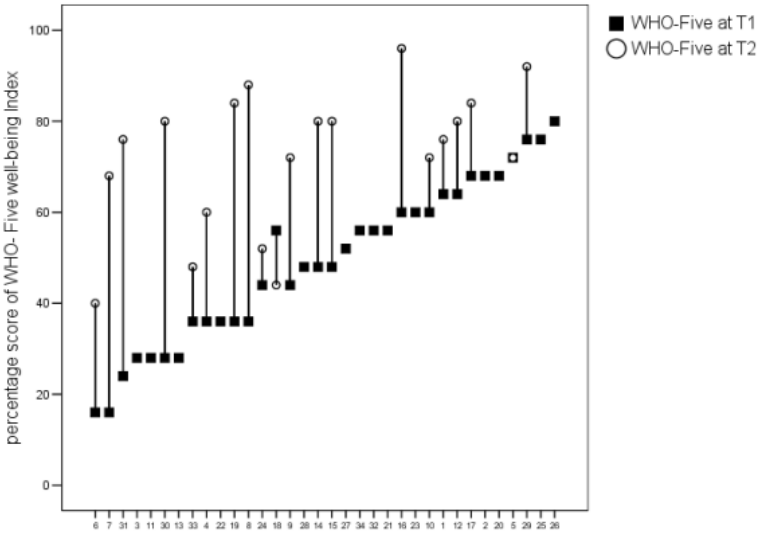

Fig. 1. Percentage scores of WHO-Five Well-being Index during summer semester examination period (T1) and summer holiday (T2)

Correlations between student psychological well-being and satisfaction with social and personal life-factors. Self-rated satisfaction with financial means, academic activities of faculty, faculty colleagues, home, general health, friends, family and his/her-own life overall was not different during the summer semester examination period (T1) and summer holiday and (T2), aspect analyzed using Wilcoxon signed-ranks test (table I). Even so, self-rated satisfaction with financial means, faculty colleagues, place of living, family and his/her-own life overall was strongly correlated with the score of WHO-Five Well-being Index during summer holiday (T2), and no correlations were found with the score of WHO-Five Well-being Index during summer semester examination period (T1) (tab. II).

TABLE I

Perceived satisfaction with social and personal life-factors, during summer semester examination period (T1) and summer holiday (T2)

Self-rated satisfaction with	T1 (median)	T2 (median)	p
Financial means	7.0	7.5	nss
Academic activities of faculty	6.0	6.5	nss
Faculty colleagues	8.0	8.5	nss
Home	9.0	10.0	nss
General health	9.0	9.0	nss
Friends	8.5	9.0	nss
Family	10.0	9.0	nss
Overall life satisfaction	8.0	9.0	nss
T1- summer semester examination period T2 – summer holiday nss-not statistically significant			

TABLE II

Correlation coefficients between the scores of WHO-Five Well-being Index and perceived satisfaction with social and personal life-factors

Self-rated satisfaction with	WHO-Five (T1)	WHO-Five (T2)
Financial means	.24	.56*
Academic activities of faculty	.11	.04
Faculty colleagues	.12	.72**
Home	-.11	.58**
General health	-.00	.22
Friends	-.07	.43
Family	.20	.57**
Overall life satisfaction	.29	.82**
T1- examination period T2 – summer holiday	** significant at the 0.01 level * significant at the 0.05 level	

DISCUSSION

These results suggest that dental students have a poor psychological well-being during summer semester examination period, much worse compared to the one during summer holiday period, during which the scores of WHO-Five Well-being Index are similar to the ones in general population (i.e. percentage score of approximately 70) (1, 6).

The significant difference of psychological well-being during semester examination period and holiday can be argued by the differences between them, as mental demand. The poor psychological well-being during summer semester examination period may be explained by the sustained mental demand, generally present during semester examination period in relation to the high number of exams taken in a relatively short time-frame. Also, during summer semester examination period it is possible that the students experience the highest stress levels, due to the cumulative effect of whole-year academic activities stress. In contrast, during summer holiday

the students have the longest period of vacation of the academic activities, which probably associates mental relaxation that promotes psychological change with improved well-being.

Academic examination stress has been related to various manifestations, such as higher level of anxiety, lower levels of self-esteem, somatic symptoms, eating disorders such as emotional eating, higher blood pressure (4, 9-11).Examinations are perceived as acting as unavoidable stressors, with possible negative consequences that depend on individual characteristics, such as personality traits, smoking habit, family medical history, like hypertension (2, 4, 9). Additionally, specific aspects related to the method of examinations have been related to the stress level. Among the aspects identified by medical students as contributing to exam anxiety, extensive course loads, long duration of exams, inadequate rest are included (12, 13).

Academic examination stress and its relation to psychological well-being should be especially considered in medical and dental students, considering that evidence show that they register higher prevalence of depression and anxiety, with levels of overall psychological distress consistently higher than in the general population, which could have an impact on their health, and also academic performance, dropout rates and professional development (3, 14, 15). Medical students register relatively frequently signs of burnout syndrome, as emotional exhaustion and depersonalization, which favors a higher degree of dysfunctional coping (5, 16, 17).In addition to this, there is limited evidence that indicates that the second year medical students experience the highest stress levels, exhibiting most frequently anxiety(18).

The results of this study also suggest that during the summer holiday, as a situation with a high degree of relaxation, there are many factors associated with student's psychological well-being, as self-rated satisfaction with their financial means, home (living conditions), family, faculty colleagues and overall life satisfaction. Even if the perception on these aspects is the same during the summer semester examination period, they are not correlated with the psychological well-being at that time. A possible interpretation of these results could be that during examination period students are extremely focused on the academic activities, other factors that typically influence their psychological well-being having a lower impact at that time.

It was noted that on the self-rated satisfaction matter, students were most dissatisfied with the academic activities of the faculty, even so, that was not related to their psychological well-being. These aspects may be in relation to their perceived opportunity to draw attention to issues that dissatisfy them about aspects related to the faculty, considering that this research was conducted in the academic settings. Additionally, these study results suggest that academic activities have an impact on students' psychological well-being, but their satisfaction with them is not a determining factor.

Study limitations are mainly related to the small convenient sample size, which may have affected the results. Also, a more accurate evaluation would include prospective follow-up during the entire academic year, in order to observe if indeed, poor well-being reaches its highest peak in the summer semester evaluation period, or it is the same with other evaluation periods. Additionally, comparison should be made

with other Faculties of Dental Medicine, in order to assess if results are not biased by specific particularities of this faculty. Even so, this research has the advantage of prospective follow-up, drawing attention to an issue that should be further investigated, considering the importance of psychological well-being and understanding its determinants.

CONCLUSIONS

Within the limitations of this study, psychological well-being is likely to be negatively influenced on a fairly large scale, by the semester examination period, with possible impact on students' health and performance on academic activities. Therefore, it is recommended to identify the most appropriate methods of examination regarding the psychological load that might be a threat to the validity of students' evaluation. Additionally, strategies for training students about adequate coping methods, designed as interventions at individual or group level, may be required.

ACKNOWLEDGEMENTS

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NEWS

IN VITRO COMPARISON OF THREE DIFFERENT IMAGE RECEPTORS FOR DETERMINING THE LENGTH OF ENDODONTIC FILES

The correct evaluation of working length is one of the most important conditions of successful endodontic treatment. Both digital and traditional radiographs have been used for these assessments. However, the accuracy of these methods is still controversial. The aim of this study was to evaluate the accuracy of endodontic file length measurements of E-speed radiographs, Digora storage phosphor plates, and Schick charge-coupled device sensors, with a secondary aim of assessing the influence of image enhancement on the measurement accuracy. Forty-seven extracted mandibular first premolar teeth were selected. ISO size 8, 10, and 15 files were inserted into each canal, and the files were fixed when the tip was seen at the apical foramen. The teeth were mounted in acrylic blocks and exposed using E-speed films, Digora storage phosphor plates, and Schick charge-coupled device sensors. Two radiologists and two endodontists measured the length of each file between the file stopper and tip on each image. Measurements were carried out on original and magnified images as well as on revealer images for Schick CDR. The actual lengths of the files were measured using a calipers to the nearest 0.01 mm and served as the "true length". Repeated measures analysis of difference and Tukey honestly significant difference tests were used to analyze the data ($P < 0.05$). E-speed films were superior to digital systems for the measurement accuracy of the size 08 file ($P < 0.05$). Revealer images gave equivalent results with E-speed films for the measurement of the size 10 file ($P > 0.05$). The most accurate results were obtained with the size 15 file regardless of the image receptor ($P > 0.05$). Size 15 or larger files should be used for endodontic working length determinations. Revealer images gave equivalent results with E-speed films and may be utilized for determining the file length of size 10 files (Betul Ilhan, Ilgın Akcay, Nesrın Dundar. In vitro comparison of three different image receptors for determining the length of endodontic files. *Journal of Dental Sciences* 2014; 9(4): 345-350).

Gianina Iovan

LASER ASSISTED *VERSUS* CONVENTIONAL FRENECTOMY IN ORTHODONTIC PATIENTS

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LASER ASSISTED VERSUS CONVENTIONAL FRENECTOMY IN ORTHO-DONTIC PATIENTS (Abstract): **Aim**: The aim of this study was to evaluate the laser assisted frenectomies versus conventional ones from the technique and the post-op recovery point of view. **Material and methods**. We selected a sample population of 20 patients with thick, low inserted maxillary midline frenum and midline diastema. We randomly divided the sample population into two groups named A and B with an equal number of patients. Patients included in group A were treated with conventional technique while patients included in group B were benefited of laser assisted frenectomy. **Results and discussion**. Our findings showed that laser assisted procedure was much faster to perform than the conventional one due to the easier protocol, absence of sutures and better cooperation from the patient side. In the same time it provided a better comfort degree to the patient and an accelerated healing**. Conclusions**. Laser assisted technique for maxillary midline frenectomy in conjunction with orthodontic treatment proved to be superior to the conventional one because it saves time; it's minimally invasive and provides a better cooperation with the child. **Keywords:** MAXILLARY MIDLINE FRENUM, DIASTEMA, FRENECTOMIES, DIODE LASER.

After Carranza et al a frenum is a fold of mucous membrane, usually with enclosed muscle fibers that attach the lips and cheeks to the alveolar mucosa and/or gingiva and underlying periosteum.

The same authors say that it becomes a problem if the attachment is too close to the marginal gingiva. Carranza at al claim that this anatomic situation may be a genetic condition of the individual or the result of recession of the gingival margin reaching the area of the frenum. Tension on the frenum may pull the gingival margin away from the tooth creating a periodontal problem. (1)

After Edwards the pretreatment relationship between a thick and low inserted maxillary midline frenum and a midline diastema showed a strong, but not absolute, correlation. (2)

The aim of this study was to evaluate the laser assisted frenectomies versus conventional ones from the technique and the post-op recovery point of view.

MATERIAL AND METHODS

We selected a sample population of 20 patients with thick, low inserted maxillary

midline frenum and midline diastema.

The average age of the sample population was 10.4 years while 55% were females and 45% males.

All patients included in the current research needed frenectomy as an adjuvant therapy to the orthodontic treatment (Fig. 1).

We randomly divided the sample population into two groups named A and B with an equal number of patients.

Patients included in group A were surgically treated through the conventional frenectomy technique using a scalpel and 4.0 silk for suture.

Patients included in group B received laser assisted surgical removal of the frenum using a diode laser with the wavelength of 940 nm and a optical fiber with the diameter of 400 μm (Fig. 2).

In order to increase the cutting effect of the laser the fiber was previously activated. (fIg. 3a-3b).

The laser was set at 1.5 W energy in CP2 and throughout the procedure the tissue was irrigated with a saline solution in order to avoid the overheating (Fig. 7).

Fig. 1. Initial aspect of the maxillary

Fig. 2. Optical fiber

Fig. 3a. Fiber activation

Fig. 3b. Complete diode laser equipment

All surgical procedures were done under infiltration anesthesia with articaine and by the same team.

RESULTS AND DISCUSSION

Suter at al. claim that closure of the maxillary midline diastema with a prominent frenum is more predictable with frenectomy and concomitant orthodontic treatment than with frenectomy alone. (3) This is why an efficient and minimally invasive management of maxillary midline frenectomy is desirable especially in children.

Our results showed that during laser assisted surgery the bleeding was nearly absent and the young patient had a better degree of comfort (Fig. 4). In the same time patients included in group B didn't need suture in order to prevent the reattachment of frenum.

Healing process was faster in the group that benefited from laser assisted surgery compared to conventional technique. (fig. 5) This fact is in accordance with fundamental research results from literature that emphasized the morphological differences identified at the gingival epithelium level and subjacent lamina propria support the value of laser therapy, stimulating an improved healing of the damaged tissues. The patients included in group B presented as well significantly diminished post operative pain and absence of edema (Fig. 6).

Fig. 4. Immediate post-op aspect after laser surgery

Fig.5. Three days follow-up

Fig. 6. Facial aspect at 3 days follow-up

Fig. 7. Laser settings

Other studies proved that the overall perception of young patients towards laser assisted periodontal surgery was very high and this is a means of reducing operating time and anxiety caused by the surgery itself. (5)

Our findings showed that laser assisted procedure was much faster to perform than the conventional one due to the easier protocol, absence of sutures and better cooperation from the patient side.

Due to the vaporization effect of the laser at the cellular level the reattachment rate of the frenum is significantly reduced and will provide a faster orthodontic tooth movement for closing the diastema.

The ideal approach for successfully treating this kind of cases is the interdisciplinary one with a tight cooperation between orthodontist, periodontist, oral surgeon and general practitioner.

CONCLUSIONS

Laser assisted technique for maxillary midline frenectomy in conjunction with orthodontic treatment proved to be superior to the conventional one because it saves time; it's minimally invasive and provides a better cooperation with the child.

The laser assisted frenectomies proved that the reinsertion rate of the midline frenum was minimal (Fig. 8-9).

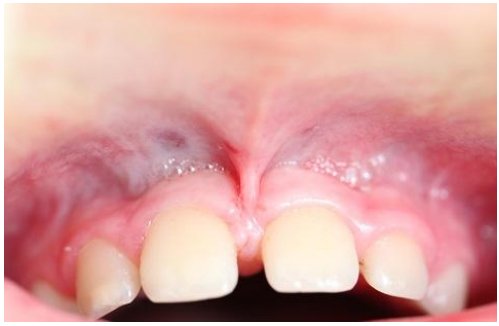

Fig 8. Initial status

Fig. 9. One month follow up status

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NEW HYDRAZONES OF FERULIC ACID: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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NEW HYDRAZONES OF FERULIC ACID: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY (Abstract): The ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a phenolic compound with important antioxidant effects and which nowadays is being extensively studied for his potential indications in inflammatory and neurodegenerative diseases, hypertension, atherosclerosis, etc. **Aim**: The synthesis of new ferulic acid compounds with potential antioxidant activity. **Material and methods**: The synthesis of the designed compounds was performed in several steps: (i) the obtaining of ferulic acid chloride by reacting of ferulic acid with thionyl chloride; (ii) the reaction between the ferulic acid chloride and hydrazine hydrate 98% to obtain the ferulic acid hydrazide; (iii) the condensation of ferrulic acid hydrazide with various benzaldehydes (2-hydroxy/3-hydroxy/4-hydroxy/2-nitro/3-nitro/4-nitro/2-methoxi/ 4-chloro/4-fluoro/4-bromo-benzaldehyde) resulting the corresponding hydrazones. **Results**: The structure of the synthesized compounds was confirmed by FT-IR spectroscopy and the evaluation of antioxidant potential was achieved by determining the total antioxidant capacity and reducing power. **Conclusions**: In this study new hydrazones of ferulic acid have been synthesized, physic-chemical and spectral characterized. The evaluation of antioxidant potential using *in vitro* methods showed the favorable influence of the structural modulation on the antioxidant effects of ferulic acid. **Keywords**: FERULIC ACID, HYDRAZONE, ANTIOXIDANT ACTIVITY

One of the most studied biological effects of ferulic acid derives from its antioxidant properties. Due to the phenolic structure, ferulic acid could easily form a phenoxy group, stabilized by resonance and which explains its antiradical effect. This allows the ferulic acid to protect the DNA and the lipids from the oxidation caused by the reactive oxygen species (ROS) (1, 2). Based on its antioxidant effect, ferulic acid can be effective in the prevention and treatment of several diseases where oxidative stress plays an important role like: Alzheimer's disease, diabetes mellitus, cancer, hypertension and atherosclerosis. At the same time the involvement of oxidative stress in inflammation suggest that ferulic acid may also be effective in the treatment of inflammatory diseases. Moreover, the specific structure gives ferulic

acid a strong UV absorption ability, making it a powerful skin protecting agent (3). Based on these considerations we have proposed the synthesis and biological evaluation of new ferulic acid hydrazones. Through their antioxidant potential, these compounds can be used in the protection of biological systems from oxidative stress.

MATERIAL AND METHODS

Ferulic acid, thionyl chloride, hydrazine hydrate, aromatic aldehydes (4-fluoro/4-bromo/ 4-chloro/ 2-nitro/ 3-nitro/ 4-nitro/2-hydroxy/3-hydroxy/4-hydroxy/2-methoxy-benzaldehy-de), organic solvents (*p.a.* quality) and standard reagents used for the antioxidant tests were purchased from Sigma Aldrich Company and Fluka Company. All the solvents and the reagents were used without prior purification. TLC plates silica gel 60 F_{254} from Merck Company were used for monitoring of the chemical reactions.

Chemistry

Synthesis of ferulic acid hydrazones. The ferulic acid was reacted with thionyl chloride to give the corresponding acid ferulic chloride. This intermediate was treated in the next step with hydrazine hydrate (98%) when the ferulic acid hydrazide it was formed, which by condensation with various aromatic aldehydes (2-hydroxy/3-hydroxy/4-hydroxy/2-nitro/3-nitro/4-nitro/2-methoxi/4-chloro/4-fluoro/4-bromo-benzaldehyde) led to the corresponding hydrazones (fig. 1). The synthesis of the designed compounds was performed by adapting some methods from literature for similar compounds (4, 5).

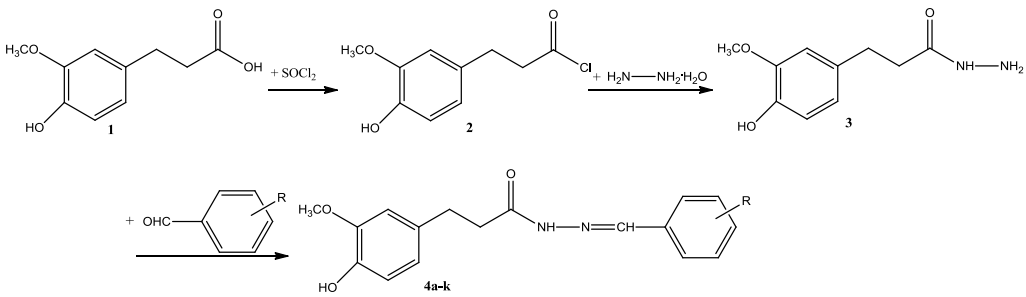

Fig. 1. The synthesis of new ferulic acid hydrazones

General procedure for the synthesis of ferulic acid hydrazones. In the first step thionyl chloride (0.15 mol, 11 mL) it was added dropwise and under stirring over ferulic acid (0.025 mol, 5 g). The reaction mixture is maintained in oil bath at a temperature of 50 ^{0}C for 4 hours. At the end of the reaction, the solution was concentrated in order to remove the excess of thionyl chloride. In the second step, over dry ferulic acid chloride (0.05 mol, 10.62 g), it was added drop wise hydrazine hydrate solution 98% (0.25 mol, 12.37 mL). The reaction mixture was maintained in ice bath for 2 hours, after which on water bath at room temperature for another 2 hours under continuous stirring. At the end of the reaction 15 mL of cold water was added and the resulting precipitate was filtered off and dried at room temperature. In the last step,

the ferulic acid hydrazide (0.015 mol, 3 g) was suspended in 100 mL of absolute ethanol and then it was added a few drops of glacial acetic acid and 0.015 mol of aromatic aldehyde (2-hydroxy/3- hydroxy/4-hydroxy/2-nitro/3-nitro/4-nitro/2-methoxi /4-chloro/4-fluoro/4-bromo-benzaldehyde). The reaction mixture was heated under reflux on water bath from 13 to 20 hours. The reaction was monitored by TLC, using a mixture of ethyl acetate: methanol: acetone: water = 5: 2: 2: 1 as solvent system.

Characterization by FT-IR spectroscopy. The FT-IR spectra of the synthesized compounds, intermediate and final compounds, were recorded using a Fourier transform spectrometer, ABB-MB 3000 FT-IR MIRacle^{TM} Single Bounce ATR-crystal ZnSe and the spectral range studied was in the range of 4000-500 cm^{-1}, at a resolution of 4 cm^{-1}, being carried out 16 scans for each sample. The spectra were interpreted using Horizont program MB^{TM} FT - IR.

Antioxidant assays

Determination of total antioxidant capacity. The total antioxidant capacity of the synthesized compounds was evaluated using phosphomolybdenum method following the protocol described by Cacic et al. (2010) with some minor modifications. The samples were dissolved in DMSO (dimethyl sulfoxide) in order to form a stock solution with the concentration of 2 mg/mL. From the stock solution it was taken different volumes (10 µL, 20 µL, 40 µL, 60 µL) and then added DMSO to have a final volume of 200 µL sample. Over the resulting mixture it was added 2000 µL reagent (28 mM phosphate, 4 mM ammonium heptamolybdate, 0.6 M sulfuric acid). After a preliminary stirring, the solutions were incubated at 95^0C for 90 minutes, cooled in cold water and centrifuged at 4,600 rpm for 15 minutes. The supernatant was separated and the absorbance was read at a wavelength of 695 nm against a blank consisting of 2000 µL reagent and 200 µL DMSO. For each compound it was calculated effective concentration 50 (EC_{50}) by linear regression and ascorbic acid (2 mg/mL) was used as a positive control. All determinations were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD).

Reducing power. Reducing power of the synthesized compounds was measured by the method described in the literature (7) with some modifications. Samples for analysis were dissolved in DMSO to give stock solutions with concentrations of 2 mg/mL. Over 1 mL sample of various concentrations obtained by serial dilutions of the stock solution (2 mg/mL) 1 mL of phosphate buffer (pH 6.6) was added. After vigorous stirring 1 mL of 1% potassium ferricyanide was added. The homogenous mixture was incubated for 20 minutes at 50 ^{0}C, then cooled in cold water and added 1 ml 10% trichloroacetic acid. After vigorous shaking the samples were centrifuged at 4500 rpm for 15 minutes. To 1 mL of the supernatant it was added 1 mL of doubly distilled water, and 0.2 mL of 0.1% ferric chloride. After 5 minutes the absorbance was read at the wavelength of 700 nm against a blank (the reaction mixture without the sample). For each compound it was calculated effective concentration 50 (EC_{50}) by linear regression and ascorbic acid (2mg/mL) was used as a positive control. All determinations were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD) (8).

RESULTS AND DISCUSSION

Physical-chemical and spectral characterization

The ferulic acid hydrazones are crystalline powders colored in different shades which vary from light yellow to bright brown, very slightly soluble in dimethylformamide (DMFA), dimethyl sulfoxide (DMSO), partially soluble in absolute ethanol, methanol, chloroform, acetone, dioxane, and insoluble in distilled water and diethyl ether. The physico-chemical characteristics and the FT-IR bands are listed in table I. The presence of the absorption band characteristic to hydrazone bond (CH=N), confirms structure of the synthesized compounds.

TABLE I

Physico-chemical and spectral characteristics of ferulic acid hydrazones (4a-k)

Comp.	R	Mol. weight	Yield (%)	m.p. (^{0}C)	FT-IR characteristic band (cm^{-1})
4a	-F(4)	314.31	66.35	189-194	3251 (-NH), 2941 (CH_{Ar}), 1633 (-CO-NH-), 1600 (-CH=N), 1095 (C-F)
4b	-Cl(4)	330.08	69.18	203-205	3253 (-NH-), 2945 (CH_{Ar}), 1595 (-CO-NH-), 1510 (-CH=N-), 813 (C-Cl)
4c	-Br(4)	375.22	68.81	210-213	3251 (-NH-), 2943 (CH_{Ar}), 1623 (-CO-NH-), 1583 (-CH=N-), 858 (C-Br)
4d	-NO_2(2)	341.32	52.04	196-198	3249 (-NH-), 2943 (CH_{Ar}), 1627 (-CO-NH-), 1606 (-CH=N-), 1569 (-C-N)
4e	-NO_2(3)	341.32	61.38	185	3083 (-NH-), 2947 (CH_{Ar}), 1598 (-CH=N-), 1508 (-C-N)
4f	-NO_2(4)	341.32	60.17	223	3114 (-NH-), 2844 (CH_{Ar}), 1595 (-CO-NH-), 1515 (-CH=N-), 1471 (-C-N)
4g	-OH(2)	312.32	55.89	186	3045 (-NH-), 3020 (-OH), 2974 (CH_{Ar}), 1646 (-CO-NH-), 1571 (-CH=N-)
4h	-OH(3)	312.32	25.85	236	3251 (-NH-), 3045 (-OH), 2947 (CH_{Ar}), 1723 (-CO-NH-), 1633 (-CH=N-)
4i	-OH(4)	312.32	84.56	179	3263 (-NH-), 3188 (-OH), 2939 (CH_{Ar}), 1606 (-CO-NH-), 1564 (-CH=N-)
4j	-OCH_3(2)	326.35	48.83	200	3251 (-NH-), 3041 (OCH_3), 2945 (CH_{Ar}), 1633 (-CO-NH-), 1598 (-CH=N-)
4k	-H	296.32	80.53	110	3190 (-NH-), 2966 (CH_{Ar}), 1587 (-CO-NH), 1594 (-CH=N-)

Antioxidant assays.

Determination of total antioxidant capacity. This method is based on the reduction of Mo^{+6} to Mo^{+5} by the tested compounds followed by the formation of the phosphate/Mo^{+5} green complexes at acidic pH. From the obtained results (tab. II, fig. 2) it can be observed that the tested compounds are more active than ferulic acid with the exception of **4c** and **4j**. The hydrazone resulted from the condensation of benzaldehyde with ferulic acid hydrazide (compound **4k**) is 1.32 times more active than ferulic acid. Regarding the influence of radicals which substitute the aromatic ring, it was observed that the most favorable influence was exerted by -OH(2),-NO_2(2) and -F(4) radicals, the corresponding compounds (**4g, 4d, 4a**) being 1.80, 1.23 and 1.11 times more active than the unsubstituted derivative (**4k**). The studied compounds have been, however, less active than the ascorbic acid used as positive control.

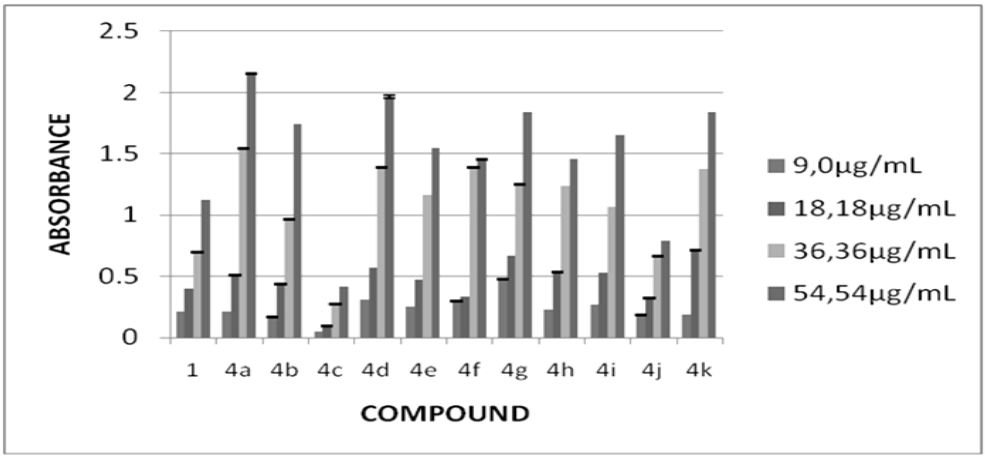

Fig. 2. The absorbance value of ferulic acid hydrazones (**4a-k**) at different concentrations

TABLE II

The total antioxidant capacity (EC_{50}, mg/mL) of ferulic acid hydrazones (4a-k)

Compound	EC_{50} µg/mL	Compound	EC_{50} µg/mL	Compound	EC_{50} µg/mL
1	23.40 ± 0.03	**4d**	14.30 ± 0.03	**4h**	19.01 ± 0.01
4a	15.78 ± 0.04	**4e**	17.72 ± 0.05	**4i**	16.62 ± 0.01
4b	18.65 ± 0.05	**4f**	16.94 ± 0.05	**4j**	32.87 ±0.12
4c	65.17 ± 0.11	**4g**	9.77 ± 0.03	**4k**	17.64 ± 0.01
ascorbic acid 5.17 ± 0.19					

1 = ferulic acid,

* the EC_{50} values are the average of three determinations ± standard deviation

Reducing power. The studied compounds have the capability to reduce Fe^{3+} from the ferricyanide complex to Fe^{2+}, changing the color of the test solution from

yellow to green depending on the reducing power of each compound. Derivatization of ferulic acid increases the reducing power, the tested compounds being more active than ferulic acid, except the compound resulting from the condensation of 4-bromobenzaldehyde (**4c**) (fig. 3, tab. III). The hydrazones resulting from the condensation of benzaldehyde with ferulic acid hydrazide **(4k)** was found to be 1.2 times more active than ferulic acid. Regarding the influence of radicals which substitute the aromatic ring it was observed that the most favorable influence was exerted by the -OH(3), the corresponding compound (**4h**) being 1.22 times more active than the unsubstituted derivative (**4k**). The compounds studied have been, however, less active than the ascorbic acid used as a positive control.

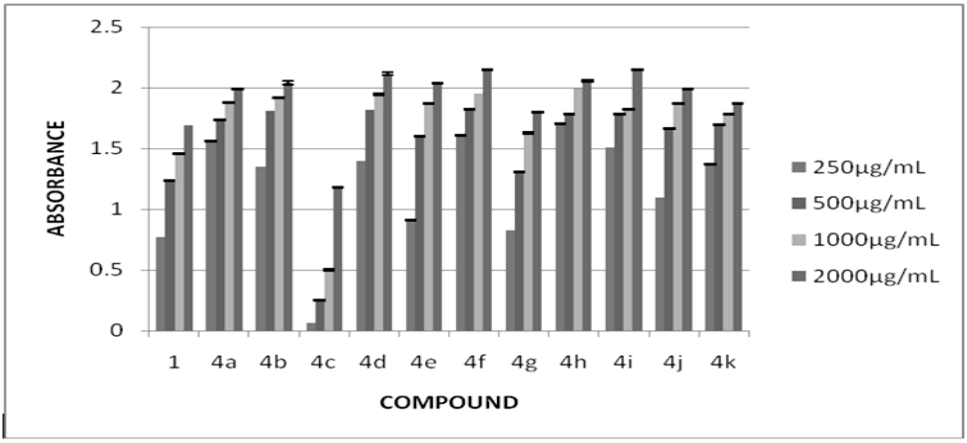

Fig. 3. Absorbance value of hydrazones of ferulic acid (**4a-k**), at different concentrations

TABLE III

The reducing power (EC_{50}, mg/mL) of ferulic acid hydrazones (4a-k)

Compound	EC_{50} µg/mL	Compound	EC_{50} µg/mL	Compound	EC_{50} µg/mL
1	189.88 ± 1.52	**4d**	153.92 ± 0.03	**4h**	128.41 ± 0.36
4a	153.8 ± 0.64	**4e**	168.50 ± 0.52	**4i**	154.54 ± 0.04
4b	158.13 ± 0.34	**4f**	143.11 ± 0.41	**4j**	163.16 ±0.49
4c	931.08 ± 2.07	**4g**	183.46 ± 0.24	**4k**	157.28 ± 0.22
ascorbic acid 5.87 ± 0.24					

1 = ferulic acid,* the EC_{50} values are the average of three determinations ± standard deviation

CONCLUSIONS

Some new hydrazones of ferulic acid have been synthesized and optimal conditions of reaction were established for obtaining compounds with high yields and advanced purity. The synthesized compounds have been characterized by their physical constants (melting point, yield,

molecular formula, molecular weight, solubility in different organic solvents) and their chemical structure was confirmed using FT-IR spectroscopy.

It was also assessed the antioxidant potential using *in vitro* methods. All the tested compounds showed total antioxidant capacity and reducing power appreciable in reference with ferulic acid, which confirms the favorable influence of structural modulation performed on the antioxidant effects of ferulic acid.

ACKNOWLEDGMENTS

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MALPRAXIS RISK MANAGEMENT IN IMPLANTOLOGY

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MALPRAXIS RISK MANAGEMENT IN IMPLANTOLOGY (Abstract): In present practice there is an increased demand from patients for prosthetic implant restoration solutions. At the same time one can notice a possible vulnerability regarding allegations of malpractice, as evidenced by the growing number of complaints of malpractice also maintained by negative reports, frequently incomplete or incorrectly documented from the press. This should require practitioners to have a professional attitude. **Keywords**: MALPRAXIS, IMPLANTO-PROSTHETIC, DENTAL PRAXIS, INFORMED CONSENT

The growing number of Romanian dentists on the medical market led to a reorientation of health services optics from providing optimal therapeutic solutions to focusing on patient satisfaction, for more profit. This led to a compromise in the application of therapeutic solutions along with a poor doctor-patient communication that has led to increased incidence of malpractice, which currently has strong media coverage. All this led to a loss of the patient`s confidence in the dentist and the quality of treatment. The patient`s reluctance towards the medical act is enhanced by continuously increasing rates of medical services so there will be much higher claims about the final result. His dissatisfaction may lead not only to the negative publicity attached but also to punitive professional claims, moral and material damages on grounds of malpractice.

The definition of malpraxis (lat. malus „bad" + gr. praxis „practice") = malpractice is the professional error committed in the exercise of medical or medical-pharmaceutical act, generating damages on the patient, involving civil liability of medical personnel and medical products and services provider, healthcare and pharmaceuticals (according Law No. 95/2006 on healthcare reform).

Dentists must responsibly provide patient care according to the standards of competence and behavior that they have assumed when choosing this profession. The dentist needs to improve and promote the patient`s oral health so as to respect his dignity, autonomy rights. Also, the practice of the profession must take place effectively and with minimum risks for the physician. Knowledge and compliance with the legislation is, from this point of view, of the utmost importance and protects the doctor, giving him the opportunity to adopt an approved therapeutic behavior which reinforces his professional dignity.

Legal regulations directly concerning the practice and conduct of the physician in our country are:

- Law No. 95/2006 on healthcare reform, as modified - art. 649 et seq.
- Order No. 482/2007 of the Minister of Public Health - "Rules for the application of Title XV: civil liability of medical personnel and medical products and services provider, healthcare and pharmaceuticals" in Law no. 95/2006 on healthcare reform. Law no. 46/2003 of patient rights
- Ministry of Health Order no. 386/2004 on approving the Implementation rules of patients' rights law no. 46/2003.
- Deontological Code of the dentist.

These rules should consider the European standards and norms to which Romanian legislation must comply with.

A number of vulnerable aspects related to malpractice that stand out and that should be taken into account are strictly medical related : a standard guide of diagnosis and treatment, the patient's informed consent, confidentiality, patient access to their medical information, patient access to non- discriminatory treatment, access to media, the right to a second opinion, limiting to their own specialty. The minimal documentation necessary in a medical office at present is the sheet of treatment, the informed consent, the general status questionnaire, the registry of consultations and treatment, the laboratory sheet, various patient agreements, internal rules displayed, the job description for employees.

Prosthetics on implants involves particular issues compared to other types of treatment in practice. This is a new, modern therapeutic solution, expensive for the patient, requiring special training and equipment from the doctor.

A first step in therapeutic success is the suggestive phrase "know the patient and his limits." The clinician should professionally evaluate the patient`s general condition, his clinical situation and decide whether the chosen therapeutic solution is up to the patient`s expectations. This requires gathering information. Retrieving general patient data is an operation that takes time and can be performed even by the latter, using a standard form that the patient fills in the waiting room along with a questionnaire where the general condition will be checked, if he suffers from other diseases, if he is under a treatment that may interfere with dental therapy. General status questionnaire must be completed thoroughly, as some medical history are absolutely not suitable for surgery such as face bone radiotherapy, bisphosphonate treatment with immunosuppressives, recent myocardial infarction (MI), certain psychiatric disorders and drug abuse. Other conditions are considered negative factors for osseointegration of implants: some forms of diabetes, multiple allergies, cardiovascular risk, a general malaise, etc. Drinking and smoking are also included here. At this point the doctor will decide if the patient needs to obtain blood marker values that help to form a complete overview of his medical condition.

The patient`s record is then completed along with the doctor with specialized data that should include the initial oral situation, diagnosis, prognosis, the description of each performed maneuvers, when it was performed, medical history, prognosis and initial costing, name and signature of the physician. After diagnosis and treatment plan development by the doctor, the patient must be explained to, as to a reasonable person, the treatment and outcomes proposed as well as the accidents and complications that may arise and provisionally

estimated costs. Subsequently, the patient must give his consent for the treatment by signing his informed consent, compulsory in writing.

*The patient`s informed consent is necessary for the application of the therapeutic solution.* It can take multiple forms: as a questionnaire with categories to be filled or checked or in the form of a declaration summarizing the risks and complications that may occur. It includes several parts: the patient`s personal data, that will be listed both in the treatment sheet, register of consultations and treatment and general status questionnaire; a part briefly describing the medical act, then the name and specialty of the medical team, another part stating that the patient has been explained the overall condition, diagnosis, prognosis, risks and complications that may occur after treatment and the cost estimates, risks that may occur if the patient does not follow the medical instructions. It will include information about: the legal representative for minors, or persons without discernment, consent for the collection, preservation and use of biological samples, the agreement for further investigation (dental records), information on the rules of the cabinet, the right to a second opinion. A section or important part is the answer to mental illnesses which the patient may present (for some conditions agreement becomes null), and then the name and signature for the agreement or disagreement of the patient or legal representative. Also the legal acts underlying the necessity of its conception and completion must be mentioned. The only situation where there is no need for the patient`s consent is life threatening situations, in which case an emergency report will be completed. In this report the circumstances, the initial state of the patient, the therapeutic emergency approach and general state after overcoming the emergency will be mentioned.

The patient`s correct information by the physician in order to obtain their consent for treatment requires communication skills from both sides. The explanation must be given by the use of a language specific for a 14 year old child, at a reasonable scientific level of patient comprehension. The patient is the only one able to decide what risks he is willing to take. In most dental offices informed consent forms are of a general nature. It is useful to develop this especially designed form for implant-prosthetic solution so as to eliminate the suspicion or the possibility that consent is considered to be formal(1, 2).

Various types of agreement are useful in practice such as: patient consent for the communication of medical data, patient agreement on the participation in higher education, agreement on communicating medical documents.

Rules of internal order are recommended for the patients to be displayed where several items are specified, such as: rules about conduct, scheduling patients, providing medical information, media access.

Medical offices, clinics, need to respect the confidentiality of patient data, here including the whole medical team: doctors, nurses, technicians. According to current laws, the physician may not disclose to any other person than the patient, data about its health. Information on the patient`s health, results of investigations, diagnosis, prognosis, treatment followed, and any information that could link the identity of a person of a certain medical information is strictly confidential, obligation of confidentiality continuing even after the patient's death. The physician / medical staff

are not allowed to provide information on the nature of the above even to the family or friends of the patient, without its consent. In these circumstances, doctors are not allowed to communicate medical data about patients to third parties.

The principle of autonomy is reflected in the practices in the obligation to ensure patient access to personal health data. At the patient`s request copies of sheet treatment and original dental scans will be made available to them (digital copies will be kept in the archive).

According to the law it is forbidden to discriminate between persons in similar circumstances on the basis of race, sex, age, ethnicity, social or national origin, and religion, political or personal dislike.

Medical malpractice. Relationship with the media is a controversial topic in the medical world, largely due to negative media campaign against Romanian medical activity and the perception of journalists by the medical world in an utterly unfavorable way. A Kompass Media report monitoring printed media revealed that during the 1 July 2007 - 17 September 2007 period, 97 articles were classified by the press as cases of "medical malpractice." All items monitored recounted discontents and complaints from patients, the tone used by publications being accusatory to the doctors, even without confirming the existence of a medical fault. According to legal regulations, no patient can be photographed or filmed in a medical facility without his express consent (without infringing the rules and regulations of the medical unit). The doctor may refuse or reschedule the meeting with the media, it is recommended to be retained in comments and treat everything with a detached tone and even positive if possible.

Of particular importance is the malpractice insurance that each of the medical staff has. It is advisable to negotiate the terms of the insurance coverage so as to obtain both material and moral damage, and cover more disputes over a longer period of time (3, 4).

The development of contracts between offices and suppliers of materials and equipment should also be considered, the certificate attesting that they have European compliance, the contracts between clinics and dental labs, in stating that prosthetic pieces have certificates of conformity and will also respect the confidentiality of patient data.

Of particular importance in supporting a correct diagnosis and optimal therapeutic option are intra and extra oral clinical examinations, followed by a series of paraclinical investigations. This can be achieved by making the initial extra oral photos that capture the smile line, the shape of the lips, and of the face, and intraoral that captures the initial dental status and the possible initial degree of dental hygiene. At the same time the dentist has to make a map of the periodontium, the periodontal probing, which is attached to the treatment sheet. Next the study designs should be made that record oral initial status and on which the mock-up of future dentures can be achieved. This mock up done in the lab is to help patients understand what the future prosthetic will be like. The physician should record the type of occlusion and the potential occlusal para functions.

Analogical or digital intra and extraoral dental X-rays, CBCT (cone bean computer tomography) are required for implant solutions. With digital radiological images processed using dedicated 3D software the volume and quality of jaw bone can be evaluated, also the distances to certain

anatomical landmarks, it can simulate various types and sizes of implant insertion axis and also determine the form of the surgery guide. Your doctor will decide if additional surgical maneuvers will be required for bone adjustment. Various makeovers will be recommended in order to establish an optimal pre implantation oral status. Digital prints together with intraoral scanners helps the doctor get a final prosthetic, close to that simulated on the computer and the mock-up. "Electronic" sheets and dedicated programs facilitate virtual presentation of the operating time and the final form of prosthetic reconstruction. It also helps in maintaining an accurate record of the patients and a close hospitalization. Hospitalization of patients should be made according to the needs of each patient so as to prevent any post therapeutic complications. Patients should understand the importance of these periodic checks, when the physician makes an assessment of oral and implant tissue, of the occlusal changes and a radiological examination of implant osseo integration (5).

Implantology is found at the intersection of two specializations: the dental alveolar surgery and prosthetic surgery. Hence, the need for the physician to achieve particular theoretical and surgical skills. Therefore, in the present practice in our country doctors are required to obtain a certificate of competence and a diploma in implantology, at which it is recommended the addition of certificates on various surgical skills. But of equal importance is the prosthetic stage, which, by unusual stress, can lead to loss of implants. Therefore, training in prosthetic rehabilitation is a very important issue, prosthetic on implants being different than on natural teeth.

To prevent potential disagreements with the patient, the doctor should mention that there are no guarantees of success and strength over time for certain maneuvers and dentures. Also it is necessary to consider the patient's expectations related to aesthetics, functionality, adaptation or resistance to various therapeutic solutions, those often going beyond the possibilities of achievement. Full preparation of the practitioner, choosing the therapeutic solution according to the particular clinical case, provides the premises for a successful treatment. Increased attention on a correct, sincere,disinterested communication, along with providing all the information from both sides, creating a partnership, may decrease the risk of conflict situations that have no relation to malpractice but that the patient perceives them as such.

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DENTAL PATIENTS' ATTITUDES TOWARDS INFECTION CONTROL

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DENTAL PATIENTS' ATTITUDES TOWARDS INFECTION CONTROL (Abstract): The level of the patients' education for health is increasing as their concern for protection during the medical treatment. The aim of this study was to assess the attitudes of the dental patients towards infection control protocols in the dental office. Material and methods: A questionnaire-based study was initiated including 210 patients aged between 16 and 68 years. Data was processed using *SPSS 14.0* and analyzed by age, gender and level of education. Results: 82.6% of the patients trust the medical staff to protect them against the spread of infection. Female subjects are twice as interested in the follow up of infection control procedures as males (53.4% respectively 25.2%). The diseases considered by the patients as presenting a high risk of transmission during dental treatment are: hepatitis B, HIV infection, hepatitis C, tuberculosis and hepatitis A. The subjects require the dentist to wear rubber gloves (89,2%), masks (61.5%) and glasses (45.5%). Conclusion: The involvement of the dental patients in their own treatment is essential in order to increase the safety and quality of the dental procedures. Keywords: DENTAL PATIENTS, SAFETY, INFECTION CONTROL

The control of infection transmission represents an important goal of the modern a safe environment for the protection of the dentistry. Using the best and updated guidelines and recommendations the dentist has the duty to ensure dental team and patients which must be involved in their own health care regarding the diagnosis, investigations and treatment (1, 2). In order to raise the public awareness on the benefits of the safety protocols it is very important to implement effective infection control programs involving the dental patients (3).

MATERIAL AND METHODS

In order to assess patients' compliance and attitudes towards infection control procedures a questionnaire-based survey was initiated involving 210 patients aged between 18 and 65 years. The confidential questionnaire included 20 questions on the patients' perception about the infection risk and infection control strategies during the dental procedures. Data was analyzed by age, gender and level of education using *SPSS 14.0* (levels of statistical significance were set at $p<0.05$).

RESULTS

Of the study group 130 (62%) were women and 80 (38%) men. In terms of education level and occupation 62 (29.6%) of patients had a high education, 66

(31.4%) a medium education, 58 (27.9%) were students and 24 (11.1%) were retired.

The results of the study reveal that the diseases considered by the patients as presenting a high risk of transmission during dental treatment are: hepatitis B (62.3%), HIV infection (47.0%), hepatitis C (44.3%), tuberculosis (12.4%) and hepatitis A (10.2%) (fig. 1).

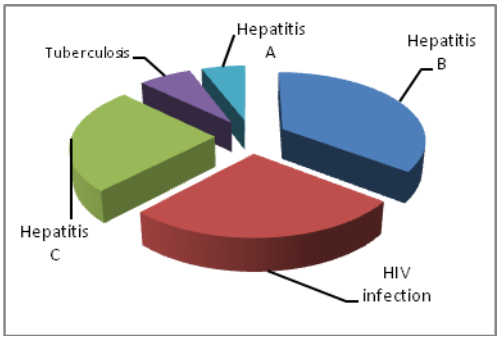

Fig. 1. Diseases considered by the patients as having a high risk of transmission

The subjects with a medium level of education showed a higher concern regarding the HIV infection (87.3%) while the subjects with high education were more worried about the viral hepatitis transmission (77.7%).

The majority of the patients (82.6%) felt to be adequately protected against the spread of infectious diseases. Men (94.6%) showed more confidence in the dental staff than women (72.3%) who manifested an increased anxiety regarding the risk of infection. 13.6% of the subjects are suspicious about their safety and therefore avoid or delay the dental treatment. Older people (79.2%) and those with medium education (70.1%) trust that appropriate measures are applied in order to protect them.

Regarding the protective equipment the patients want the dentist to wear rubber gloves (89.2%), masks (61.5%) and glasses (45.5%) during the dental treatment (fig. 2). 98.2% of the subjects , without significant differences by gender or education level, appreciate that those equipments decrease the risk of infectious diseases transmission.

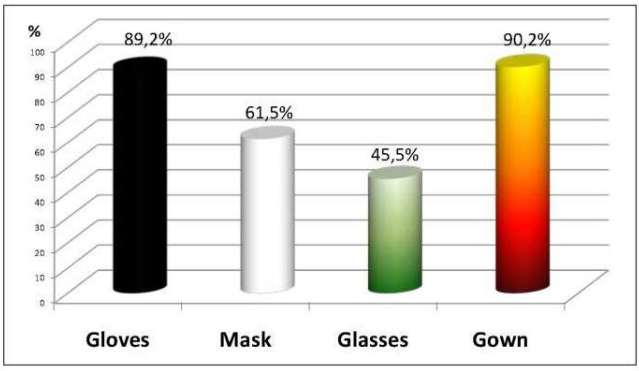

Fig. 2. Patients options regarding the dentist's protective equipment

The patients consider that the main strategies to prevent the spread of the infectious diseases are: hands hygiene (74.2%), disinfection of the surfaces after each patient (52.5%) and the sterilization of the instruments (43.6%).

The desire of the patients to be involved in the infection control procedures differs

by age and gender. Half of them (47.3%) are interested in the application of the procedures like surfaces disinfection, changing the glass for oral rinses, hand cleaning, using of an individual set of instruments, replacement of the dentist protective equipment for each patient. Of those 43.2% patients belonged to the age group of 19-35 years and 37.2% to the age group of 36-64 years.

Female subjects are twice as interested in the follow up of infection control procedures as males (53.4% respectively 25.2%). 10.7% of women and 23.4% of men consider that they do not have the necessary knowledge in this domain and 6.1% of women and 9.1% of men do not want to "offend" the medical staff. 6.2% of female subjects and 12.4 of male subjects are not interested in these issues and protocols (fig. 3).

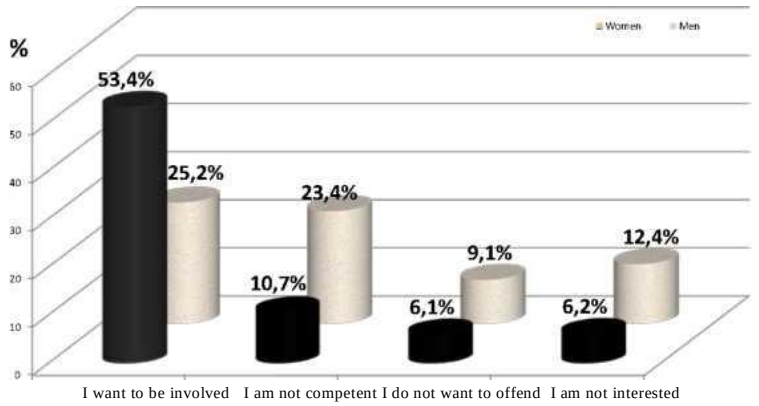

Fig. 3. Patients' involvement in the infection control procedures

DISCUSSION

The evaluation of the patient's perception on infection control procedures is particularly important in motivating the dentists to adopt the specific procedures for safety.

The assessment of the patients' attitude and perspective towards infection control revealed that majority of them trust the medical team and feel protected in the dental office (4). In this study concerns about safety procedures used by dentists are expressed especially by young people while older patients claim that they do not have the required competence to evaluate those aspects. The differences by gender were significant, over one third of men claiming that the infection control procedures are not of their concern. A high level of infection control awareness was seen in the female group of patients.

The study results reveal that the majority of the patients want the dentist to wear protective equipment in order to reduce the risk of infection transmission. Almost 90% of the subjects consider that protective gloves are indispensable, results comparable to those reported by other authors (5). The masks and goggles are also recommended to be worn by the dental team but less important for the patients, findings close to those presented in other studies (6, 7). Patients also expressed firm attitudes regarding the importance of routinely followed infection control procedures like hands hygiene, disinfection of the surfaces

after each patient and the sterilization of the instruments.

The educational level influenced the patients' wiliness to be involved in their own health care. The high level of education resulted in a high patients' implication in their own protection with major benefits on the safety of the dental treatment.

The relative high proportion of patients not willing to be involved in assessing the safety protocols during the dental treatment may be due to the confidence in the medical team but also to the lack of knowledge about the risks of infection and the measures required to reduce it.

CONCLUSIONS

The majority of the patients, especially men, feel protected against infection transmission but they require the medical team to wear protective equipment and adopt the safety protocols. The patients with high level of education desire to be involved in evaluation of the recommended infection control measures considering that an effective communication with the dentist guaranties an increased quality level of the dental treatment.

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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF SOME ANTIOXIDANTS

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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF SOME ANTIOXIDANTS (Abstract). Measurement of free radical scavenging capacity of antioxidants is influenced by a number of parameters that depend on reaction rate. **Aim**: A new method for assessing the free radical scavenging activity in which the influence of antioxidant concentration and reaction rate on chlorpromazine radical cation absorbance are simultaneously monitored. **Materials and methods**: The change in radical solution absorbance at 525 nm within a fixed time at different concentrations of the standard antioxidant – ascorbic acid (AA) are determined and percent inhibition is calculated. **Results**: The percent inhibition of color was plotted versus time, and the area under the curve was calculated for each concentration of the standard antioxidant. The calibration curve was obtained by plotting the area under the curve versus ascorbic acid concentration. The antioxidant activity of the samples was calculated using the regression line equation ($r^2 = 0.9991$) and expressed as ascorbic acid molar equivalents (AAE) depending on the unit of measurement chosen for the tested product. **Conclusions:** The proposed method takes into account the two parameters influencing the kinetics of the reaction between antioxidant and radicals, namely the antioxidant concentration and fixed time for measuring absorbance. **Keywords:** FREE RADICAL SCAVENGING, AREA UNDER THE CURVE.

To assess the antioxidant activity, some researchers have proposed a standardized protocol consisting of a series of steps aimed at identifying and quantifying the antioxidant action (1).

An important step in this protocol is the evaluation of free radical scavenging activity of the tested product. Free radical scavenging activity of the antioxidants is based on their ability to donate one hydrogen atom (HAT) or transfer an electron to free radical (ET).

The analytical methods for assessing the free radical scavenging capacity are based either on the detection of stable or unstable free radicals scavenging. Stable free radical chromophores are obtained in advance by a controlled oxidation reaction; their color diminishes following the interaction with the free radical scavenging antioxidant.

The most commonly used free radicals are: the $ABTS^{\bullet+}$ radical cation, obtained by chemical oxidation of 2,2'–azino–bis (3–ethylbenzothiazoline–6–sulfonic acid), which has maximum absorptions on VIS

and IR spectra at 415 nm, 645 nm, 734 nm, and 815 nm, respectively, and DPPH radical (1,1–diphenyl–2 picryl hydrazyl) having an absorption maximum at 516 nm due to the unbound electron on the nitrogen atom. These methods use Trolox, a synthetic, water-soluble vitamin E analogue, as a reference standard antioxidant. The main drawback of these methods is the use of artificial radicals that have not been identified in any biological system (2-5).

In living organisms, oxidation reactions produce short-lived, unstable free radicals as intermediates during oxidation process such as $OH^{\bullet}$ (hydroxyl radical), $R–O–O^{\bullet}$ (peroxyl radical), $O_2^{\bullet-}$ (superoxide radical anion) and NO (nitric oxide) or $ONOO^-$ (peroxynitrite).

The development of in vitro methods for assessing the ability of detecting such radicals includes, first of all, the generation of model-radicals on which the antioxidant to intervene. The literature cites various options for obtaining these short-lived radicals and evaluating the ability of some compounds to quench these radicals (6,7).

This paper proposes an improved method for the evaluation of free radical quenching activity using as chromophore indicator the radical cation obtained by controlled oxidation of chlorpromazine with potassium persulfate ($CP^{\bullet+}$).

Chlorpromazine radical cation is involved in a number of oxidation processes in biological systems and has enough stability to monitor color change under the action of antioxidants. The proposed method combines the influence of two factors involved in the kinetics of free radical quenching reaction: antioxidant concentration and reaction rate. The standard antioxidant is ascorbic acid.

MATERIAL AND METHODS

***Reagents*:**

- Chlorpromazine (Sigma) maleate solution 10^{-2} M;
- Potassium persulphate (Merck) solution 10^{-3} M;
- Hydrochloric acid (Farmitalia) solution 1 M;
- L (+)–scorbic acid (Merck) solution 2.10^{-4} M prepared at the time of use.

***Equipment*:**

- Hewlett Packard 8453 diode-array UV–VIS spectrophotometer;
- Scaltec analytical balance.

Chlorpromazine radical cation is prepared by oxidation of chlorpromazine maleate with potassium persulfate in aqueous solution acidified with hydrochloric acid (8).

Twenty minutes after the initiation of reaction, at a volume of 2 ml pre-formed radical solution 1 ml of standard ascorbic acid solution in different concentrations (10–100 μM/L) is added and then analyzed by VIS spectrophotometry at $\lambda = 525$ nm in a 1 cm cuvette against water. Absorbance of the solutions is determined at 10 minutes and 20 minutes after the addition of ascorbic acid.

Percent inhibition is calculated using the relationship:

$$\% \text{ Inhibition } = \frac{A_r - A_s}{A_r} \cdot 100$$

where A_s – sample absorbance
A_r – radical absorbance

Plot the variation of percent inhibition change during the time interval considered for each concentration value. The area under the curve is calculated. The calibration curve is obtained by plotting the values of the area under the curve against ascorbic acid concentration.

RESULTS AND DISCUSSION

Percent inhibition of radical solution absorbance was calculated at 10 minutes and 20 minutes after the addition of standard ascorbic acid solution and the area under the curve was calculated for each concentration in the studied range (10–100μM / L) (tab. I).

TABLE I

Area under the curve calculated in $CP^{\bullet+}$ – ascorbic acid (AA) system

AA (μM/L)	Absorbance		Inhibition (%)		AUC
	10 min.	20 min.	10 min.	20 min.	
0	0.53103	0.49920	–	–	–
10	0.49834	0.46651	6.155961	6.548478	63.52219
20	0.48185	0.45002	9.261247	9.851763	95.56505
30	0.46253	0.43070	12.89946	13.72196	133.1071
40	0.44335	0.41152	16.51131	17.56401	170.3771
50	0.42005	0.38822	20.89901	22.23157	215.6529
60	0.39976	0.36793	24.71988	26.29607	255.0798
70	0.38394	0.35211	27.69900	29.46514	285.8207
80	0.36262	0.33079	31.71384	33.73598	327.2491
90	0.34415	0.31232	35.19199	37.43590	363.1394
100	0.32653	0.29470	38.51007	40.96554	397.3781

Profile variation of the area under the curve against the concentration of standard antioxidant (ascorbic acid) is shown in figure 1.

By plotting the values of the area under the curve depending on ascorbic acid concentration the calibration curve was obtained (fig. 2).

Regression line was calculated statistically (tab. II).

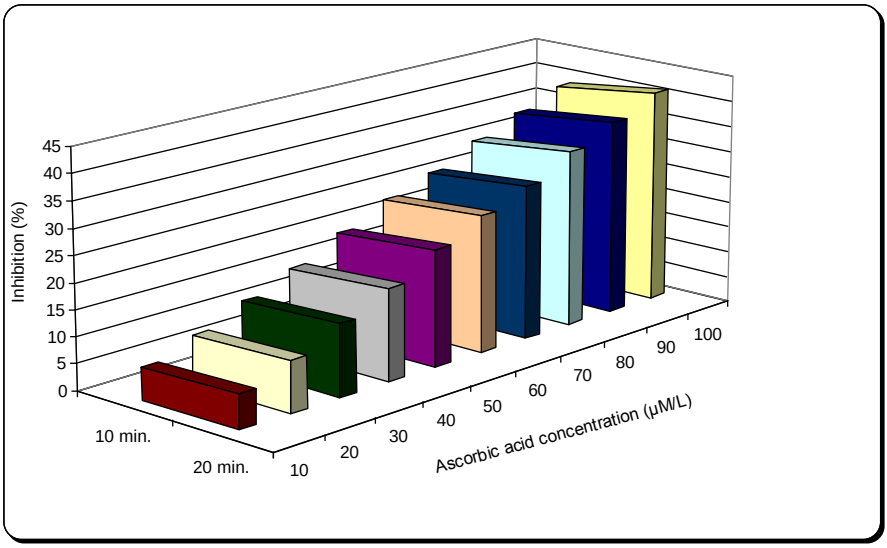

Fig. 1. Profile of area under the curve at different ascorbic acid concentrations

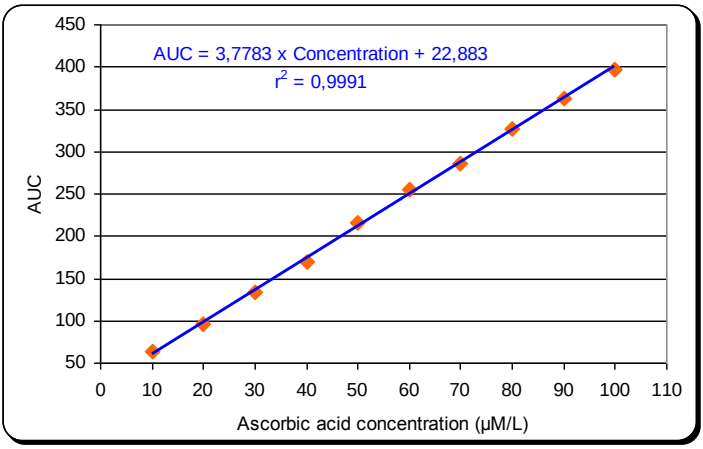

Fig. 2. Area under the curve (AUC) against ascorbic acid concentration

TABLE II

Statistical calculation of regression line

Parameters	AUC)
Equation for the regression line	AUC = 3.7783 x C + 22.883
Regression coefficient (r^2)	0.9991
Intercept	22.883
Slope	3.7783

In order to determine free radical scavenging activity of specific products the absorbance of the sample (obtained by the appropriate processing of the product) and radical cation has to be measured twice (previously fixed time), the same as in the case of the standard. The percent inhibition is calculated and then the area under the curve is determined by plotting the variation of percent inhibition against the fixed time interval. The equation of the calibration curve is used to calculate the antioxidant activity of the samples, expressed as ascorbic acid molar equivalents of (AAE) according to the unit of measurement chosen for the sample.

$$\text{Concentration } (\mu M/L) = \frac{AUC - 22.883}{3.7783}$$

The use of chlorpromazine radical cation in the development of a spectrometric method for determining the free radical scavenging capacity of antioxidants has two advantages. On the one hand, this radical is involved in oxidative processes in living organisms, and, on the other hand it is sufficiently stable that its change in absorbance in the presence of the antioxidant to be monitored with good accuracy.

By the proposed improved method the free radical scavenging activity is determined using a specific parameter for the evaluation of antioxidant activity, namely the area under the curve, as a measure of the contribution to total antioxidant activity of the analyzed products. This parameter combines the influence of variable factors that can alter the accuracy of measurements: fixed time measurement and antiox-

idant concentration.

CONCLUSIONS

We propose a spectrometric method for the evaluation of free radical scavenging activity based on the discoloration reaction of chlorpromazine radical cation chromophore (radical indicator) by ascorbic acid (standard antioxidant). This improved method uses as specific parameter the area under the curve that combines the variable factors influencing the kinetics of the reaction between radicals and antioxidants, namely antioxidant concentration and the time interval during which the determination is made. This parameter is a complete measure of the ability of antioxidants to capture free radicals. The proposed method is simple, requires standard equipment and can be adapted to assess the free radical scavenging activity of various products: herbs, cosmetics, food.

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BIOACTIVITY SCREENING OF *PINUS BRUTIA* BARK EXTRACTS: SUPEROXIDE DISMUTASE-LIKE AND NITRIC OXIDE SCAVENGING EFFECTS

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BIOACTIVITY SCREENING OF *PINUS BRUTIA* BARK EXTRACTS: SUPEROXIDE DISMUTASE-LIKE AND NITRIC OXIDE SCAVENGING EFFECTS (Abstract): In the Eastern Mediterranean region the wood of *Pinus brutia* Ten. is used as building material, the bark being the main waste of the wood production processes. A study was designed to explore possible medicinal applications for the bark waste. This paper reports on the superoxide dismutase-like and NO scavenging effects of bark extracts. **Material and methods:** Bark extracts (a raw hydromethanolic extract and its fractions) were initially screened regarding their ability to scavenge DPPH radical. The superoxide dismutase-like and NO scavenging effects were further evaluated. Catechin and quercetin were the positive controls in all antioxidant assays. **Results and discussion:** According to the EC_{50} values, all extracts (11.8 ± 0.1 – 21.10 ± 0.05 μg/ml) efficiently scavenged DPPH radical in comparison to quercetin (3.13 ± 0.05 μg/ml) and catechin (6.36 ± 0.05 μg/ml). The raw extract, diethyl ether and ethyl acetate fractions (449.46 ± 1.75, 115.43 ± 0.25 and 278.3 ± 2.3 μg/ml, respectively) exhibited higher superoxide dismutase-like effects in comparison to catechin (> 645.1 μg/ml). In NO scavenging assay, the raw extract, ethyl acetate and aqueous fractions (160.63 ± 0.85, 162.96 ± 0.52 and 160.23 ± 2.35 μg/ml, respectively) showed a scavenging ability similar to that of quercetin (156.76 ± 5.05 μg/ml) and higher than the one developed by catechin (242.66 ± 7.65 μg/ml). **Conclusions:** As superoxide anion and NO are important mediators in inflammation, our results support a possible use of *Pinus brutia* bark waste to develop nutraceuticals with efficiency in disorders involving oxidative and inflammatory stress.
Keywords: *PINUS BRUTIA* TEN., SUPEROXIDE DISMUTASE, SUPEROXIDE ANION, NITRIC OXIDE.

Both acute and chronic inflammations are characterized by a high reactive oxygen species (ROS) production. Besides pro-inflammatory mediators, leukocytes (neutrophils, macrophages) release ROS to destroy the source of inflammation (1). There are numerous studies supporting the involvement of superoxide anion, nitric oxide and their reaction product, peroxynitrite, in inflammation. Superoxide anion triggers inflammation by several mechanisms: damage of endothelial cells, increase in micro-

vascular permeability, generation of chemotactic factors such as leukotriene B4, infiltration of neutrophils at the site of inflammation *via* up-regulation of adhesion molecules such as ICAM-1 and P-selectin, generation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), induction of lipid peroxidation, poly-ADP-ribose polymerase activation. Regarding the latter mechanism, poly-ADP-ribose polymerase mediates inflammation by inducing cellular necrosis and NF-kB-dependent transcription. Many synthetic superoxide dismutase mimetics are tested on their potential to serve as candidates for the development of new anti-inflammatory agents (1, 2). Despite its well-known benefic effects (relaxation of blood vessels, inhibition of vascular smooth muscle cells proliferation and platelet aggregation and adhesion, decrease of expression of pro-inflammatory genes), NO is an important mediator in inflammatory processes. Pro-inflammatory cytokines induce the production of NO *via* inducible nitric oxide synthase (iNOS). NO generates higher nitrogen oxides which are mainly responsible for DNA damage. In addition, NO inhibits catalase, cytochrom P-450, ribonucleotide reductase (enzyme responsible for DNA synthesis) and couples to superoxide generating peroxynitrite. Peroxynitrite is a highly pro-inflammatory and cytotoxic reactive species; it nitrates tyrosine residues in proteins, depletes mitochondrial energy and induces DNA strand breaks (3, 4). In this respect, removal of superoxide or NO are important therapeutic means to reduce the inflammatory processes.

Pinus brutia Ten. (Pinaceae) is widely spread in the Mediterranean region, its wood being used as raw material in furniture industry or as building material. The aim of the present study was to explore possible therapeutic uses for the bark waste. Therefore a raw extract and four fractions were isolated from pine bark. This study investigated the DPPH, superoxide and NO scavenging effects of the raw extract and its fractions.

MATERIAL AND METHODS

Plant material. *P. brutia* bark was supplied by the Cyprus Department of Forests of the Ministry of Agriculture, Natural Resources and Environment in March 2008. Dried bark powder was extracted with 80% methanol (v/v). A crude extract (PbE) and four extractive fractions, namely diethyl ether (PbE1), ethyl acetate (PbE2), n-butanol (PbE3) and aqueous (PbE4) fractions, were obtained according to a procedure described previously (Elena Cretu, submitted).

DPPH radical scavenging assay. The ability to scavenge free radicals was assessed by the DPPH assay (5). Briefly, a DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol; the solution was further diluted with methanol to an absorbance of 1.00 ± 0.05 at 517 nm. Different concentrations of *P. brutia* extracts (50 µL) were mixed with DPPH solution (2.95 ml). The mixture was vortexed vigorously and the decay in absorbance at 517 nm was measured after 5 min reaction time. Catechin and quercetin were used as positive controls. The ability to scavenge the DPPH radical was calculated using the following formula: DPPH scavenging activity (%) = $100 \times (A_{start} - A_{end}) / (A_{start})$; A_{start} is the absorbance before adding the test sample and A_{end} is the absorbance after 5 min reaction time.

Superoxide dismutase-like activity assay. The superoxide dismutase-like (SOD-like) activity was evaluated using pyrogal-

lol system for the generation of superoxide anion radical (6). The reaction mixture consisted of 0.05 M Tris-HCl buffer (pH 8.0) containing 1 mM EDTA (2.8 ml), 6 mM pyrogallol (0.2 ml) and samples to be tested (0.1 ml). The absorbance of the mixture at 325 nm was measured every 30 s for 4 min. The dependence of absorbance on time was plotted for every sample (extract, positive control). Catechin and quercetin were the positive controls. The superoxide dismutase-like activity, expressed as superoxide anion radical scavenging activity, was calculated using the equation: SOD-like activity (%) = 100 × (1 – $slope_{sample}/slope_{control}$); $slope_{sample}$ and $slope_{control}$ are the slopes of the curves expressing the dependence absorbance *vs.* time for samples (*P. brutia* extracts, quercetin, catechin) and control, respectively.

Nitric oxide scavenging assay. In this assay, NO is generated by the spontaneous decomposition of sodium nitroprusside in an aqueous solution. The assay was performed according to a previously reported method with minor changes (7). Different concentrations of samples (*P. brutia* extracts, positive controls) (0.5 ml) were mixed with 10 mM sodium nitroprusside (0.5 ml) and phosphate saline buffer (0.5 ml). The mixture was incubated at 30ºC in dark. After 180 min 1% sulfanilamide in 5% phosphoric acid (0.5 ml) was added. The solution was left at 30ºC for 10 min followed by the addition of 0.1% naphtylethylenediamine dihydrochloride. The absorbance of the resulting chromophore was measured at 546 nm. Catechin and quercetin were used as positive controls. The ability to scavenge nitric-oxide was calculated using the formula: NO scavenging activity (%) = 100 × ($A_{control} – A_{sample}$)/$A_{control}$; $A_{control}$ is the absorbance of control and A_{sample} is the absorbance in presence of samples (*P. brutia* extracts, positive controls).

Statistical analysis. All assays were carried out in triplicate. The results were expressed as means ± SD. The EC_{50} values were calculated by linear interpolation between values above and below 50% activity.

RESULTS AND DISCUSSION

DPPH radical scavenging assay. This assay is widely used to screen the free radical scavenging ability of antioxidants. DPPH radical is a stable free radical showing a strong absorbance at 517 nm. Antioxidants scavenge DPPH radical by either hydrogen donation or electron transfer with a consequent decrease in absorbance at 517 nm (8). All *P. brutia* extracts scavenged DPPH radical in a concentration dependent manner. At 5.20 μg/ml the scavenging activity ranged from 17.44 ± 0.22% to 25.95 ± 0.04% while at 83.33 μg/ml all extracts showed scavenging effects higher than 80%. Within this assay, quercetin was more active than catechin (75.12 ± 0.51 *vs.* 41.39 ± 0.42% at 5.20 μg/ml; 96.98 ± 0.57 *vs.* 95.14 ± 0.55% at 83.33 μg/ml). According to the EC_{50} values, the DPPH radical scavenging activity decreased in the following order: quercetin > catechin > PbE2 > PbE3 > PbE > PbE4 > PbE1 (tab. I).

Superoxide dismutase-like activity assay. Within this assay, only PbE, PbE1 and PbE2 scavenged superoxide anion radical dose-dependently thus exhibiting SOD-like activity. As compared by the EC_{50} values, the most active was PbE1 (115.43 ± 0.25 μg/ml) followed by PbE2 (278.3 ± 2.3 μg/ml) and PbE (449.46 ± 1.75 μg/ml). PbE3 and PbE4 showed no activity in this assay. In the same experimental conditions,

catechin had weaker scavenging effects than *P. brutia* extracts. At 645.1 µg/ml, catechin showed only 20.41 ± 0.24% scavenging activity while PbE, PbE1 and PbE2 scavenged superoxide anion radical by 84.16 ± 0.62, 94.80 ± 0.66 and 91.38 ± 0.87%, respectively (tab. II). The scavenging activity of quercetin could not be evaluated due to its low solubility in the reaction medium.

TABLE I

DPPH radical scavenging activity of *Pinus brutia* bark extracts

Extract/ Positive control	Concentration (µg/ml)	% DPPH scavenging activity	EC_{50} (µg/ml)
PbE	5.20	17.44 ± 0.22	14.8 ± 0.2
	10.41	29.28 ± 0.53	
	20.83	70.28 ± 1.33	
	41.66	85.42 ± 0.29	
	83.33	92.03 ± 0.03	
PbE1	5.20	17.58 ± 0.77	21.10 ± 0.05
	10.41	30.11 ± 0.61	
	20.83	49.68 ± 0.05	
	41.66	68.91 ± 0.21	
	83.33	82.65 ± 0.23	
PbE2	5.20	25.95 ± 0.04	11.8 ± 0.1
	10.41	45.49 ± 0.32	
	20.83	70.80 ± 0.49	
	41.66	88.05 ± 0.35	
	83.33	92.55 ± 0.32	
PbE3	5.20	24.01 ± 0.24	12.6 ± 0.1
	10.41	43.15 ± 0.42	
	20.83	67.87 ± 0.20	
	41.66	87.16 ± 0.56	
	83.33	92.58 ± 0.31	
PbE4	5.20	22.42 ± 0.19	18.36 ± 0.35
	10.41	35.66 ± 0.47	
	20.83	53.16 ± 0.47	
	41.66	72.76 ± 0.38	
	83.33	87.89 ± 0.24	
Catechin	5.20	41.39 ± 0.42	6.36 ± 0.05
	10.41	71.54 ± 1.08	
	20.83	90.17 ± 0.28	
	41.66	91.33 ± 0.18	
	83.33	95.14 ± 0.55	
Quercetin	2.60	40.85 ± 0.92	3.13 ± 0.05
	5.20	75.12 ± 0.51	
	10.41	94.47 ± 0.83	
	20.83	96.54 ± 0.86	
	41.66	97.18 ± 0.87	
	83.33	96.98 ± 0.57	

TABLE II

Superoxide dismutase-like activity of *Pinus brutia* bark extracts

Extract/ Positive control	Concentration (µg/ml)	% Superoxide dismutase-like activity	EC_{50} (µg/ml)
PbE	161.2	1.99 ± 0.30	449.46 ± 1.75
	241.9	5.90 ± 0.99	
	322.5	12.95 ± 0.81	
	403.2	35.24 ± 0.55	
	483.8	60.00 ± 0.52	
	564.5	81.14 ± 0.21	
	645.1	84.16 ± 0.62	
PbE1	96.7	28.04 ± 0.41	115.43 ± 0.25
	129	63.80 ± 0.21	
	161.2	83.89 ± 0.44	
	241.9	90.89 ± 0.79	
	322.5	94.88 ± 0.51	
	403.2	90.97 ± 0.67	
	483.8	91.34 ± 0.99	
	564.5	90.04 ± 0.75	
	645.1	94.80 ± 0.66	
PbE2	161.2	5.71 ± 0.63	278.3 ± 2.3
	241.9	31.59 ± 0.88	
	322.5	69.39 ± 1.31	
	403.2	88.34 ± 1.23	
	483.8	92.52 ± 0.45	
	564.5	93.83 ± 0.48	
	645.1	91.38 ± 0.87	
PbE3	inactive at all concentrations		n.d.
PbE4	161.2	0.00 ± 0.00	n.d.
	241.9	0.00 ± 0.00	
	322.5	0.00 ± 0.00	
	403.2	0.00 ± 0.00	
	483.8	0.00 ± 0.00	
	564.5	0.00 ± 0.00	
	645.1	2.86 ± 0.81	
Catechin	161.2	6.8 ± 0.9	n.d.
	241.9	9.23 ± 0.54	
	322.5	12.68 ± 0.24	
	403.2	14.53 ± 0.23	
	483.8	18.39 ± 0.63	
	564.5	18.86 ± 0.88	
	645.1	20.41 ± 0.24	
Quercetin	n.t.	n.d.	n.d.

n.d.=not determined; n.t.=not tested.

Nitric oxide scavenging assay. NO, generated by the decomposition of sodium nitroprusside, interacts with oxygen generating nitrite and nitrate ions which can be spectrophotometrically quantified by Griess reaction. In the presence of NO scavengers, the production of nitrite ions is significantly reduced (7). The NO scavenging effects of *P. brutia* extracts were concentration dependent. PbE, PbE2 and PbE4 exhibited similar scavenging effects (EC_{50} = 160.63 ± 0.85, 162.96 ± 2.65 and 160.23 ± 2.35 µg/ml, respectively). The scavenging activity of PbE3 was significantly lower (EC_{50} = 219.26 ± 5.00 µg/ml). In case of PbE2, the scavenging activity could not be determined due to the low solubility of its constituents in the reaction medium. It is worthy to note that, according to the EC_{50} values, the scavenging effects of PbE, PbE2 and PbE4 were similar to that of quercetin (156.76 ± 5.05 µg/ml) but higher than the effect showed by catechin (242.66 ± 7.65 µg/ml). The NO scavenging effects decreased as follows: quercetin > PbE4 > PbE > PbE2 > PbE3 > catechin (tab. III).

TABLE III

Nitric oxide scavenging activity of *Pinus brutia* bark extracts

Extract/ Positive control	Concentration (µg/ml)	% Nitric oxide scavenging activity	EC_{50} (µg/ml)
PbE	156.25	48.67 ± 0.24	160.63 ± 0.85
	312.5	82.00 ± 0.37	
	625	88.01 ± 0.69	
	1250	92.92 ± 0.45	
PbE1	-	n.d.	n.d.
PbE2	156.25	48.60 ± 0.55	162.96 ± 0.52
	312.5	71.69 ± 0.50	
	625	85.46 ± 0.65	
	1250	91.74 ± 0.52	
PbE3	156.25	35.86 ± 0.74	219.26 ± 5.00
	312.5	64.83 ± 1.19	
	625	82.25 ± 0.35	
	1250	90.03 ± 0.61	
PbE4	156.25	49.08 ± 0.54	160.23 ± 2.35
	312.5	74.65 ± 0.76	
	625	85.32 ± 0.30	
	1250	92.35 ± 0.77	
Catechin	156.25	40.83 ± 0.83	242.66 ± 7.65
	312.5	55.27 ± 0.56	
	625	72.82 ± 0.50	
	1250	81.58 ± 0.63	
Quercetin	156.25	49.93 ± 0.66	156.76 ± 5.05
	312.5	64.19 ± 0.40	
	625	74.29 ± 0.29	
	1250	n.d.	

n.d.=not determined.

CONCLUSIONS

This study shows that several extracts isolated from *Pinus brutia* bark have high SOD-like and NO scavenging effects when compared to well known antioxidants such as catechin and quercetin. Both the raw extract (PbE) and two extractive fractions (PbE1, PbE2) exhibited significantly higher SOD mimic effects in comparison to catechin. Fractions PbE2 and PbE4, but also PbE showed NO scavenging potential similar to that of quercetin and higher than the one developed by catechin. These results support a possible use of *Pinus brutia* bark waste to develop nutraceuticals with efficiency in disorders involving oxidative and inflammatory stress. As our previous studies revealed high polyphenolic contents in all extracts, it is possible that, besides other phytochemicals, certain polyphenols mediate SOD-like and NO scavenging effects. Further *in vivo* studies are needed to a better assessment of the potential therapeutic uses of *Pinus brutia* bark extracts.

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15-LIPOXYGENASE INHIBITION, SUPEROXIDE AND HYDROXYL RADICALS SCAVENGING ACTIVITIES OF *CEDRUS BREVIFOLIA* BARK EXTRACTS

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15-LIPOXYGENASE INHIBITION, SUPEROXIDE AND HYDROXYL RADICALS SCAVENGING ACTIVITIES OF *CEDRUS BREVIFOLIA* BARK EXTRACTS (Abstract): *Cedrus brevifolia* (Hook. f.) Henry, a species endemic to Cyprus, has not been studied regarding its constituents and potential biological activities. **Material and methods:** A crude extract from *Cedrus brevifolia* bark and its four fractions (diethyl ether, ethyl acetate, n-butanol and aqueous fractions) were investigated regarding their capacity to inhibit 15-lipoxygenase and scavenge reactive oxygen species (superoxide anion and hydroxyl radicals). Catechin was used as positive control in all antioxidant assays. **Results and discussion:** In the superoxide anion radical scavenging assay, the crude extract showed the highest activity (EC_{50} = 57.73±1.25 µg/mL) comparable to that of the positive control, catechin (EC_{50} = 52.60±1.65 µg/mL). All other fractions exhibited noticeable scavenging effects against superoxide radical, their EC_{50} values ranging from 76.33±3.50 to 91.06±4.45 µg/mL. The ethyl acetate and n-butanol fractions were the most active in the hydroxyl radical scavenging (EC_{50} = 580.20±18.72 and 792.10±15.36 µg/mL, respectively) and 15-lipoxygenase inhibition assays (EC_{50} = 34.0±0.9 and 40.96±0.45 µg/mL, respectively). **Conclusions:** *Cedrus brevifolia* bark is a valuable source of compounds with reactive oxygen species and 15-lipoxygenase inhibitory activities. These results support further studies for the identification and isolation of the bioactive constituents. **Keywords:** *CEDRUS BREVIFOLIA*, 15-LIPOXYGENASE, SUPEROXIDE ANION RADICAL, HYDROXYL RADICAL

In living cells, reactive oxygen species (ROS) (superoxide anion, hydroxyl, peroxyl and alkoxyl radicals, hydrogen peroxide, singlet oxygen, hypochlorous acid) are produced during normal activities such as mitochondrial respiration, peroxisomal beta-oxidation, cytochrome P450 metabolism. Pathological processes such as inflammation, exposure to ultraviolet and gamma-irradiation are additional sources of ROS (1). In low levels, ROS have important physiological roles (defence against pathogens, cellular signaling) while in high concentrations they become harmful as they damage cells constituents such as lipids, proteins, carbohydrates, DNA (oxidative stress). Oxidative stress plays a key role in the development of cancer, cardiovascular, neurodegenerative and rheumatic diseases, diabetes mellitus (2, 3). One of

the most important factors associated with carcinogenesis is DNA damage produced by ROS. During cell division, these oxidative lesions lead to mutations of the genes that regulate cell growth and differentiation. Lipid peroxidation indirectly induces DNA mutagenic lesions. Final products of lipid peroxidation (epoxides, aldehydes) diffuse from the site of production and bind covalently to DNA, leading to exocyclic adducts with mutagenic potential. The overexpression of NOX enzymes (NADPH oxidases that transfer electrons across membranes generating superoxide anion radical and other ROS) is implicated in ischaemia/reperfusion injury, hypertension and neurodegeneration but also in age-associated hearing loss, cataract and retinal degeneration (1-3). In rheumatoid arthritis, ROS damage the cartilage, extracellular colagen and DNA (4). In diabetes mellitus, the increased production of superoxide anion radical activates polyol and hexosamine pathways, increases the production of advanced glycation end products (AGEs) and the expression of their receptors thus leading to diabetes complications (atherosclerosis, cardiomyopathy) (5). Antioxidants are able to delay or prevent diseases associated with oxidative stress (2, 3). Therefore, there is an increasing interest in finding new antioxidants, especially from natural sources.

Cedrus brevifolia (Hook. f.) Henry (Cyprus cedar) is a conifer belonging to: division-*Pinophyta*, class-*Pinopsida*, order-*Pinales*, family-*Pinaceae*, genus-*Cedrus*. The present natural geographic distribution of *Cedrus* species is limited to the Himalayan Mountains (1500-3200 m) and the Mediterranean coast (1000-2200 m) (6). Few studies on *C. brevifolia* reported an increased sensitivity and a limited growth rate in drought conditions in comparison with other species of cedar (*C. libani*, *C. atlantica*) (7, 8). A genetic diversity study using AFLP (*Amplified Fragment Length Polymorfism*) fingerprinting technique was performed on 17 populations of cedar. For *C. brevifolia*, found in small and fragmented populations, one of the highest levels of intrapopulational diversity was noticed. This unexpected pattern of diversity and differentiation suggests a population in evolution rather than one in decline (9).

The present study was designed to evaluate the antioxidant potential (15-lipoxygenase inhibition, superoxide and hydroxyl radicals scavenging activities) of *C. brevifolia* bark extracts.

MATERIAL AND METHODS

Chemicals. (+) Catechin hydrate, nitrobluetetrazolium (NBT), phenazine methosulphate (PMS), iron (II) sulfate heptahydrate, linoleic acid, lipoxidase from soybean (15-LO), hydrogen peroxide solution were obtained from Fluka (Steinheim, Germany). Sodium salicylate and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Steinheim, Germany). Tris (hydroxymethyl)aminomethane (Tris) and beta-nicotinamide adenine dinucleotide reduced (NADH) were supplied by Merck (Darmstadt, Germany). All other solvents and reagents were of analytical grade.

Equipments. For UV/VIS measurements, a Jasco V-550 spectrophotometer was employed.

Plant material.C. brevifolia bark was supplied by the Cyprus Department of Forests of the Ministry of Agriculture, Natural Resources and Environment in March 2008.

Preparation of bark extracts. Dried and powdered bark was extracted with 80%

methanol (v/v); the crude extract (CbE) was further fractionated by successive liquid-liquid partition. The diethyl ether (CbE1), ethyl acetate (CbE2), n-butanol (CbE3) and aqueous (CbE4) fractions were obtained according to a methodology described previously (Elena Crețu, submitted). The polyphenolic content and profile of *C. brevifolia* bark extracts were studied and reported (Elena Crețu, submitted).

15-Lipoxygenase inhibition assay. Extracts and catechin (positive control) were dissolved in DMSO in concentrations ranging from 0.31 to 5 mg/mL. The reaction mixture consisted of extracts/catechin and 0.2 M borate buffer (pH 9.0) containing 15-LO (10000 U/mL). After 10 min incubation at 25°C, the substrate (linoleic acid in borate buffer) was added. Absorbance of the reaction mixture was measured at $\lambda = 234$ nm after 30 and 90 s reaction time. Percent 15-LO inhibition was calculated using the formula:

Inhibition of 15-LO (%) = $100 \times [(A_{control,\ 90} - A_{control,\ 30}) - (A_{sample,\ 90} - A_{sample,\ 30})/(A_{control,\ 90} - A_{control,\ 30})]$, where $A_{control,\ 30}$ and $A_{control,\ 90}$ are the absorbances of the control after 30 and 90 s reaction time, respectively. $A_{sample,\ 30}$ and $A_{sample,\ 90}$ are the absorbances of the mixtures containing extracts/catechin after 30 and 90 s reaction time, respectively (10). The assay was carried out in triplicate and the results were expressed as mean values ± SD.

Superoxide anion radical scavenging assay. Extracts and catechin (positive control) were dissolved in DMSO in a concentration range of 0.039 − 1.25 mg/mL. The reaction mixture consisted of extracts/catechin and 16 mM Tris-HCl buffer (pH 8.0) containing 557 μM NADH, 45 μM PMS and 108 μM NBT. Absorbance of the reaction mixture was measured at $\lambda = 560$ nm after 5 min incubaton at 25°C. Percent superoxide anion radical scavenging activity was calculated as follows:

Superoxide anion radical scavenging activity (%) = $100 \times (A_{control} - A_{sample})/A_{control}$, where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance in presence of extracts/catechin (11). The assay was carried out in triplicate and the results were expressed as mean values ± SD.

Hydroxyl radical scavenging assay. Extracts and catechin (positive control) were dissolved in DMSO in different concentrations ranging from 4 to 12 mg/mL. The final reaction mixture contained extracts/catechin, 1.5 mM iron (II) sulfate heptahydrate, 20 mM sodium salicylate and 6 mM hydrogen peroxide. The mixture was incubated at 37°C for 30 min. After cooling, the absorbance was measured at $\lambda = 562$ nm. Percent hydroxyl radical scavenging activity was calculated using the formula:

Hydroxyl radical scavenging activity (%) = $100 \times (A_{control} - A_{sample})/A_{control}$, where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance in presence of extracts/catechin (12). The assay was performed in triplicate and the results were expressed as mean values ± SD.

RESULTS AND DISCUSSION

15-Lipoxygenase inhibition assay. 15-LO plays a major role in the development of atherosclerosis. The enzyme oxidizes circulating low-density lipoproteins (LDL) initiating the formation of the atherosclerotic plaques in the arterial wall. 15-LO is also responsible for other processes involved in atherogenesis such as endothelial injury, proinflammatory cytokine release and cytolysis by modulating numerous cellular signaling pathways (13). In this

assay, all extracts inhibited 15-LO in a concentration-dependent manner. At the highest concentration tested (5 mg/mL), CbE3 (97.16±0.34%) and CbE2 (97.08±0.76%) were more active than the positive control, catechin (88.30±1.48%) (fig. 1). The EC_{50} values confirmed that CbE2 (34.0±0.9 µg/mL) and CbE3 (40.96±0.45 µg/mL) were the most active among *C. brevifolia* extracts but slightly less active than catechin (29.43±0.55 µg/mL) (tab. I).

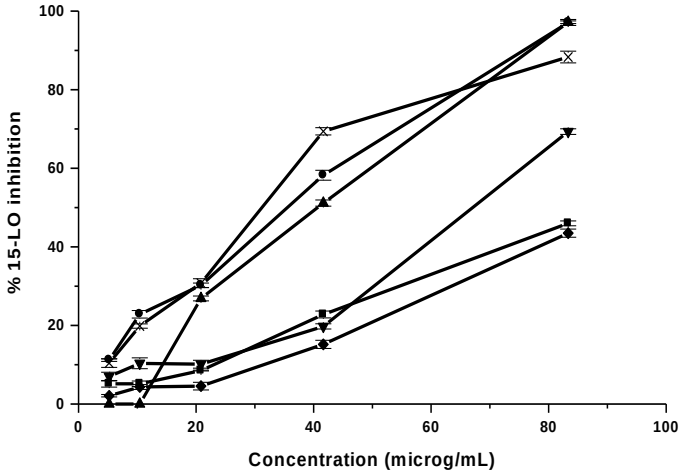

Fig. 1. 15-Lipoxygenase inhibitory effects of *Cedrus brevifolia* bark extracts (▼CbE; ■ CbE1; ● CbE2; ▲ CbE3; ◆ CbE4; × catechin)

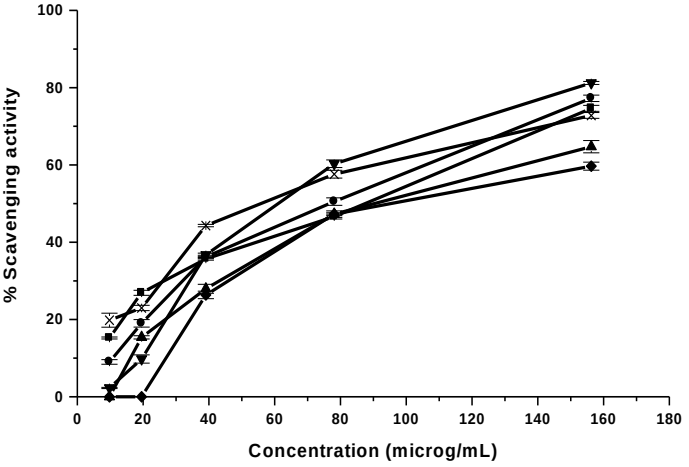

Fig. 2. Superoxide anion radical scavenging activity of *Cedrus brevifolia* bark extracts (▼CbE; ■ CbE1; ● CbE2; ▲ CbE3; ◆ CbE4; × catechin)

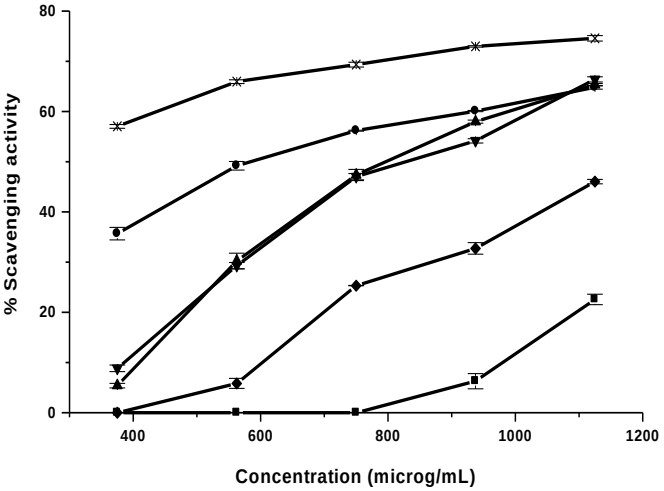

Fig. 3. Hydroxyl radical scavenging activity of *Cedrus brevifolia* bark extracts (▼CbE; ■ CbE1; ● CbE2; ▲ CbE3; ◆ CbE4; × catechin)

TABLE I

Antioxidant activities of *Cedrus brevifolia* bark extracts

Extract/ Positive control	EC_{50}(µg/mL)		
	15-LO inhibition assay	Superoxid anion radical scavenging assay	Hydroxyl radical scavenging assay
CbE	63.6 ± 0.6	57.73 ± 1.25	822.9 ± 13.9
CbE1	> 83.33	84.93 ± 1.35	> 1125
CbE2	34.0 ± 0.9	76.33 ± 3.50	580.20 ± 18.72
CbE3	40.96 ± 0.45	87.20 ± 1.95	792.10 ± 15.36
CbE4	> 83.33	91.06 ± 4.45	> 1125
Catechin	29.43 ± 0.55	52.60 ± 1.65	318.40 ± 4.45

Superoxide anion radical scavenging assay. Superoxide anion radical is generated *in vivo* during mithocondrial respiration; it is also produced by phagocytic cells to kill pathogens. Superoxide radical plays important roles in many physiological processes such as gene transcription, cell growth and proliferation, apoptosis. Despite its beneficial effects, superoxide radical gives rise to more harmful ROS. It can react with nitric oxide to form peroxynitrite anion, a highly cytotoxic species. In addition, it reacts with hydrogen peroxideyielding hydroxyl radical (Haber-Weiss reaction); the latter is very deleterious for all biomolecules in or near its generation site. Superoxide radical-induced oxidative stress is involved in the development of many pathological conditions and diseases (inflammation, cardiovascular and neurodegenerative disorders, cancer, ageing) (14-18). As shown in fig. 2, the superoxide radical scavenging activities of *C. brevifolia* bark extracts were concentration-

dependent. At 1.25 mg/mL, CbE was the most active showing 81.21±0.38% scavenging activity, followed by CbE2 (77.25±0.83%), CbE1 (74.63±0.77%), CbE3 (64.74±1.58%) and CbE4 (59.68±1.02%); at the same concentration, catechin showed 72.81±0.76% scavenging activity. According to the EC_{50} values, CbE(57.73±1.25 µg/mL) was the most active, its scavenging effect being comparable to that of catechin (52.60±1.65 µg/mL) (tab. I).

Hydroxyl radical scavenging assay. Hydroxyl radical is generated *in vivo* by the transition metal-induced reduction of hydrogen peroxide (Fenton reaction). It is considered to be the most aggressive ROS leading to oxidative damage of polyunsaturated fatty acids, polypeptide chains and DNA bases. It is worthy to note that hydroxyl radical is responsible for lipid peroxidation, a process that alters both the structure and functions of the cell membranes causing cell damage. Besides, hydroxyl radical is known to be involved in quinone toxicity, radiation injury, ischemia-reperfusion injury, diabetes, cancer and ageing. (17-20). At 12 mg/mL the hydroxyl radical scavenging activity of CbE reached 66.39±0.52%. Similar activities were recorded for CbE2 (64.81±0.36%) and CbE3 (65.34±0.24%) while CbE1showedonly 22.57±1.03% activity (fig. 3). With regard to the EC_{50} values, CbE2 (580.20±18.72 µg/mL) was the most active hydroxyl radical scavenger; its activity was 1.8 times lower than that of catechin (318.40±4.45 µg/mL) (tab. I).

CONCLUSIONS

The results of this study show that *C. brevifolia* bark is an important source of compounds with 15-LO inhibitory and reactive oxygen species scavenging effects. The fractionation of the crude extract (CbE) led to fractions with higher activity in 15-LO inhibition and hydroxyl radical scavenging assays. The ethyl acetate (CbE2) and n-butanol (CbE3) fractions showed remarkable 15-LO inhibitory activity in comparison with catechin. In addition, CbE2 and CbE3 were highly active as hydroxyl radical scavengers. The fractionation did not increase the scavenging ability towards the superoxide anion radical as CbE was the most active among the bark extracts. However, it is worthy to note that all fractions showed high scavenging effects towards the superoxide anion radical. All these findings confirm the potential therapeutic value of *C. brevifolia* bark and support further studies for the identification and isolation of the bioactive constituents.

ACKNOWLEDGEMENTS

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PREVENTIVE MEDICINE - LABORATORY **ORIGINAL PAPER**

TRIPLEX REAL-TIME PCR FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND PANTON-VALENTINE LEUKOCIDIN IN NORTH-EAST ROMANIA

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TRIPLEX REAL-TIME PCR FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND PANTON-VALENTINE LEUKOCIDIN IN NORTH-EAST ROMANIA (Abstract): The aim of the present study was to investigate *S. aureus* isolates for the presence of methicillin-resistance and Panton-Valentine leukocidin (PVL) genes and to further characterize positive strains by means of antibiotic resistance patterns. **Material and Methods**: We used a triplex Real-Time PCR method for simultaneous detection of *nuc*, *mec*A and *pvl* genes in clinical isolates from 188 patients admitted to "Sf. Parascheva" Infectious Diseases Hospital Iaşi, during a 3 year period (2008-2010). **Results** The study revealed a relatively high rate of PVL-producing strains (23.93%), mainly community-associated (CA-MRSA) (51.11%). Most *pvl*-positive CA-MRSA isolates were resistant to erythromycin (91.3%), but none was resistant to clindamycin, fluoroquinolones, rifampicin, chloramphenicol or fusidic acid. **Conclusions:** Antibiotic susceptibility testing showed a high rate of multidug-resistance among strains classified as CA-MRSA (54.83%), but not among PVL-producers (4.44%). Although resistance to fusidic acid was previously proposed as a marker for PVL-producing CA-MRSA, our data suggest that we cannot rely on resistance to fusidic acid to screen for PVL-producing CA-MRSA in our setting. **Keywords**: MRSA, MECA, PVL.

Staphylocccus aureus is an important hospital and community-acquired pathogen that causes a broad spectrum of diseases, for which the currently available treatments are at risk of becoming less effective, as this bacterium is able to rapidly acquire resistance to antibiotics. The mobile element SCC*mec* carries *mec*A gene encoding a low-affinity PBP2a that confers resistance to beta-lactam antibiotics (1). Methicillin-resistant *S. aureus* (MRSA) is a common agent of nosocomial infections (HA-MRSA) and is now increasingly implicated in infections among persons in the community without established risk factors for MRSA acquisition (CA-MRSA) (2). *S. aureus* pathogenicity is related to a wide variety of virulence factors that allow it to colonize, invade and evade the immune system. These factors include Panton-Valentine leukocidin (PVL), a pore-forming cytotoxin, encoded by *lukF-PV* and *lukS-PV* genes. The toxin targets polymorphonuclears and contributes to tissue necrosis. Infec-

tions caused by PVL-producing strains are characterized by an increased systemic inflammatory response, decreased survival in patients with community pneumonia and increased severity of localized lesions (3). PVL has been linked to skin and soft tissue infections (SSTIs), necrotizing pneumonia, bone and joint infections and was associated with CA-MRSA infections that affect young healthy individuals with no exposure to healthcare centers (4).

Our aim was to evaluate the prevalence of PVL-producing MRSA strains in "Sf. Parascheva" Infectious Diseases Hospital Iaşi, using a triplex RT-PCR technique that allows rapid and simultaneous detection of *nuc*, *pvl* and *mec*A genes. This study constitutes a preamble to the use of multiplex RT-PCR directly on clinical specimens. We also analyzed the phenotypic antimicrobial resistance profiles among CA-MRSA, HA-MRSA and methicillin-susceptible *S. aureus* (MSSA). Multi-drug resistance (MDR) among MRSA was defined as concurrent resistance to three or more non-beta-lactam antimicrobial agents.

MATERIAL AND METHODS

Clinical isolates. In total, 188 non-duplicate *S. aureus* isolates from "Sf. Parascheva" Infectious Diseases Hospital Iaşi were evaluated in the present study. The strains were isolated from pus (102 strains), blood-cultures (BC) (46 strains), cerebrospinal fluid (CSF) (6 strains), sputum (18 strains) or from multiple specimens (16 strains), from 2008 to 2010.

Triplex Real Time PCR. The isolates were analyzed for the presence of *mec*A, *pvl* and *nuc* genes using a triplex RT-PCR method previously described (5). Nucleic acid was extracted using *GenElute Bacterial Genomic DNA kit* (Sigma Aldrich, Germany). Primers and probes were synthesized based on oligonucleotide sequences previously described by McDonald *et al.*(6), and were used in concentrations of: 0.30 μM *mec*A primers, 0.1 μM *mec*A probes, 0.40 μM *pvl* primers, 0.1 μM *pvl* probes, 0.05 μM *nuc* primers, 0.05 μM *nuc* probes. Reactions were performed with *Brilliant Multiplex QPCR Master Mix* (Agilent Technologies, USA) under the following thermal cycling conditions: 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 55°C for 1 min. Simultaneous amplification of target genes was detected with Stratagene MX3005P system (Agilent Technologies, USA). *S. aureus* ATCC 25923, 33592 and 49775 were used as controls of amplification.

Antibiotic susceptibility testing. Antimicrobial susceptibility patterns were evaluated using ATB STAPH 5 strips (bioMérieux, France) and disk diffusion method, as recommended by CLSI guidelines 2008-2010. We tested the sensitivity to the following antibiotics: penicillin (P), oxacillin (OX), cephoxitin (FOX), clindamycin (CM), erythromycin (E), tetracycline (TE), gentamicin (G), rifampicin (RIF), trimethoprim/sulfamethoxazole (SXT), fluoroquinolones (FQ), chloramphenicol (C), linezolid (LZD), teicoplanin (TEC) and fusidic acid (FUS). Inducible resistance to CM was assessed by double-disk (E and CM) diffusion method (D-zone test). Minimum inhibitory concentrations (MICs) for vancomycin (VA) and OX were determined using E-test method (bioMérieux, France).

RESULTS

We detected *mec*A gene in 86 isolates (45.74%), while phenotypic testing failed to detect methicillin-resistance in 5 cases.

Thus, the sensitivity of 1 µg oxacillin disk testing was 95.34% and the sensitivities of 30 µg cephoxitin disk testing and ATB STAPH 5 strips were 97.67%, compared to *mec*A detection as gold standard. MICs for OX varied between 0.094-256 µg/ml, with a heteroresistance rate of 21.27% of the total number of strains. Of the total number of tested strains, 45 were *pvl*-positive (23.93%), representing 33.72% of MRSA isolates. Most *pvl*-positive strains were isolated from pus (82.22%), the rest being isolated from BC (6), CSF + BC (1) and CSF + BC + sputum (1). Clinical evolution for those patients was as follows: death (2 cases), aggravated (1), unchanged (6), improved (26) and healed (10). Out of all *pvl*-positive strains, 16 were MSSA, with 6 different resistance patterns, the most common being resistance to P (8 strains); one strain was susceptible to all antibiotics (tab. I).

TABLE I
Antibiotic resistance patterns for MSSA *pvl*-positive strains

Resistant strains	P	G	CM	E	TE
8	+				
3	+	+			
1	+			+	
1	+		+		
1	+				+
1	+	+		+	+

Among PVL-producing MRSA (29 strains), 7 resistance profiles were described, the most common being single-drug resistance to E (22 strains); of those strains, two were MDR (tab. II). None of the *pvl*-positive strains showed inducible CM resistance and all of them were susceptible to C, LZD, TEC, VA and FUS. Among PVL-non producing strains,44 different resistance profiles were detected, most of them being MSSA resistant to P (27.97%) and MRSA multidrug-resistant to E, CM and TE (11.18%).

TABLE II
Antibiotic resistance patterns for MRSA *pvl*-positive strains

Resistant strains	G	CM	E	TE	FQ	SXT	RIF
22			+				
2	+		+				
1	+						
1	+						+
1				+	+		
1		+	+	+			
1	+		+	+		+	

Community-onset infections were defined as those episodes with a positive culture within 48h of admission, without health care-associated risk factors (history of hospitalization in the past year, surgery, renal dialysis, residence in a long-term care facility, presence of percutaneous medical devices or positive MRSA culture within the preceding 1 year). Based on this definition, 32.97% of the total number of isolates and 72.09% of MRSA strains were considered CA-MRSA; out of those, 37.09% were PVL-producing strains. A comparison of antibiotic resistance between CA-MRSA and HA-MRSA strains reveals a higher resistance rate for TE, G, RIF and FQ in the case of HA-MRSA isolates, while resistance rates to SXT, C and FUS were similar (fig. 1).

Out of 86 MRSA strains, 54 were MDR, the majority being classified as CA-MRSA (34/54), with the most frequent resistance profile: E, CM and TE (10 strains). Most

MDR strains classified as HA-MRSA were additionally resistant to G, RIF and FQ (9 strains). Only 2 *pvl*-positive strains were MDR, representing 3.7% of MDR strains.

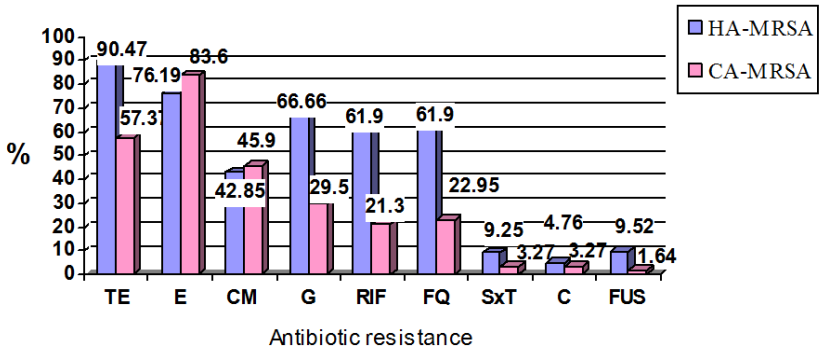

Fig. 1. Antibiotic resistance of CA-MRSA *versus* HA-MRSA strains

DISCUSSION

MRSA rate determined by this study (45.74%) is concordant with previously published data by Dorneanu *et al* (47%)(7) and with reports from EARS-Net, placing Romania on one of the first places on the incidence of invasive MRSA infections (39.1%)(8). Correct identification of methicillin-resistance is needed for effective monitoring of MRSA spread. Since conventional methods of antibiotic susceptibility testing sometimes fail to detect hetero-resistant strains, molecular techniques were developed as standard tests for MRSA confirmation. These techniques are not accessible for routine testing in clinical laboratories, so evaluation of sensitivities for most frequently used phenotypic methods is imposed. Our data regarding MRSA detection were similar to those reported by Anand *et al*. (9) and Pottumarthya *et al*. (10), showing a sensitivity of 30 µg cephoxitin disk testing of 100% and 99.0% respectively, while a weaker concordance was observed when 1 µg oxacillin disk was used (sensitivity 87.5% and 94.7% respectively).

The emergence and community dissemination of new and virulent MRSA strains harboring PVL constitutes a public health concern (11, 12). Some authors anticipate the replacement of HA-MRSA strains by community strains infiltrating the hospital environment due to their colonization properties (13). The majority of CA-MRSA causes SSTIs, but certain cases can progress to invasive tissue infections and bacteremia (14). Usually, these strains are sensitive to most non-beta-lactam antibiotics and have a low level of methicillin-resistance, often expressed heterogeneously. Our study found an oxacillin heteroresistance rate in CA-MRSA of 45.16% and a prevalence of MDR among these strains of 54.83%, with resistance to: TE (94.11% of MDR strains), E (91.17%), CM (79.41%), G (44.11%), FQ (41.17%), RIF (38.23%), SXT (5.88%), C (5.88%), FUS (5.88%). These data are concordant with observations that indicate a blurring of the boundaries between community and hospital, with CA-MRSA becoming more resistant to antibiotics (15).

The role of PVL toxin to the virulence of *S. aureus* has been disputed due to inconclusive results of *in vivo* studies using mice. But epidemiological data and studies on rabbit model, whose neutrophils are as

sensitive to PVL action as human cells, are linking necrotizing skin infections with PVL-production (16). More than 250 articles published during 2002-2007 identified an association between PVL and CA-MRSA disease outbreaks (17). Hedin *et al*. (18) showed that PVL is a stable genetic marker for CA-MRSA infections, 96% of *pvl*-positive strains being CA-MRSA, while 81% of *pvl*-negative strains were HA-MRSA. Fang *et al.*(19) reported that 56 % of 104 CA-MRSA strains carried *pvl* genes and a similar rate (66%) was detected by Berglund *et al*.(20). Our study showed a relative high rate of *pvl*-positive isolates (23.93%), when compared to the results of Holms *et al.* (21) that noted a low rate of PVL-producers among *S. aureus* isolates (1.6%).

Most of *pvl*-positive strains analyzed were CA-MRSA (23 / 45, 51.11% respectively). Only two PVL-producing CA-MRSA strains were MDR, showing resistance to E, G, TE, one strain being also resistant to SXT. Most *pvl*-positive CA-MRSA isolates (21/23) were resistant to E, but none was resistant to CM, FQ, RIF, C or FUS. The 100% sensitivity to FUS among those strains is discordant with reports suggesting that fusidic acid resistance can be used for identification of PVL-producing CA-MRSA (22). In our study none of the PVL-producing strains showed resistance to FUS.

CONCLUSIONS

Multiplex RT-PCR is a useful tool for rapid detection of MRSA and identification of genes encoding the synthesis of virulence factors. The introduction of this technique in our laboratory allowed us to verify the results of phenotypic testing, which showed good sensitivity in the detection of MRSA (95.34 – 97.67%). Detection of *pvl* genes revealed a relatively high rate of PVL-producing strains (23.93%) that were associated mostly with CA-MRSA strains and a particular antibiotic resistance profile with resistance to E and sensitivity to CM, FQ, RIF, C and FUS. According to our data, resistance to fusidic acid, although previously proposed as a marker for PVL-producing CA-MRSA, cannot be used to screen for those strains in our setting. Antimicrobial susceptibility testing showed a high rate of MDR among CA-MRSA (54.83%), but not among PVL-producers. This raises the problem concerning the tendency of community strains to spread into hospitals and gain resistance.

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DIVALENT CATIONS LEVELS CHANGES IN NEPHROTIC SYNDROME

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DIVALENT CATIONS LEVELS CHANGES IN NEPHROTIC SYNDROME (Abstract): Divalent cations (calcium, magnesium, zinc, cooper, manganese) play an important role in various biological processes. Different acute or chronic renal disorders in children or adults modify the urinary excretion of these cations and may influence their concentrations in organism. **Aim:** Evaluation of the changes of some divalent cations levels (Cu, Zn, Mg, Ca) in acute renal diseases. **Material and methods:** We measured plasma concentrations and urinary excretion of cations in pediatric patients with acute urinary infections. We also evaluated malondyaldehide (MDA) and total antioxidant capacity (TAC) plasma levels. **Results:** The obtained results show that serum levels of Ca, Cu, Zn are decreased in patients with acute urinary infections compared with a control group of healthy children, while urinary excretion of Cu and Zn there were higher in group study compared with control group. There are no significant differences of the serum magnesium concentration. Increased plasma MDA levels and decreased plasma TAC, Cu and Zn concentrations indicate an increased oxidative stress in patients with acute renal diseases. **Conclusions:** Our preliminary data show that in some acute urinary conditions, such as lower urinary tract infection and pyelonephritis, appear disturbances of plasma and urinary concentrations of divalent cations. We consider that trace elements should be measured routinely in children with renal disorders. **Key words**: ZINC, COPPER, MAGNESIUM, NEPHROTIC SYNDROME

Divalent cations (calcium, magnesium, zinc, copper, manganese) play multiple roles in all human tissues and systems. These cations can be found both intracellular and extracellular and there are complex systems for their transport through the cellular membrane, and also for their concentration regulation in the human body (1, 2). The activity of more than 300 enzymes is magnesium dependent, and a similar number of enzymes are dependent of zinc.

Copper (Cu) is an essential trace element that acts as a critical cofactor when is incorporated into specific copper-enzymes that catalyze electron transfer reactions required for cellular respiration, iron oxidation, neurotransmitter biosynthesis, antioxidant defense. Copper is both a pro-oxidant and antioxidant. Its antioxidant activity has been attributed to increased CuZnSOD activity.

Zinc (Zn) is an intracellular signaling molecule and it plays an important role in cell-mediated immune functions and oxidative stress. It is also an anti-inflammatory agent and an antioxidant (3).

The human body contains 2-3 g zinc,

most of which is bound to proteins. Over 300 enzymes have been shown to contain zinc, either directly involved in catalysis, as a cofactor, or for structural stabilization. Another large group of zinc containing proteins is transcription factors. Despite its important function, the body has limited zinc stores that are easily depleted and cannot compensate longer periods of zinc deficiency.

Zinc deficiency is a cause of immune dysfunction and infection. Previous human studies have shown that the activation of the acute phase response alters zinc metabolism.

The kidney plays a significant role in magnesium homeostasis. Proximal tubular magnesium reabsorption is proportional to sodium reabsorption, and a reduction in sodium reabsorption during long-term intravenous fluid therapy may result in magnesium deficiency.

Different acute or chronic renal disorders in children or adults modify the urinary excretion of these cations and may influence their concentrations in organism (4, 5). At present there are little data about how the therapy administrated in nephrotic syndrome influences the concentration of divalent cations. Kidney is the main elimination route for all divalent cations.

In this study we investigated the changes of some divalent cations levels (Cu, Zn, Mg, Ca) in pediatric patients with acute renal diseases.

MATERIAL AND METHODS

The study group was consisted of 18 children aged from 2 to 17 years, with acute nephropathies such as urinary tract infection and acute pyelonephritis, admitted in the nephrology clinic of Sf. Maria Children's Hospital Iasi. We collected urine and blood samples and we measured the urine and plasma levels of magnesium, zinc and copper cations through atomic absorption method. We also measured the levels of serum MDA and TAC. Serum MDA levels were measured by thiobarbituric acid method and for TAC determination we used RANDOX kit. The same measurements were carried out as well for a control group consisted of 10 children aged from 2 to 15 years, with a normal health status.

RESULTS

Plasma levels of copper were decreased with 11% in children with renal disorders in comparison with control group, and plasma levels of zinc were decreased with 18% ($p<0.01$). Urinary levels of Cu and Zn there were significantly higher in group study compared with control group. There were found no significant modifications of the plasma or urinary magnesium concentrations between the study group and control group. MDA plasma levels (nmol/mL) were increased in patients with acute nephropathies compared with control group (2.12 ± 0.27 vs. 0.48 ± 0.15). Levels of serum TAC (mmol/L) were found significant decreased in study group in comparison with control group (1.16 ± 0.09 vs. 1.71 ± 0.14).

DISCUSSION

Our data are consistent with the literature data concerning clinical studies of divalent cation levels in renal diseases. There are few data about seric and urinary divalent cations changes related to renal diseases.

A study performed on 105 pediatric patients with nephrotic syndrome or nephritis-nephrosis which evaluated plasma zinc

concentration and quantitative urinary zinc excretion showed that the plasma level was decreased with 39% in patients with nephrotic syndrome compared with the control group. The rate of urinary zinc excretion was not different from that of the controls, with the exception of patients whose disease was in the polyuric phase of beginning remission where it was fivefold higher and those who had achieved a long-lasting remission of the disease (6).

Another study that investigated the effect of proteinuria on urinary zinc and copper excretion in children with nephrotic syndrome (NS) revealed that clearance, fractional excretion, and urinary excretion of zinc and copper were significantly higher in children with relapse of NS than in children with remission of NS or in healthy children. The authors found a linear correlation between proteinuria and urinary zinc and copper excretion in relapse of NS. The results of the study suggested that zinc and copper deficiency in NS may be related also to increased urinary zinc and copper losses (7). Since about two thirds of the plasma zinc is bound to albumin, an unchanged zinc-albumin ratio would reflect an equal zinc and albumin loss.

Some authors evaluated copper, zinc, calcium and magnesium concentrations in plasma, erythrocytes and urine in patients with chronic renal failure. They revealed significant plasma hypozincaemia and hypozincuria in uremic patients (though the erythrocytes zinc concentration was significantly higher than in controls), increased erythrocytes copper levels, significant hypocalcemia in erythrocytes and plasma, magnesium levels being increased (8).

The causes of Zn deficiency in kidney disease are not clear. Decreased dietary Zn intake and intestinal absorption, increased endogenous Zn loss, and increased urinary Zn excretion (as in the nephrotic syndrome) all may contribute to altered Zn metabolism (9).

The benefit of the administration of Zn doses requires further evaluation under controlled conditions. Some researchers showed that in children (1-16 years) with steroid-sensitive nephrotic syndrome, zinc administration (10 mg/day) reduced by 20% the frequency of relapses. The patients which received zinc developed a significantly higher likelihood of sustained remission (10).

Another study performed on 14 children with nephrotic syndrome showed that the mean blood zinc level was significantly lower than that of 113 control children. The blood albumin/zinc ratio was also lower in nephrotic children than in healthy children. There was no correlation between blood albumin and blood zinc levels. The authors suggested that abnormally low zinc levels are partly due to deficient zinc uptake and may explain some of the disorders described in children with persistent nephrotic syndrome (11).

The comparison of the status of zinc and copper in chronic hemodialysed patients with and without diabetes showed that the alteration in the distribution of zinc in patients with chronic kidney disease is independently of the presence of diabetes and also the status of copper seems not to be influenced by chronic kidney disease, but only by the metabolic derangements associated with diabetes (12).

Oxidative stress has been proposed as one of the possible mechanism involved in the nephrotic syndrome. Peroxidation of lipid membranes raises the concentration of their byproducts malonyldialdehyde (MDA) and

the consequent lowering of antioxidants (13). Total antioxidant capacity (TAC) is involved in antioxidant protection in nephrotic syndrome (14). In the kidney, oxygen radical production has been detected in vascular cells, juxta glomerular cells, tubular cells, podocytes, mesangial cells and isolated glomeruli. Free radicals have a negative influence on renal tissue in nephrotic syndrome.

There are studies that investigated oxidant and antioxidant status of copper, zinc and homocysteine (HCY) in nephrotic syndrome patients and the effect of antioxidants, mineral and B-complex vitamins on oxidant and antioxidant status. Results showed a significant statistically decreased level of serum TAC, Cu, Zn, plasma Vit C while serum malonyldialdehyde and homocysteine level were increased in nephrotic syndrome patients compared with control. The level of serum homocysteine and MDA were significantly decreased and activity of serum TAC, Cu, Zn was significantly increased after 3 months with Zincovit (15).

Data suggest that it can appear a relative deficit of oxidant/antioxidant balance in nephrotic syndrome, which could predispose to increased oxidative stress.

In many diseases, zinc deficiency may complicate the clinical features; affect adversely immunological status, increases oxidative stress and increase the generation of inflammatory cytokine. It is therefore important that the status of zinc to be evaluated. Alterations in zinc metabolism in some disease conditions must be studied taking into account the links between homeostatic perturbation and cellular biology, for to characterize zinc distribution and for elucidate the regulatory processes and the factors to which they respond (16).

Copper deficiency has been documented in conditions predisposing to excessive copper losses. Urinary losses of ceruloplasmin-bound copper likely play a significant role in predisposing patients with nephrotic syndrome to copper deficiency (17). Renal patients undergoing continuous ambulatory peritoneal dialysis may also experience excessive losses of ceruloplasmin-bound copper via dialysis exchanges.

Copper imbalances in humans not only impair normal function of ceruloplasmin, superoxide dismutase or of other copper-dependent proteins, but can also lead to disease states (18).

Hypomagnesemia is occasionally observed in chronic renal failure due to an obligatory renal magnesium loss. It is also seen during the diuretic phase of acute renal failure, in post-obstructive diuresis and after renal transplantation. Patients on continuous ambulatory peritoneal dialysis develop hypomagnesaemia when low magnesium dialysis fluid is used.

CONCLUSIONS

Our data show that in acute urinary diseases occur some cationic imbalances which may influence the clinical manifestations and development of the renal disease and these imbalances should be corrected.

Oxidative stress may be one of the possible mechanisms involved in the nephrotic syndrome.

We consider that the decrease of plasma zinc concentration may be involved in the immune dysfunction associated with nephrotic syndrome.

The knowledge of bivalent cations misbalances may open new ways in the treatment of nephrotic syndrome and in the reduction of some side effects of drugs usually used in treatment.

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FACTORI IMPLICAŢI ÎN COLONIZAREA MICROBIANĂ A RĂŞINILOR ACRILICE

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FACTORS INVOLVED IN MICROBIAL COLONIZATION OF ACRYLIC RESINS (Abstract): The objective of this study was to evaluate the effect of polishing technique and the polymerisation type on the microbial colonization of acrylic resins. **Material and methods:** The studied resins were: Castapress/Vertex, Prothyl Hot/Zermack, Duracryl Plus/Spofa Dental, Vertex-Soft/Vertex, Superacryl Plus/Spofa Dental. Ten specimens, two for every resin, presenting dimensions of 50/25/2 cm were done. One sample of each resin was polished with extrahard tungsten carbide burs, the other sample being polished with extrahard extrafine and diamond burs. The final polishing was done using a conventional method: pumice, water and lathe bristle brush for 90 seconds, 1500 rpm and soft leather polishing wheel for 90 seconds, 3000 rpm. One sample of Duracryl Plus was glazed after polishing with Glaze™/Bosworth. Vertex Soft specimens were not polished because it is a resilient material. The samples were immersed in human saliva for 24 hours and after that were colored with May Grunwald Giemsa (MGG). The total germs number (TGN) was determined by Buiuc method. **Results:** The lowest value of the microbial colonization was related to the heat-curing resins treated by conventional polishing combined with glazing techniques. **Conclusions:** The results noticed that the type of acrylic resin (autopolymerizing and heat-cured) and the polishing techniques have a significant influence on the microbial colonization. Specimens of self curing resin had a higher porosity than heat curing resin after the same surface treatment, generating a higher TGN. **Key words**: ACRYLIC RESINS, TGN, POLISHING TECHNIQUE, MICROBIAL COLONIZATION

Pentru prevenirea colonizării bacteriene şi a acumulării plăcii pe suprafeţele protezelor dentare trebuie acordată o importanţă deosebită stării de suprafaţă a acestora, în mod special feţei mucozale, sursă frecventă a stomatitelor paraprotetice. În acest scop protezele amovibile trebuie să aibă suprafeţe netede şi bine lustruite pentru a menţine confortul şi sănătatea ţesuturilor orale (1).

După introducerea în cavitatea orală proteza este rapid acoperită de o peliculă salivară dobândită (maximum 2 ore), compusă dintr-un strat selectiv de componente salivare adsorbite (în special glicoproteine) care au un rol important în modularea colonizării microorganismelor.

Scopul studiului a constat în analiza prin microscopie optică a gradului de colo-

nizare bacteriană a cinci răşini acrilice, cu regim de polimerizare diferit (autobaropolimerizare şi termopolimerizare), care au fost supuse la prelucrări mecanice diverse.

MATERIALE ŞI METODE

Răşinile studiate au fost alese dintre cele mai frecvent utilizate în protetica dentară: *Castapress/Vertex, Prothyl Hot/Zermack, Duracryl Plus/Spofa Dental, Vertex-Soft/Vertex, Superacryl Plus/Spofa Dental*. S-au realizat zece probe, câte două pentru fiecare tip de material.

Probele au fost realizate sub formă de plăcuţe paralelipipedice cu lungimea de 50 mm, lăţimea 25 mm şi grosimea 2 mm. Machetele plăcuţelor au fost transformate în probe finale respectând algoritmul tehnologic specific şi indicaţiile firmei producătoare pentru fiecare tip de răşină (tab. I). Probele au fost notate cu literele A-E, respectiv A'-E'.

TABELUL I

Regimul de polimerizare al răşinilor studiate

Proba	Denumirea comercială	Regimul de polimerizare
A, A'	Castapress/ Vertex	30 minute la 55º C şi 2.5 bar
B, B'	Prothyl hot/Zermack	5 minute de la 80° la 100°C + 20 minute la 100°C
C, C'	Duracryl Plus/Spofa Dental	30 - 40°C / 0,2 - 0,3 MPa.
D, D'	Vertex Soft	3 ore la 70º C, urmat de 30 de minute la 100ºC
E, E'	Superaryl Plus (Spofa Dental)	Temperatura creşte în 30 minute până la 70ºC, 30 minute se menţine la 70ºC, urcă în 30 minute până la 100ºC, 30 minute rămâne la 100ºC

Prelucrarea şi lustruirea celor două probe din fiecare tip de răşină s-a făcut utilizând tehnici diferite, viteza şi timpul de prelucrare fiind însă identice (30 de secunde, 1500 rpm).

Prelucrarea mecanică s-a făcut cu o freză extradură din carbură de tungsten cu granulaţie fină (galbenă) pentru probelele: A, B, C, D şi E iar pentru probelele A', B', C', D' şi E' prelucrarea s-a făcut atât cu o freză extradură cu granulaţie extrafină (53-63 μ) de formă conică, cât şi cu o freză diamantată cu granulaţie fină (roşie) de formă cilindrică, cu vârful rotunjit.

Lustruirea finală a avut ca obiectiv obţinerea unui "luciu de oglindă" şi s-a realizat cu praf abraziv pentru lustruit (*Polishing powder/Astar Fedent*) şi pastă abrazivă (*High lustre polishing paste*) acţionate de perii şi filţuri.

Probele D şi D' fiind dintr-un material rezilient, care se utilizează doar pe suprafaţa mucozală a protezei, au beneficiat de o prelucrare uşoară cu praf *Vertex™ Soft*. Lustruirea a fost făcută cu praf abraziv (*Astar-Fedent*) şi pastă abrazivă, încărcate pe un con din pâslă (filţ) şi puf de bumbac.

Proba C a fost acoperită cu o peliculă de glazură fotopolimerizabilă pentru răşini acrilice şi materiale compozite (*Bell Coat/ K B Mutsumi*), care a fost aplicată prin pensulare în strat subţire pe suprafaţa uscată a probei şi apoi fotopolimerizată patru minute.

Saliva a fost obţinută de la un singur pacient, în vârstă de 43 de ani, sănătos din punct de vedere clinic, care nu a urmat nici o terapie medicamentoasă în ultimele trei luni, şi nu a utilizat nici soluţii pentru clătiri orale, care ar fi putut modifica compoziţia şi secreţia salivară.

Obiectivele urmărite au vizat determinarea încărcăturii microbiene a salivei recoltate şi determinarea încărcăturii microbiene a biofilmului salivar de pe supra-

fețele probelor luate în studiu.

Metoda de lucru a constat în:

- dezinfecția probelor prin imersie timp de 1 oră în soluție Zeta 1 Ultra
- menținerea probelor în salivă timp de 24 ore
- aprecierea gradului de contaminare microbiologică a probelor analizate s-a realizat prin metoda cultivării în plăci Petri (2), utilizându-se agar nutritiv (Merck, Germany). Obținerea inoculului s-a realizat prin spălarea repetată a probelor cu 5 ml apă distilată sterilă. Pentru inoculare s-a utilizat un volum de 100 µl inocul/placă Petri, iar în cazul periei s-a efectuat însămânțarea atât prin spălare cât şi prin amprentare.
- incubarea (24 ore la 37°C) a fost urmată de o apreciere cantitativă a încărcăturii microbiene.
- colorarea panoptică May Grunwald Giemsa (MGG) a probelor pentru vizualizarea adeziunii bacteriene cu ajutorul microscopiei optice (M.O).

REZULTATE ŞI DISCUȚII

Valoarea NTG/ml din saliva recoltată a fost de 46 x 10^{10} iar valorile NTG probelor au fost cuprinse între 0,16 x 10^8 UFC/cm^2 – 12,1 x 10^8 UFC/cm^2 (fig. 1).

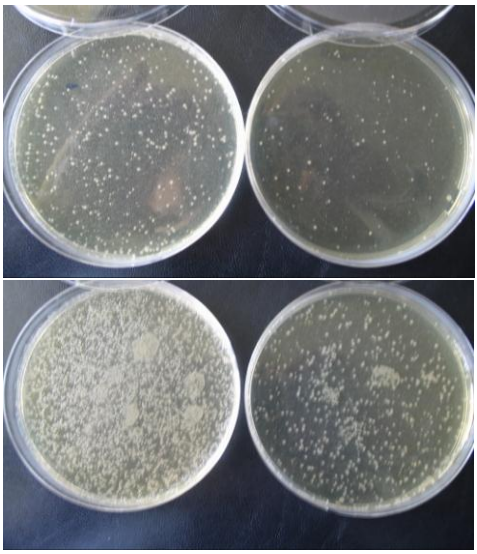
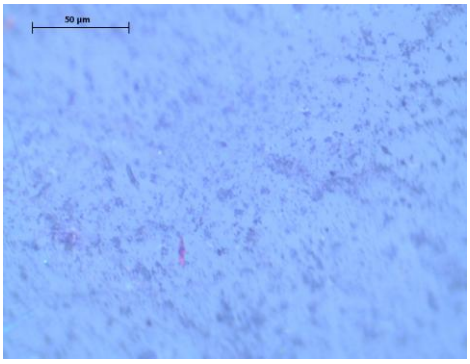


Fig. 1. Determinarea NTG-ului

Cea mai mică valoare (0,16 x 10^8) s-a întâlnit la proba C (glazurată), ceea ce demonstrează faptul că deşi glazurarea nu poate preveni colonizarea bacteriană, modifică semnificativ cantitatea microorganismelor care aderă la suprafața respectivă (fig. 2).

a. 

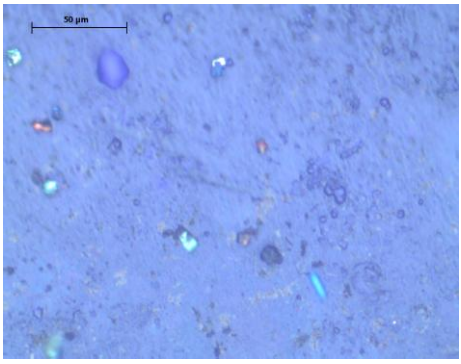
b. 

Fig.2.a. Proba C = 0,16 x 10^8 UFC/cm^2 b.Proba C'=1,65 x 10^8 UFC/cm^2 (magnificație X 50)

Corelând rezultatele colonizării bacteriene cu metoda de prelucrare folosită, se observă diferențe între probele care au fost prelucrate numai cu freza extradură şi cele care au fost prelucrate atât cu freza extradură cât şi cu freza diamantată, chiar dacă metoda de lustruire a fost aceeaşi pentru toate probele (tab. II).

TABELUL II

Corelația încărcătură bacteriană/regimul de polimerizare a rășinilor studiate

Proba	Tipul de polimerizare	Colonizarea bacteriană UFC/cm^2
A	Vertex castapress autopolimerizare	1.76 x 10^8
A'	autopolimerizare	1.68 x 10^8
B	Prothyl Hot termopolimerizare	1.46 x 10^8
B'	termopolimerizare	1.11 x 10^8
C	Duracryl autopolimerizare, glazurare	0.16 x 10^8
C'	Duracryl autopolimerizare	1.65 x 10^8
D	Vertex soft termopolimerizare, rezilienţă	12.1 x 10^8
D'	Vertex soft termopolimerizare	12.1 x 10^8
E	Superaryl Plus (Spofa Dental)	1.01 x 10^8
E'	Superaryl Plus (Spofa Dental)	0.83 x 10^8

Valoarea cea mai mare înregistrată în cazul probelor din *Vertex Soft* (12.1 x 10^8 UFC /cm^2), este în acord cu datele din literatură care precizează că rășinile reziliente au textură spongioasă, ce antrenează creşterea porozității şi implicit, a colonizării bacteriene, limitând longevitatea clinică a materialului (3).

De asemenea, studiind corelația dintre regimul de polimerizare şi colonizarea microbiană, am observat că rășinile acrilice autopolimerizabile prezintă valori mai mari ale încărcăturii microbiene .

În ceea ce priveşte colonizarea rășinilor acrilice glazurate, rezultatele obținute au fost net superioare probelor neglazurate. Sesma N. şi col., 2005 (4) a demonstrat printr-un studiu clinic că protezele prezentau la o lună după glazurare o peliculă mai subțire de placă bacteriană comparativ cu cele neglazurate. Reevaluarea după 3 luni, a arătat că glazura a fost fisurată, generându-se arii microretentive ce au determinat acumularea plăcii bacteriene. Aceste constatări au condus la concluzia că eficiența glazurării este condiționată de păstrarea integrității stratului depus.

Totuşi, literatura de specialitate prezintă opinii diferite cu privire la relația de dependență (directă sau indirectă) între rugozitatea suprafeței şi colonizarea bacteriană. Astfel Von Fraunhofer şi col., 2009 (5) afirmă că „este posibil" ca rugozitatea suprafeței să ofere un mediu care promovează atracția inițială a colonizării timpurii. Oricum, formarea biofilmului depinde de mai mulți factori, cu o importanță specială fiind speciile bacteriene, natura substratului şi factorii de mediu înconjurător. Există însă şi numeroase studii care demonstrează o relație liniară între aceşti doi parametri (6, 7, 8, 9).

Analiza încărcăturii microbiene a probelor face apel la un număr de variabile cunoscute ale materialului (rugozitate, umectabilitate, tip de polimerizare) care trebuie corelate cu multitudinea variabilelor ce definesc „mediul salivar”.

Prin urmare este dificilă compararea rezultatelor diferitelor studii, datorită protocoalelor diferite utilizate: perioadelor diferite de incubație, utilizării de salivă totală sau parțială, condițiile unui flux salivar stimulat sau nestimulat, temperaturilor diferite ale salivei, precum şi prezența sau absența nutrienților care pot interfera cu viabilitatea celulară şi capacitatea ei de aderare.

CONCLUZII

Cea mai mică valoare a încărcăturii microbiene ($0{,}16 \times 10^8$) s-a întâlnit la proba C (glazurată), ceea ce demonstrează că glazurarea modifică semnificativ cantitatea microorganismelor care aderă la suprafața protezei.

Cea mai crescută valoare a încărcăturii microbiene (12.1×10^8 UFC/ cm^2) s-a întâlnit la proba D (răşină rezilientă) datorită texturii spongioase a răşinii.

Valorile încărcăturii microbiene sunt mari în cazul răşinilor acrilice autopolimerizabile, în timp ce răşinile termopolimerizabile prezintă valori mai mici.

Probele care au beneficiat de prelucrare cu freza extradură au valori mai crescute ale încărcăturii microbiene comparativ cu cele prelucrate cu freza extradură şi diamantată.

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FACTORI PROGNOSTICI ÎN CANCERUL MAMAR

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PROGNOSTIC FACTORS IN BREAST CANCER(Abstract): Survival in breast cancer is determined by many factors, their influence is different. The aim of the study is to assess the impact on survival of various prognostic factors. **Material and methods**: We studied a total of 198 cases of histologically confirmed breast cancer patients, and we evaluated the impact of prognostic factors on survival. **Results:** The most important factor influencing survival is the preset of axillary metastasis(p: 0.0001). Other important prognostic factors are: tumor size(p: 0.003), tumor *grading*(p: 0.0025), N stage(p: 0.0001.) lymphovascular invasion (p 0.0005) and Nottingham Prognostic Index(p: 0.0109). The factors with no impact on survival were: histological type(p: o,82), number of lymph nodes affected(p: 0,23), tumor necrosis(p: 049) and inflammatory infiltration (p:0,2). **Conclusions:** The most important prognostic factors are: axillary metastasis followed by tumor *grading*, NPI and lymphovascular invasion.
Key words: BREAST CANCER, PROGNOSTIC FACTORS, SURVIVAL.

Cancerul mamar reprezintă o problemă de larg interes pentru sănătatea populațională. Metodele de depistare precoce, tratamentul chirurgical şi oncologic au reuşit să îmbunătățească prognosticul pacienților suferinzi de neoplazie mamară (1, 2).

Factorii care influențează prognosticul în cancerul mamar sunt multipli, iar importanța lor este diferită. Stabilirea riscului de recidivă şi metastazare este important nu doar pentru a putea prevedea evoluția pacientului, ci permite cuantificarea beneficiului pentru tratamentul adjuvant. Pacienții cu risc major de recidivă şi metastazare au o indicație majoră de a urma tratamentul complementar chimio şi radioterapic, cu un raport risc/beneficiu minim. Noile metode de investigație genetică vor permite definirea clară a pacientelor care necesită tratament adjuvant şi inclusiv chimio şi radiosensibilitatea acestora, ceea ce va diminua riscurile supratratamentului (3, 4).

Se deosebesc două tipuri de factori care pot influența evoluția pacienților cu cancer mamar: factorii prognostici care prin definiție sunt aceia care ne oferă informații despre evoluția potențială a pacienților în absența tratamentului adjuvant sistemic şi factorii predictivi care reprezintă răspunsul la un anumit tratament sistemic (de exemplu receptorii estrogenici pentru terapia antihormonală) (5, 6).

MATERIAL ŞI METODE

Studiul a inclus 300 de paciente cu cancer mamar confirmat histologic şi care au suferit o intervenție chirurgicală radicală sau conservatoare. Datele au fost obținute din registrul cu rezultate histopatologice şi din foile de observație, iar pacientele au

beneficiat de control în urma unei scrisori de invitație. În cazul decesului pacientelor, am solicitat date referitoare la cauzele şi data decesului. Procentul de răspuns a fost în jurul cifrei de 70%. De asemenea, s-au acumulat date din registrele Fundației "Destine Comune" a pacientelor operate de cancer mamar precum şi cele privind supraviețuirea şi cauzele de deces obținute de la Clinica de Oncologie, Târgu Mureş.

Pentru 198 de paciente am putut obține date satisfăcătoare. În studiul retrospectiv am comparat influența diverşilor factori prognostici asupra supraviețuirii: vârsta, tipul histologic, *grading*-ul tumoral, dimensiunea tumorii, metastazarea limfatică axilară, indicele prognostic Nottingham, infiltratul inflamator, necroza tumorală, invazia limfo-vasculară şi grupa sangvină. Rezultatele studiului au fost analizate statistic cu ajutorul programului Graphpad demo folosind curbele de supraviețuire Kaplan Meier.

REZULTATE

1. Vârsta pacientelor. Lotul de studiu a avut limita de vârstă 40 - 50 de ani, fără a se dovedi influența asupra prognosticului. Semnificația statistică a evidențiat un p de 0,94 pentru 40 respectiv 0,13 pentru 50 de ani (fig. 1).

2. Tipul histologic. Cel mai frecvent întâlnit a fost carcinomul ductal invaziv prezent la 141(71,21%), urmat de carcinomul lobular prezent la 51(25,75%) din cazuri. Supraviețuirea la 5 ani a fost de 74,46% pentru carcinomul ductal şi de 68,4% pentru cel lobular invaziv. Datele nu prezintă semnificație statistică (p 0,82). Am studiat influența asocierii dintre carcinomul ductal invaziv şi cel *in situ*, supraviețuirea acestor forme fiind mai mare la 5 ani (86%) decât la formele invazive pure (72%)(p = 0,04) (fig. 2).

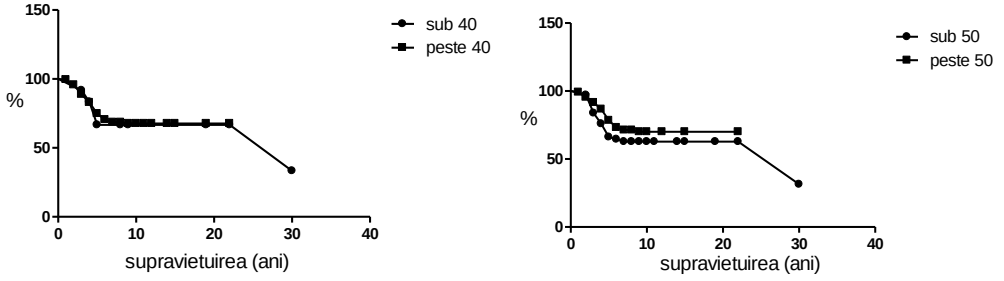

Fig. 1. Nivelurile supraviețuirii celor două grupuri de vârstă din lotul luat în studiu.

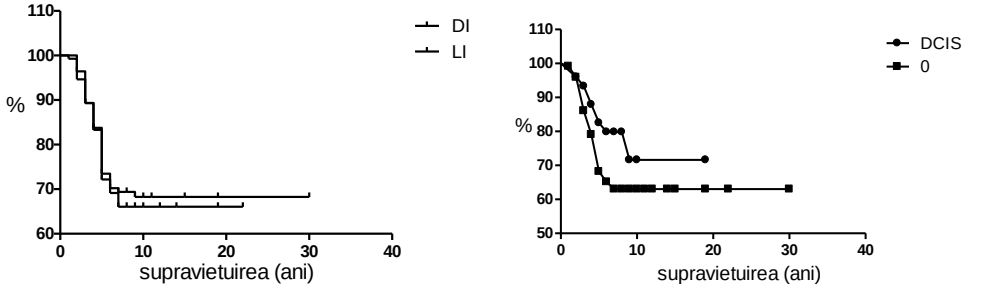

Fig. 2. Evaluări comparative a supraviețuirii la pacientele incluse în studiu.

3. Grading-ul tumoral. Din punct de vedere al supraviețuirii, *grading*-ul tumoral a avut un impact hotărâtor, astfel, supraviețuirea la 5 ani în Gr I a fost de 92,8%, Gr II 78,4 %, iar Gr III 64,7 %, datele având o înaltă semnificație statistică cu un p de 0.0025 (fig. 3), iar supraviețuirea la 5 ani în relație cu stadiul tumoral este redată în tabelul I.

TABELUL I

Stadiul tumoral	Supraviețuirea la 5 ani %
0, I	100
II A	90
II B	80,7
III A	65
III B	52
IV	0

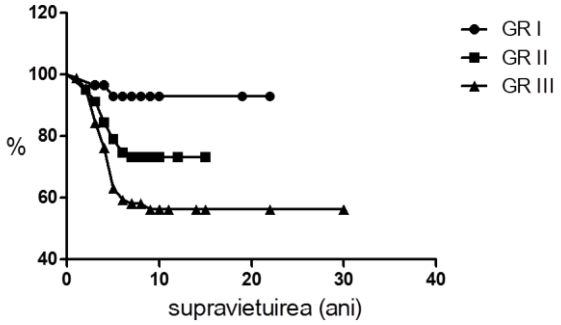

Fig. 3. Nivelurile supraviețuirii în relație cu *grading*-ul tumoral.

4. Metastazele ganglionare. În lotul studiat, un număr de 79 paciente (39,89%) nu au prezentat adenopatii metastatice, în timp ce acestea au fost întâlnite la 119 paciente (60,1%). Într-un singur caz nu s-a practicat limfadenectomie, pacienta prezentând metastaze la distanță, gestul terapeutic s-a limitat la mastectomie de curățire. Supraviețuirea la 5 ani în lotul N0 a fost de 94,5 %, iar N+ a beneficiat de o supraviețuire de 62,18%(p de 0,0001). Comparând supraviețuirea pacientelor din punctul de vedere a stadiului N am găsit o diferență semnificativă statistic între loturile N1 şi N2 (p = 0,0001) (fig. 4).

Impactul asupra supraviețuirii în raport cu numărul de ganglioni afectați având ca reper cifra 3, respectiv 5, nu a evidențiat diferențe semnificative statistic (p = 0,23; respectiv 0,07) (fig. 5).

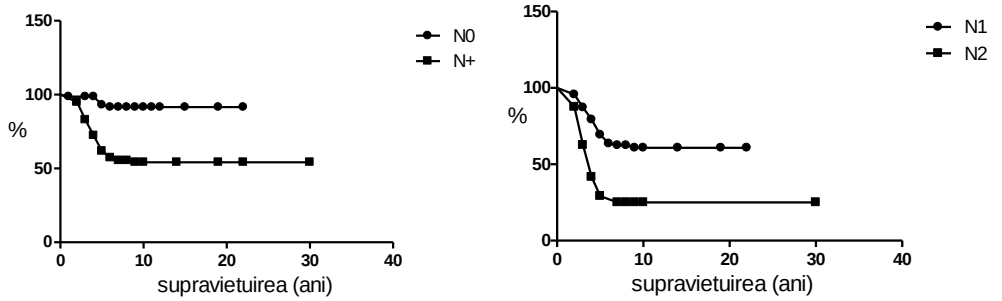

Fig. 4. Evaluarea privind supraviețuirea la 5 ani pe două loturi de paciente.

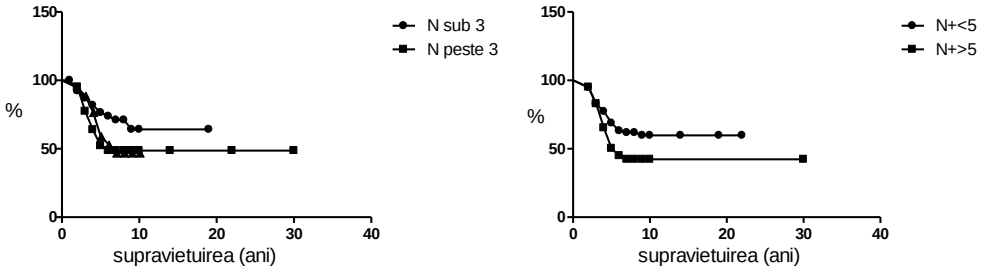

Fig. 5. Analiza duratei de supraviețuire în relație cu numărul ganglionilor afectați de metastaze.

5. Dimensiunea tumorii şi stadiul T. Datele obținute de noi au fost controversate, astfel s-a observat o semnificație statistică mai ales pentru stadiul T4 şi T1(p = 0,003), iar pacientele cu tumori aflate în stadiul T2 şi T3 au avut curbe de supraviețuire aproximativ superpozabile (fig. 6).

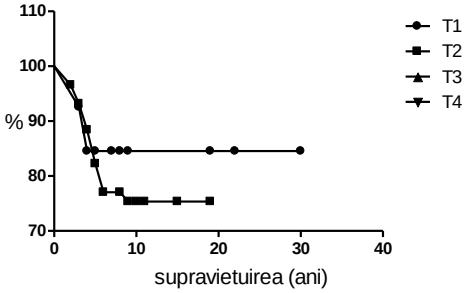

Fig. 6. Comparații între stadiile evolutive ale tumorii.

6. Indicele prognostic Nottingham. S-a folosit formula de calcul al NPI: 2 x dimensiunea tumorii în cm + stadiul N (1-3) + *grading*-ul tumoral (1-3). Lotul studiat a fost subîmpărțit în NPI sub şi peste 5, diferența rezultând că pacientele cu NPI peste 5 au o mortalitate la 5 ani mai mare, semnificativ statistic (p 0,0109).

Infiltratul inflamator peritumoral este considerat un factor de protecție fiind manifestarea unui răspuns imun puternic. În cazuistica studiată formele cu infiltrat inflamator au avut un prognostic mai bun dar fără semnificație statistică (p 0,2) (fig. 7).

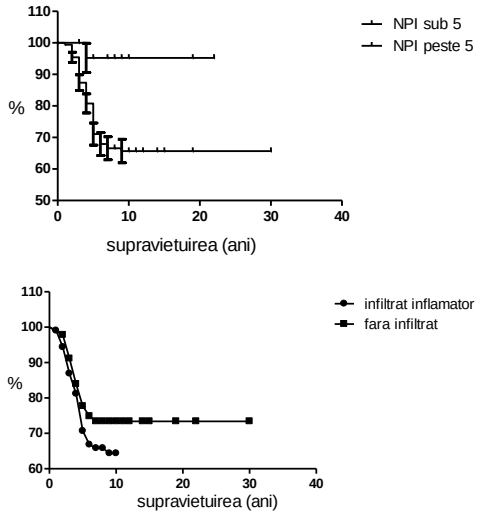

Fig. 7. Evaluarea asupra unor caracteristici ale tumorii cu valoare prognostică.

Prezența invaziei vasculare are o semnificație negativă atât asupra prognosticului imediat cât şi a celui la distanță. Pe lotul studiat, un număr de 55 (27,77%) paciente au prezentat invazie vasculară iar la 143 (72,22%) aceasta a fost absentă. Cu ajutorul curbelor de supraviețuire s-a stabilit o corelație importantă între invazia limfo-vasculară şi supraviețuirea la 5 ani (p = 0,0005).

Necroza tumorală se datorează creşterii rapide a unei tumori care depăşeşte capacita-

tea acesteia de a se vascularizа, ca urmare, este considerată a fi un tip tumoral cu evoluţie nefavorabilă (indice mitotic crescut). Cazurile cu necroză din lotul studiat au beneficiat de o durată de supravieţuire mai redusă fără semnificaţie statistică (p = 0,49) (fig. 8).

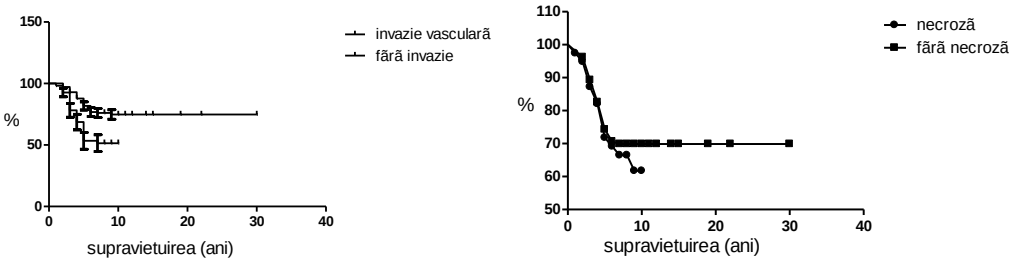

Fig. 8. Aspecte ale prognosticului dependent de invazia vasculară şi de necroza tumorală.

Relaţia dintre grupul sangvin asupra supravieţuirii pacientelor cu cancer mamar nu a evidenţiat rezultate cu semnificaţie statistică (p = 0,71).

Studiul multifactorial a determinat care dintre factorii clasici de prognostic: stadiul T, metastazarea axilară sau *grading*-ul tumoral au o influenţă mai importantă asupra supravieţuirii pacientelor cu cancer mamar. S-a evidenţiat faptul că deşi toţi factorii enumeraţi influenţează statistic semnificativ supravieţuirea, impactul cel mai important îl are metastazarea limfatică axilară (tab. II).

TABELUL II

Term	Odds ratio	95%	C. I.	Coeficient	S. E.	Z-Statistic	P-Value
Grading	2,1620	1,2481	3,7449	0,7710	0,2803	2,7507	0,0059
N	6,5025	2,6842	15,7522	1,8722	0,4514	4,1472	0,0000
T	1,6592	1,1735	2,3460	0,5064	0,1767	2,8656	0,0042
CONSTANT	*	*	*	-5,4245	0,9741	-5,5687	0,0000

DISCUŢII

Han et al (1) a demonstrat faptul că vârsta sub 35 de ani la debutul neoplaziei mamare reprezintă un semnal de alarmă atât pentru recidivă cât şi pentru supravieţuirea tardivă. După El-Saghir et al (2) vârsta tânără nu pare să fie un factor decisiv pentru recidivă la grupul cu ganglioni negativi, dar influenţează major recidiva, metastazarea şi supravieţuirea la pacientele cu adenopatii tumorale. În lotul studiat vârsta nu s-a dovedit un factor semnificativ pentru supravieţuire luând în consideraţie că numărul de cazuri cu vârstă sub 40 de ani a fost redus.

Black et al.(3) într-un studiu cu variabile multiple au corelat tipul histologic cu gradul de malignitate şi au constatat ca fiind mai important din punct de vedere al semnificaţiei prognostice *grading*-ul tumoral. De asemenea, McCready et al(4) subliniază importanţa *grading*-ului tumoral pentru evaluarea supravieţuirii la 5 ani a pacientelor cu cancer mamar. Studiul nostru apreciază *grading*-ul tumoral ca fiind un factor prognostic cu o semnificaţie statistică importantă dar în ceea ce priveşte tipul histologic nu relevă o diferenţă de supravieţuire între formele ductale şi lobulare invazive. Nu putem explica de ce asocierea cu formele *in situ* oferă un plus de supravieţuire la pacientele cu carcinom ductal invaziv.

Prezenţa metastazelor axilare este considerat un semn de boală avansată cu efecte

importante asupra supraviețuirii. Deşi numărul de ganglioni limfatici afectați de metastaze nu a avut o influență semnificativă asupra supraviețuirii gradul N, respectiv extensia extracapsulară, au un impact important asupra supraviețuirii fapt confirmat şi de studiile lui Maksimovici et al (5).

Din studii prospective largi s-a demonstrat că pacientele cu NPI mai mic de 3-4 au o mortalitate anuală egală cu populația generală, ca urmare, nu necesită tratament oncologic suplimentar, pe când cele cu valori ale indicelui de peste 5 au o rată de supraviețuire mult mai redusă, ca urmare, beneficiază de tratament oncologic. Studiul nostru a confirmat importanța NPI în evaluarea prognosticului (6).

Majoritatea studiilor actuale consideră metastazele axilare ca fiind factorul prognostic cel mai important, fapt confirmat de evaluarea multifactorială care conferă acestui factor cea mai importantă semnificație statistică.

CONCLUZII

Principalul factor de prognostic, cu semnificația statistică cea mai ridicată s-a dovedit a fi statusul ganglionar N, astfel cazurile cu metastaze ganglionare au beneficiat de o supraviețuire mai redusă decât cele N0. Numărul ganglionilor afectați nu pare a avea semnificație, dar stadiul afectării ganglionare N1 sau N2 (deci invazia extraganglionară) s-a dovedit a fi un factor important asupra supraviețuirii.

Tipul histologic ductal sau lobular nu s-a dovedit important din punct de vedere al supraviețuirii dar are importanță pentru enunțarea deciziei terapeutice

Carcinomul *in situ* pare a avea un efect protector, deşi componenta *in situ* extensivă ridică probleme deosebite pentru abordarea conservatoare datorită extensiei de-a lungul canalelor galactofore peste limita palpabilă.

Dimensiunea tumorii (în cm) nu este un factor semnificativ dar mai important este stadiul tumoral T, respectiv invazia tegumentului sau peretelui toracic.

Grading-ul tumoral este un factor prognostic independent cu influență hotărâtoare asupra supraviețuirii.

Invazia limfo-vasculară influențează atât apariția metastazelor ganglionare cât şi supraviețuirea la distanță dar necroza tumorală şi infiltratul inflamator peritumoral nu pare a avea o influență semnificativă statistic.

În lotul studiat s-a dovedit că factorii cu cel mai nefavorabil impact asupra supraviețuirii sunt: metastazele ganglionare, *grading*-ul tumoral, stadiul T şi invazia limfovasculară.

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CONTRIBUŢII LA EVALUAREA STATUSULUI HEMODINAMIC ÎN SINDROMUL METABOLIC

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CONTRIBUTIONS TO THE ASSESSMENT OF HEMODYNAMIC STATUS IN METABOLIC SYNDROME (Abstract): The concept of normohemodynamics is based on the fact that the main function of the cardiovascular system is the transport of oxygen. The cardiovascular system is adapting to a new hemodynamic status with every heartbeat. Only one class, called the normohemodynamic state, containing a simultaneous normotension and normodynamic flow, can serve as therapeutic goal. Metabolic syndrome includes a constellation of clinical and biological features that confer an increased cardiovascular risk. Clutter hemodynamic modulators in metabolic syndrome and assessment of hemodynamic status both overall and by its components, open new perspectives in the management of patients with hypertension and metabolic syndrome. **Material and methods**: In the current study were included 32 patients meeting the criteria for metabolic syndrome and hypertension and 32 controls. Patients were monitored and assessed for the global hemodynamic status and for the modulators of hemodynamics, intravascular volume, vasoactivity and inotropy, respectively. **Results and conclusions:** There are no significant differences in the global hemodynamic status between the study group and the control group; in the study group there is a clear weathering of hemodynamic modulators. The improvement of these factors opens a new perspective in the global cardiovascular assessment and therapeutic directions. **Key words**: HEMODYNAMIC STATUS, DISORDERS, MODULATORS, METABOLIC SYNDROME, HYPERTENSION

Rolul esenţial al sistemului cardiovascular este să asigure transportul de oxigen şi nutrienţi către ţesuturi, nivelurile adecvate ale DO2I (Oxygen Delivery Index) fiind corelate cu supravieţuirea şi calitatea vieţii (1). Transportul oxigenului se află în relaţie cu fluxul sangvin şi nu cu presiunea sangvină deoarece sistemul cardiovascular se adaptează la un nou status hemodinamic cu fiecare bătaie a cordului iar hemodinamica evaluează elementele inseparabile flux/presiune (2).

Variaţiile indexului sistolic (IS) reprezintă cauza principală a modificărilor hemodinamice continui; variaţiile frecvenţei cardiace reprezintă răspunsul compensator cronotrop pentru a menţine fluxul de perfuzie constant (3).

Măsurarea indexului cardiac se realizează prin termodiluţie, metodă invazivă care exprimă valoarea medie a debitului cardiac pe perioada de diluţie (aproximativ 12 bătăi ale cordului), din care poate fi calculată

valoarea medie a IS sau prin bioimpedanţa electrică toracică (BET), metodă noninvazivă bazată pe rezistenţa ţesuturilor la frecvenţa ridicată a unui curent electric de magnitudine redusă. Principiul acestei tehnici se bazează pe faptul că sângele este substanţa cu cea mai bună capacitate conductoare din punct de vedere electric din corp.

Parametrii măsuraţi de BET sunt: conductivitatea fluidului toracic (CFT), variaţia impedanţei cu timpul (dZ/dt max), acceleraţia fluxului aortic în relaţie cu inotropismul şi fracţia de ejecţie, frecvenţa cardiacă (FC), timpul de ejecţie ventriculară (TEV), frecvenţa respiratorie (FR). Cu ajutorul formulelor automate de calcul, se obţin valori pentru: VEPT (volumul ţesutului toracic participant electric), EPCI (index de contractilitate a fazei de ejecţie), indexul stării inotrope, debitul cardiac (cardiac output, performanţa cordului), suprafaţa corporală (SC), indexul cardiac (IC), fracţia de ejecţie şi volumul per bătaie (SV), calculat conform ecuaţiei lui Sramek.

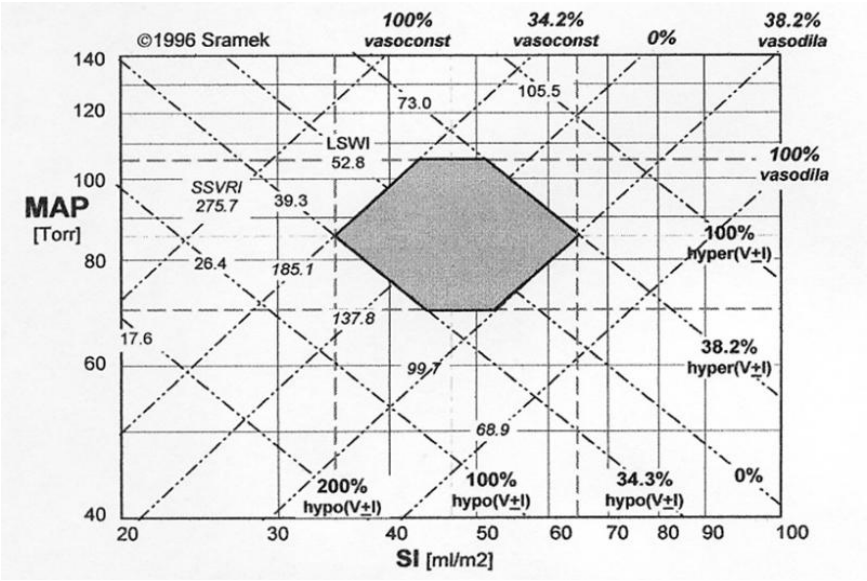

Fig. 1. Harta hemodinamică (după Sramek B.)

Relaţiile dintre statusul hemodinamic şi modulatorii hemodinamici, pentru un adult în repaus, se exprimă grafic prin harta hemodinamica ce prezintă 2 coordonate:

- sistemul ortogonal aparţinând statusului hemodinamic: presiunea arterială medie (PAM) şi indexul sistolic (IS);
- sistemul diagonal al modulatorilor hemodinamici: volemia, inotropismul şi vasoactivitatea.

Centrul hărţii reprezintă statusul hemodinamic ideal, normohemodinamica şi simultan, normovolemie, normoinotropism şi normovasoactivitate (fig. 1).

Determinarea prin BET a acestor modulatori permite identificarea dezordinilor hemodinamice şi deschide perspectiva unei terapii care să direcţioneze pacientul într-o stare de normohemodinamică şi de normoperfuzie, schimbând conceptul de medicină cardiovasculară reactivă (tratarea pacientului când afectarea hemodinamică este dia-

gnosticată) într-un nou concept de medicină cardiovasculară proactivă (5,6,7,8).

Lucrarea de faţă îşi propune să evalueze posibilităţile de determinare a statusului hemodinamic la pacienţii cu hipertensiune arterială şi sindrom metabolic.

MATERIAL ŞI METODE

Lotul de studiu a inclus 32 de pacienţi cu HTA şi sindrom metabolic iar lotul martor a fost reprezentat de 32 de pacienţi cu sindrom metabolic dar normotensivi. Criteriile de excludere au fost: obezitatea extremă, prezenţa blocului de ram drept sau a fibrilaţiei atriale, anemia sau poliglobulia. Pacienţii au fost evaluaţi prin bioimpedanţă electrică toracică.

Rezultatele obţinute au fost prelucrate şi interpretate folosind programul *SPSS 16.0* iar pentru comparaţia loturilor s-a utilizat testul "Independent Sample."

REZULTATE ŞI DISCUŢII

1. Evaluarea statusului hemodinamic global a evidenţiat că: 28 (87,5%) pacienţi din lotul de studiu au fost normohemodinamici; 2 (6,25%) hiperdinamici; 2 (6,25%) hipodinamici. Pentru lotul martor, s-a constatat că: 29 (90,62%) pacienţi au fost normodinamici; 2 (6,25%) hiperdinamici; 1 (3,12%) hipodinamic. Se observă astfel că în ceea ce priveşte statusul hemodinamic global, nu s-au înregistrat diferenţe semnificative între lotul de studiu şi cel martor.

Pentru a verifica dacă între gupurile de pacienţi există diferenţe semnificativ statistic s-a utilizat *testul t* (corelaţia mediilor studiate), care a demonstrat existenţa unei corelaţii nesemnificative pentru statusul hemodinamic global (tab. I, II).

2. Evaluarea modulatorilor hemodinamici. În lotul de studiu 11 (34,4%) pacienţi prezentau ca dezordine predominanta hipervolemia; 20 (62,5%) vasoconstricţia; 1 (3,1%) parametri normali. În lotul martor 21 (65,6%) pacienţi au prezentat parametri normali în ceea ce priveşte volemia, inotropismul şi vasoactivitatea; 3 (9,4%) hipervolemie; 8 (25%) vasoconstricţie. Pentru a verifica dacă între grupurile de pacienţi există diferenţe semnificativ statistic în ceea ce priveşte modulatorii hemodinamici s-a utilizat *testul t*. S-a observat astfel că HTA şi sindromul metabolic se corelează cu dezordini ale modulatorilor hemodinamici, deşi la o primă evaluare a statusului hemodinamic global pacienţii ar putea fi încadraţi în categoria celor normodinamici (tab. III, IV).

TABELUL I

Statusul hemodinamic global, comparativ pentru lotul de studiu şi cel martor

Status hemodinamic	Lot de studiu	Lot martor	Valoare t	Valoare p
normohemodinamici	28	29	0,39	0,43
hiperdinamici	2	2	0	1
hipodinamici	2	1	0,58	0,24

TABELUL II

Rezultatele utilizării testului "Independent Sample" pentru evaluarea statusului hemodinamic global

Status hemodinamic	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
normohemodinamic	,628	,431	,395	62	,694	,03125	,07918	,1270	,18952
			,395	61,03	,694	,03125	,07918	-,1270	,18957
hiperdinamic	,000	1,00	,000	62	1,000	,00000	,06148	,1229	,12290
			,000	62,00	1,000	,00000	,06148	,1229	,12290
hipodinamic	1,39	,243	-,584	62	,562	-,03125	,05354	-,1382	,07578
			-,584	56,28	,562	-,03125	,05354	-,1384	,07599

TABELUL III

Evaluarea modulatorilor hemodinamici

Modulatori	Lot de studiu	Lot martor	Valoare t	Valoare p
Hipervolemie	11	3	2,4	<0,01
Vasoconstricţie	20	8	3,2	0,039
Limite normale	1	21	6,8	<0,01

TABELUL IV

Rezultatele utilizării testului "Independent Sample" pentru evaluarea modulatorilor hemodinamici

Dezordine predominantă	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
hipervolemie	31,39	,000	2,498	62	,015	-,25000	,10009	,4500	-,04993
			2,498	51,45	,016	-,25000	,10009	,4508	-,04911
vasoconstrictie	4,429	,039	3,215	62	,002	-,37500	,11666	,6081	-,14181
			3,215	61,24	,002	-,37500	,11666	,6082	-,14175
modulatori he-modinamici in limite normale	97,25	,000	6,880	62	,000	,62500	,09085	,4434	,80660
			6,880	39,17	,000	,62500	,09085	,4412	,80873

CONCLUZII

Sindromul metabolic este o construcţie clinic utilă în medicina prevenţională. Acesta este alcătuit dintr-o constelaţie de elemente clinice şi biologice care conferă indivizilor un risc cardiovascular crescut.

Hipertensiunea arterială este cea mai frecventă componentă individuală a sindromului metabolic şi totodată un factor cu mare susceptibilitate de a fi modificat.

S-a dovedit că bioimpedanţa electrică toracică permite evaluarea noninvazivă atât a statusului hemodinamic global cât şi a modulatorilor hemodinamici (volemie, inotropism, vasoactivitate).

S-a stabilit că HTA şi sindromul metabolic se corelează cu dezordini ale modulatorilor hemodinamici.

Evaluarea prin BET deschide perspectiva unei abordări terapeutice hemodinamice a pacienţilor cu HTA şi sindrom metabolic, care să vizeze dezordinea hemodinamică predominantă.

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REZISTENȚA LA ANTIBIOTICE A *STREPTOCOCCUS PNEUMONIAE*, 2003-2009

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ANTIMICROBIAL RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE*, 2003-2009 (Abstract). *Streptococcus pneumoniae* is the most important respiratory tract pathogen in otitis, sinusitis, bronchitis, and community-acquired pneumonia. **Material and methods**: A total of 155 patient isolates of *S. pneumoniae* were collected between February 2003 and December 2009 from the Iaşi „Sf. Parascheva" Infectious Diseases Hospital. Susceptibility to antibiotics was assessed by disk diffusion method (CLSI 2003-2008); minimum inhibitory concentrations (MIC) to penicillin and cefotaxime were determined by broth dilution method or E-test method. **Results**: Of these isolates, 32.6% were penicillin non-susceptible (MIC ≥ 0.12 µg/mL for nonmeningeal isolates before 2008 and all meningeal isolates and MIC ≥ 2 µg/mL for nonmeningeal isolates after 2008). The percentage of penicillin resistance increased from 0% to a maximum of 50% in 2008. Resistance rates (intermediate and resistant strains) among non-beta-lactam agents were as follows: macrolides, 30.9%; clindamycin, 9%; tetracycline, 34.3%; chloramphenicol, 2.1%; and trimethoprim-sulfamethoxazole, 57.5%. No resistance to vancomycin, linezolid and quinupristin-dalfopristin was observed. Resistance to non-beta-lactam antibiotics was higher among penicillin-resistant strains than penicillin-susceptible strains. Overall, antimicrobial resistance was higher among isolates from respiratory tract comparing with isolates from cerebrospinal fluid and blood. **Conclusions**: Resistance rates with *S. pneumoniae* have markedly increased in Romania during the last years. Pneumococcal isolates from Iaşi, Romania are often resistant to beta-lactams and available non-beta-lactam agents, especially if penicillin non-susceptible. **Key words**: *STREPTOCOCCUS PNEUMONIAE*, RESISTANCE, PENICILLIN

Streptococcus pneumoniae este cel mai important patogen al tractului respirator, care produce otite, sinuzite, bronşite şi pneumonie comunitară (1). În ultimele decade s-a remarcat creşterea concentraţiei minime inhibitorii (CMI) la penicilină (2); scăderea sensibilităţii la penicilină este însă diferită de rezistenţa la penicilină. Scăderea sensibilităţii la penicilină a apărut în toată lumea datorită diseminării mai multor clone rezistente la penicilină şi, în mai mică parte, datorită consumului excesiv de antibiotice (1).

Cu excepţia tulpinilor cu rezistenţă de nivel înalt, tratamentul bacteriemiei, sinuzitei, otitei, bronşitei sau pneumoniei comunitare produse de *S. pneumoniae* rezistent la penicilină rămâne penicilina sau orice alt beta-lactam. Doar pentru meningi-

ta pneumococică produsă de tulpini rezistente la penicilină clinicianul trebuie să aleagă un alt antibiotic, cu bună penetrabilitate în LCR şi rată de omorâre adecvată (1).

În ciuda creşterii ratei de rezistenţă a pneumococului la penicilină şi la macrolide în toată lumea, relevanţa clinică a acestui fenomen este încă necunoscută (3).

MATERIAL ŞI METODĂ

Tulpini bacteriene. Am inclus în studiu 155 tulpini non-duplicat de *S. pneumoniae* cu semnificaţie clinică de infectant, izolate din probe de spută, LCR sau hemocultură recoltate de la pacienţi internaţi în Spitalul de Boli Infecţioase „Sf. Parascheva" Iaşi în perioada 1.02.2003-31.12.2009 (tab. I). Izolatele au fost identificate ca *S. pneumoniae* prin caracterele de cultură pe agar-sânge şi testul de sensibilitate la optochin.

TABELUL I

Proveniența tulpinilor de *S. pneumoniae* incluse în studiu

	Spută	LCR	Hemocultură	Total
2003	4	7	1	12
2004	8	5	4	17
2005	12	20	5	37
2006	8	9	6	23
2007	9	12	4	25
2008	10	4	4	18
2009	12	7	4	23
Total	63	64	28	155

Testarea sensibilităţii la antibiotice. Pentru evaluarea sensibilităţii pneumococilor la antibiotice (eritromicină, E, clindamicină, CM, trimetoprim-sulfametoxazol, SXT, ofloxacin, OFX, levofloxacin, LVX, cloramfenicol, C, tetraciclină, TE, rifampicină, RIF, vancomicină, VA, linezolid, LZD) am utilizat metoda difuzimetrică pe agar Mueller Hinton cu 5% sânge de berbec (CLSI 2003-2008).

Pentru aprecierea sensibilităţii tulpinilor testate la penicilină (P) şi cefotaxim (CTX) am determinat CMI pentru aceste antibiotice folosind metoda diluţiilor în bulion Mueller Hinton cu concentraţie ajustată în cationi şi cu minim 2,5% sânge de cal lizat, conform recomandărilor CLSI, efectuată în duplicat (tulpini 2003-2007) şi metoda E-test (tulpini 2008-2009).

Controlul de calitate al testelor de sensibilitate la antibiotice a fost realizat folosind tulpina *S. pneumoniae* ATCC 49619.

Prelucrarea statistică. Baza de date şi prelucrarea datelor s-a realizat cu ajutorul programului EPIINFO 2005. Pentru compararea datelor s-a utilizat testul chi pătrat, fiind considerate semnificative valorile lui $p \leq 0,05$. Riscul relativ estimat Odds Ratio (OR) a fost calculat cu ajutorul intervalului de încredere IC95.

REZULTATE

Am analizat retrospectiv 155 tulpini non-duplicat de *S. pneumoniae* izolate între 2003-2009, cu un maxim de 37 tulpini izolate în 2005; 63 tulpini au fost izolate din probe de spută, 64 tulpini din LCR şi 28 tulpini din hemoculturi. Niciuna dintre tulpinile studiate nu a fost rezistentă la vancomicină sau linezolid. Rezistenţa la clindamicină, fluorochinolone, cloramfenicol, rifampicină a fost sub 10% (fig. 1).

Se observă o rată a tulpinilor non-sensibile (rezistente şi intermediare) la antibiotice mai mare pentru tulpinile izolate din probe respiratorii decât pentru cele izolate din produse normal sterile. Remarcăm în mod deosebit, prezenţa unui procent semnificativ mai mare de tulpini non-rezistente la cefotaxim (antibiotic de elecţie în tratamentul infecţiilor pneumococice invazive) în probe de spută comparativ cu celelalte produse patologice investigate. Peste 40% din pneumococii implicaţi în

infecții respiratorii joase au fost non-sensibili la penicilină, antibioticul de elecție în tratamentul acestor infecții. Majoritatea tulpinilor izolate din infecții pulmonare sunt rezistente la cotrimoxazol. Şi pentru eritromicină şi tetraciclină se observă rate înalte de rezistență între izolatele respiratorii de pneumococ (fig. 1).

Rezistența la penicilină a pneumococilor cauzatori de infecții respiratorii joase a avut o tendință continuu ascendentă, cu excepția anului 2009, când remarcăm scăderea bruscă, semnificativă ($p<0,05$) a procentului de tulpini rezistente (fig. 2).

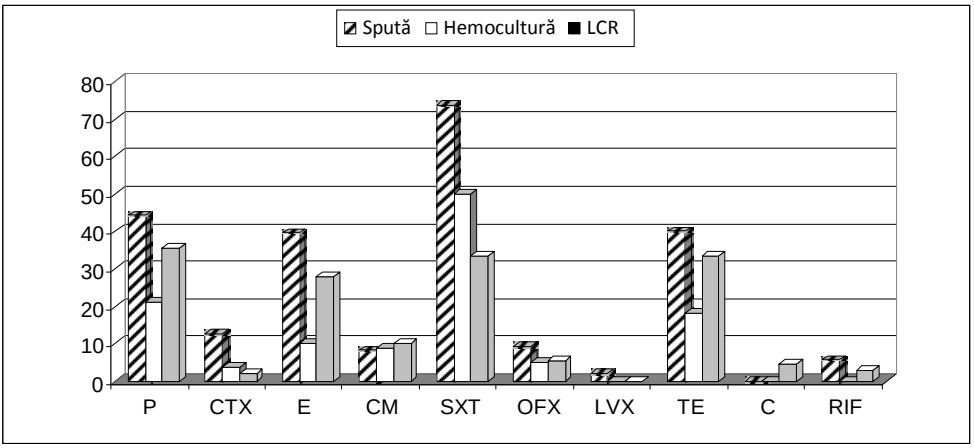

Fig. 1. Tulpini (%) de *S. pneumoniae* non-sensibile la antibiotice (2003-2009), în funcție de produsul patologic din care au fost izolate.

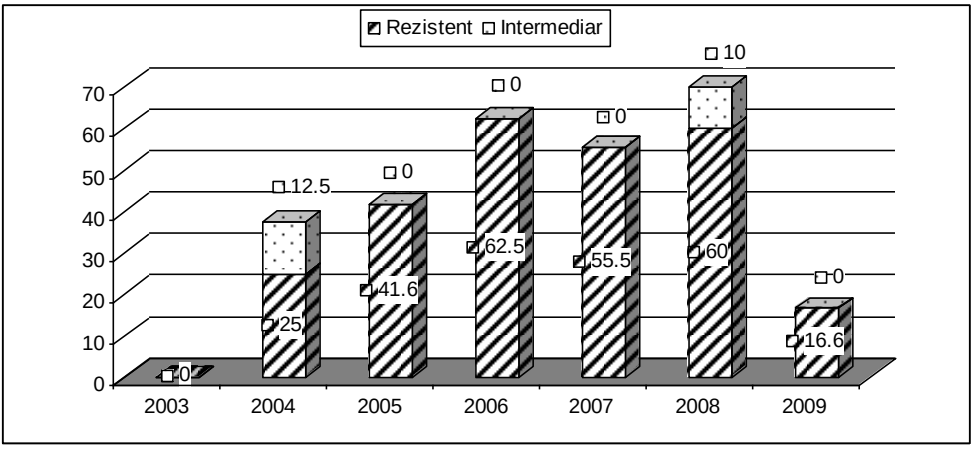

Fig. 2. Evoluția rezistenței la penicilină a pneumococilor izolați din probe de spută, 2003-2009.

Pentru penicilină şi cefotaxim am apreciat sensibilitatea la antibiotice prin determinarea CMI. Remarcăm că, în ciuda scăderii procentului de tulpini de pneumococ izolate din spută, rezistente la penicilină, în anul 2009, CMI_{90} pentru aceste tulpini s-a menținut la acelaşi nivel ca în anii precendenți (tab. II). Acest lucru este

explicat de modificarea punctelor de ruptură pentru penicilină în ianuarie 2008 (4, 5). Dacă am aplica punctele de ruptură CLSI 2008 şi tulpinilor anterioare acestei date, rata non-sensibilităţii pneumococilor izolaţi din infecţii respiratorii joase la penicilină ar fi diminuată. Tulpini de pneumococi rezistente la cefotaxim au fost izolate din probe de spută doar în 2007-2008.

În 2009 am izolat, de la un pacient cu meningită, o tulpină de *S. pneumoniae* rezistentă la penicilină şi cefotaxim, cu valori CMI foarte mari, >32 µg/ml pentru penicilină şi 16 µg/ml pentru cefotaxim.

TABELUL II

CMI_{50} şi CMI_{90} (µg/ml) pentru penicilină şi cefotaxim pentru izolate de *S. pneumoniae* din spută şi LCR

			2003	2004	2005	2006	2007	2008	2009
Spută	Penicilină	CMI_{50}	0,015	0,03	0,03	2	2	1	0,25
		CMI_{90}	0,03	2	4	8	16	8	8
	Cefotaxim	CMI_{50}	0,06	0,06	0,125	0,125	0,25	0,5	0,125
		CMI_{90}	0,125	0,5	1	0,5	8	4	1
LCR	Penicilină	CMI_{50}	0,015	0,015	0,015	0,015	0,015	0,015	0,015
		CMI_{90}	0,015	0,03	0,25	2	2	2	>32
	Cefotaxim	CMI_{50}	0,03	0,03	0,03	0,06	0,06	0,06	0,06
		CMI_{90}	0,06	0,06	0,06	0,125	0,25	0,25	16

Din cele 155 tulpini testate, 22 (14,2%) au prezentat rezistenţă multiplă la antibiotice (i.e., rezistenţă la cel puţin 3 clase de antibiotice), cu un maxim de 38,9% tulpini în 2008. Dintre acestea, cele mai multe (14) au fost izolate din probe de spută (22,2%). Tot o tulpină izolată din infecţii respiratorii, în 2008, a fost rezistentă la 8 antibiotice. Cea mai frecventă combinaţie de rezistenţă multiplă la antibiotice a fost de penicilină, cotrimoxazol şi eritromicină.

DISCUŢII

Streptococcus pneumoniae a fost demonstrat a fi cea mai frecventă cauză a pneumoniei comunitare (PC) (6). La sfârşitul anilor 1970 au fost descrise tulpini de pneumococi rezistente la penicilină în Africa de Sud şi Spania. Până la începutul anilor 1990, clone rezistente la penicilină se răspândiseră rapid în Europa şi apoi în întreaga lume. În plus, în paralel cu rezistenţa la penicilină apăruse şi rezistenţa la macrolide şi alte antibiotice. Şase clone internaţionale (serotipurile 6A, 6B, 9V, 14, 19F, 23F) au fost responsabile pentru majoritatea acestor izolate rezistente. Actualmente, 20-30% din tulpinile de pneumococ din întreaga lume prezinta rezistenţă multiplă la antibiotice (7); aceste date sunt similare celor din studiul nostru.

Rezistenţa la antibiotice a pneumococului generează greutăţi în alegerea unui tratament empiric în pneumonie. În cazul tulpinilor cu rezistenţă intermediară la penicilină sunt recomandate doze crescute ale acestui antibiotic. Infecţiile cu tulpini cu rezistenţă de nivel înalt la penicilină trebuie tratate cu alte antibiotice, cum ar fi cefalosporine de generaţia a treia sau noile fluorochinolone antipneumococice. În ariile cu rezistenţă de nivel înalt la macrolide, cum este şi România, monoterapia empirică cu un macrolid nu este optimă pentru tratamentul pacienţilor spitalizaţi cu PC moderată sau moderat-severă. Fluorochinolo-

nele sunt considerate antibiotice excelente în tratamentul PC pneumococice la adult, dar recomandarea lor se face cu reținere pentru a evita dezvoltarea rezistenței (6).

A fost estimat că în anul 2004, în România au fost 10.000 cazuri de pneumonie pneumococică care au necesitat spitalizare şi 38.200 cazuri tratate în ambulatoriu. Costul total al acestor îngrijiri medicale a fost de 30 milioane RON, dintre care 86% au fost cheltuiți pentru cazurile spitalizate şi 14% pentru cele din ambulatoriu (8).

În România, rezistența la penicilină a pneumococilor izolați din probe de spută a crescut de la 33,3% în perioada 1973-1978 la 56,6% în perioada 1985-1989. Rezistența acestora la eritromicină a crescut de la 38,4% (1973-1978) la 66,6% (1979-1984). Din cele 83 de tulpini de pneumococi izolate din infecții umane între 1990-1995 1,2% au fost rezistenți la ceftriaxonă, 3,6% la cloramfenicol, 44,5% la cotrimoxazol (9). Între 2004-2005, tulpinile de *S. pneumoniae* izolate în Institutul "Marius Nasta" Bucureşti au fost în proporție de 13,4% cu rezistență înaltă la penicilină şi 39,3% cu rezistență de nivel jos la penicilină. Rata rezistenței la alte antibiotice a fost: 64,0% la cotrimoxazol, 18,8% la eritromicină, 8,3% la cloramfenicol. Toate tulpinile au fost sensibile la levofloxacin (10).

În studiul nostru, incidența tulpinilor de pneumococi izolați din probe de spută non-sensibili la penicilină, între 2003-2009 este mai redusă (41% rezistență de nivel înalt şi 3,2% rezistență de nivel jos) decât în studiile anterioare efectuate în România. Există discrepanțe mari privind rata rezistenței la eritromicină în studiile anterioare (66,6% *versus* 18,8%); în studiul nostru aceasta a fost de 39,6% în probe respiratorii. Remarcăm creşterea incidenței rezistenței la cotrimoxazol față de studiile anterioare, 71% rezistență a pneumococilor izolați din probe de spută.

Într-un studiu privind rezistența la antibiotice a *S. pneumoniae* din Sydney, Australia, Gosbell *et al.* (11) au ajuns la concluzia că rezistența la antibiotice non-beta-lactamice a fost mai frecventă la tulpinile rezistente sau intermediare la penicilină. Rezistența multiplă a fost găsită la 87% din tulpinile rezistente la penicilină. Rezistența la eritromicină a fost observată la 89% din tulpinile rezistente la penicilină. Unele izolate nu au fost sensibile la quinupristin/dalfopristin.

Într-un studiu efectuat pe 6991 tulpini de *S. pneumoniae* izolate în Canada între 1997-2002, 20,2% din tulpini nu au fost sensibile la penicilină. Proporția tulpinilor multidrog-rezistente a crescut de la 2,7% la 8,8% în cei 5 ani de studiu. În ciuda scăderii consumului de antibiotice în Canada în cei 5 ani, ratele rezistenței de nivel înalt şi a multirezistenței a *S. pneumoniae* au crescut (12).

În ciuda creşterii dramatice a ratei rezistenței la antibiotice la pneumococi, impactul clinic al rezistenței la antibiotice este greu de definit. Dezbaterile despre rolului rezistenței în creştere a pneumococului la beta-lactamine, macrolide şi fluorochinolone asupra ratei de eşec terapeutic continuă. Rezultatele *in vitro* nu par a fi predictive pentru rezultatele *in vivo* (3). Eşecurile clinice reflectă frecvent factori independenți de sensibilitatea la antibiotice a microorganismelor infectante. Factori care țin de gazdă (e.g., vârsta extremă, imunosupresia, boala de fond, comorbidități) sau factori care afectează virulența intrinsecă a microorganismelor (e.g., subtipul capsular) influențează puternic prognosticul. Rata mortalității este mai mare în prezența implicării multilobare, a insuficienței renale, a necesității de îngrijire în secția de terapie inten-

sivă, a hipoxemiei, dereglării severe a parametrilor fiziologici şi comorbidităţi (13).

CONCLUZII

Concentraţia minimă inhibitorie a penicilinei faţă de tulpini de *S. pneumoniae* izolate din probe de spută a avut o tendinţă crescătoare în perioada 2003-2008, în contrast cu CMI ale tulpinilor izolate din infecţii invazive, cu tendinţă stagnantă.

Nu am evidenţiat tulpini de *S. pneumoniae* rezistente la vancomicină sau linezolid, indiferent de sediul infecţiei.

Pneumococii izolaţii din infecţii în Spitalul Clinic de Boli Infecţioase „Sf. Parascheva" Iaşi sunt frecvent rezistenţi la antibiotice beta-lactamice, dar şi la alte antibiotice disponibile, mai ales dacă sunt tulpini non-sensibile la penicilină. Incidenţa tulpinilor de pneumococ cu rezistenţă multiplă la antibiotice este mai mare între izolatele din spută, cea mai frecventă asociere fiind penicilină-eritromicină-cotrimoxazol.

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CERCETĂRI HISTO-ANATOMICE PRIVIND DOUĂ SUBSPECII DE *LAVANDULA ANGUSTIFOLIA* MILL.

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HISTO-ANATOMICAL RESEARCHES OF TWO SUBSPECIES OF *LAVANDULA ANGUSTIFOLIA* MILL. (Abstract): **Aim:** The structure of the inflorescences of two subspecies of *Lavandula angustifolia* ş*L. angustifolia* ssp. *angustifolia* and. *L. angustifolia* ssp. *pyrenaica* (D.C.) Guineaţ was studied to determine the range of variation in certain histo-anatomical characters. **Methods**: The flower and leaf structure has been analysed on cross and superficial section using the usual techniques and methods applied in plant histo-anatomical research. **Results**: In cross-section, inflorescence axis has a squared-shape contour, with four proeminent ribs. On the epiderme of inflorescence axis there are present stomata, trichoms and secretory hairs. The trichoms are pluricelullar and T-branched. The secretory hairs have a short pedicel and a uni-or bicellular head. **Conclusions**: The study revealed that there are only quantitative differences, referring to the degree of sclerification and lignification of mechanical elements and the size of the vascular bundles. **Key words**: ANATOMY, INFLORESCENCES, *LAVANDULA ANGUSTIFOLIA*

Continuând investigaţiile noastre privind histo-anatomia şi compoziţia chimică a unor plante medicinale (1, 2), în studiul de faţă ne-am propus să comparăm structura anatomică a inflorescenţelor de *Lavandula angustifolia*.

În Flora Europaea (3) sunt descrise 7 specii de *Lavandula*, *Lavandula angustifolia* având 2 subspecii: *ssp. angustifolia* şi *ssp. pyrenaica* (D.C.) Guinea, care diferă între ele astfel:

- ssp. *angustifolia* prezintă bractei, de obicei, mai scurte decât caliciul, ce au o lungime de 4,5-6 mm, dintele mai înalt al caliciului fiind neclar;
- ssp. *pyrenaica* (D.C.) Guinea prezinta bractei, de obicei, mai înalte decât caliciul, ce au o lungime de 6-7 mm, dintele mai înalt al caliciului fiind vizibil.

Date sumare de ordin histo-anatomic referitoare la levănţică se întâlnesc în diferite lucrări, unele cu caracter de monografie (4, 5, 6, 7). De structura anatomică a unor clone de levănţică s-au ocupat la noi în ţară Toma şi Niţă (8), din al căror articol am preluat parte din descriere. După cum se ştie, inflorescenţa este spiciformă, lungă de 15-20 cm, alcătuită din dichazii scurt pedicelate dispuse în verticile ± spaţiate şi axilare, fiecare cu câte 4-6 flori aproape sesile (9). Florile au: **bractei ovate**, membranoase, lungi de 4-5 mm; **caliciu cilindric** (tubulos), lung de 4-5 mm, cu 10-15 nervuri paralele şi cu 5 dinţi (dintre care cel posterior este mai lung); **corolă tubuloasă**, zigomorfă, bilabiată, mai lungă decât caliciul, pubescentă şi glanduloasă; **androceu didinam**, cu cele 4 stamine având antere reniforme; **gineceu bicarpelar**,

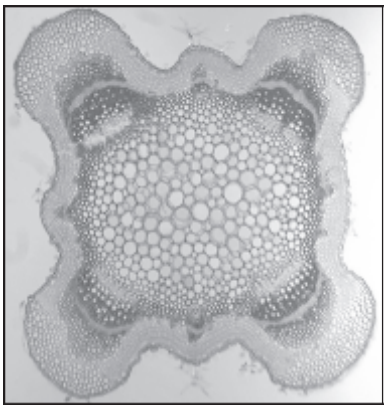

Fig. 1. Secţiune transversală prin axa inflorescenţei (nivel superior) de *Lavandula angustifolia* ssp. *angustifolia* (imagine de ansamblu).

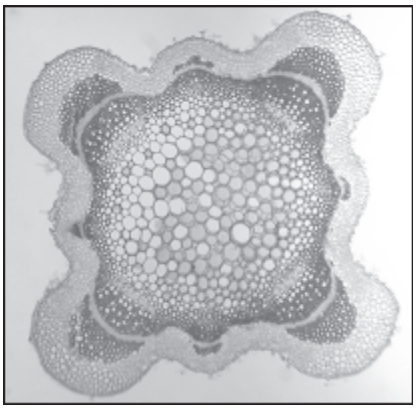

Fig. 2. Secţiune transversală prin axa inflorescenţei (nivel superior) de *Lavandula angustifolia* ssp. *pyrenaica* (imagine de ansamblu).

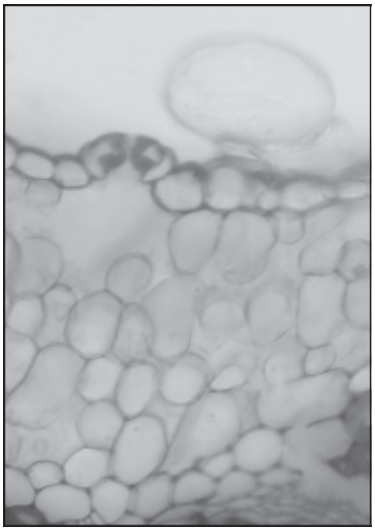

Fig. 3. Secţiune transversală prin axa inflorescenţei (nivel superior) de *Lavandula angustifolia* ssp. *angustifolia* : peri secretori

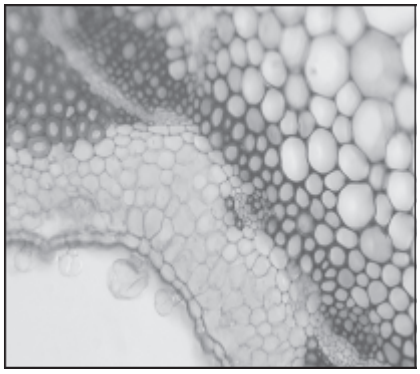

Fig. 4. Secţiune transversală prin axa inflorescenţei (nivel median) de *Lavandula angustifolia* ssp. *pyrenaica* – păr secretor

cu stil ginobazic, stigmat bifid şi disc nectarifer tetralobat la baza ovarului.

Fructele sunt tetranucule (nucule ovoidale, lungi de 2 mm, cafenii), iar seminţele sunt exalbuminate.

MATERIAL ŞI METODĂ

Materialul vegetal inclus în studiu a fost constituit din inflorescenţe de *Lavandula angustifolia* ssp. *angustifolia* şi *Lavandula angustifolia* ssp. *pyrenaica* (D.C.) Guinea. Inflorescenţele au fost fixate şi conservate în etanol 70%, iar secţiunile prin axa inflorescenţei şi florii s-au realizat cu ajutorul unui microtom manual, fiind colorate cu verde de iod şi roşu de ruteniu şi incluse în gelatină glicerolată. Secţiunile permanente au fost analizate la un microscop Optika (WF 10X-18MM) prevăzut cu obiective (4x/0,1 ; 10x/0,25 ; 40x/0,65 ; 100 x/1,25 cu o cameră foto încorporată (Canon PSA 540, zoom optic 4x 6.0 megapixeli) şi program de prelucrare Optika Vision Lite 2007.

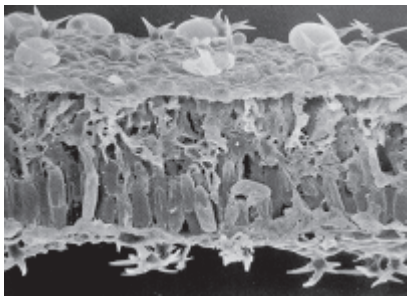

Fig. 5. Peri secretori şi tectori de la nivelul limbului foliar de *Lavandula angustifolia*

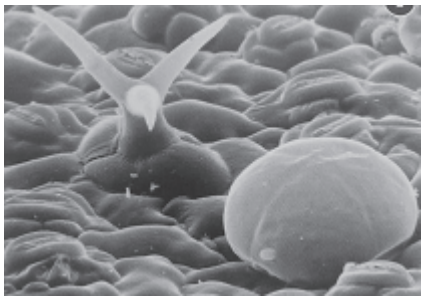

Fig. 6. Peri secretori şi tectori de la nivelul limbului foliar de *Lavandula angustifolia*

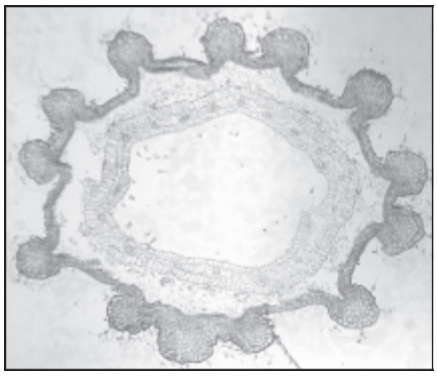

Fig. 7. Secţiune transversală prin floarea de *Lavandula angustifolia* ssp. *angustifolia :* caliciu şi corolă (imagine de ansamblu)

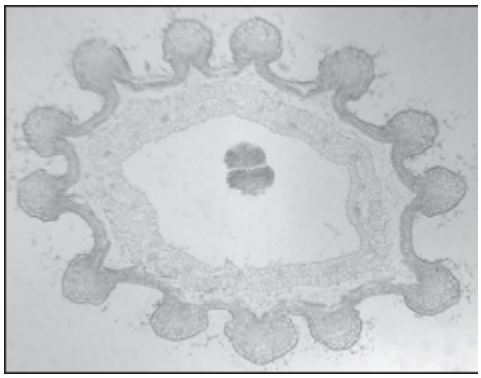

Fig. 8. Secţiune transversală prin floarea de *Lavandula angustifolia* ssp. *pyrenaica :* caliciu şi corolă (imagine de ansamblu)

REZULTATE ŞI DISCUŢII

Analizând particularităţile de structură ale axei pe care se inseră verticilele de flori, am constatat că axa inflorescenţei este prismatică, de contur pătratic în secţiune transversală, cu 4 coaste foarte proeminente; spre baza inflorescenţei, acestea alternează cu altele mai puţin proeminente (fig. 1, 2).

La nivelul epidermei axei inflorescenţei se disting stomate, peri tectori şi peri secretori. Atât frecvenţa perilor secretori, cât şi a celor tectori scade de la vârful către baza inflorescenţei. Perii glandulari, secretori au celula bazală mai mică decât celulele epidermice care o înconjoară, constituind pedicelul unicelular pe care se găseşte glanda uni- sau bicelulară. Perii tectori, aproape totdeauna pluricelulari şi ramificaţi în formă de T, sunt morţi la maturitate, plini de aer şi formează un toment albicios cu rol în protecţia plantei împotriva unei transpiraţii excesive.

Valoarea inflorescenţelor este dată de uleiul volatil sintetizat de perii glandulari secretori (fig. 3, 4), dar şi de papilele epidermice ale petalelor. Perii secretori se găsesc în tot lungul tulpinii, pe toate frunzele (fig. 5, 6), pe axul inflorescenţei şi pe flori.

Pe lângă perii secretori, perii tectori, ramificaţi sunt evidenţi atât pe axa inflorescenţei, cât şi pe caliciu şi corolă.

În ceea ce priveşte structura florii (fig. 7, 8, 9, 10), periantul interesează cel mai mult prin numărul mare de peri secretori (alături de peri tectori) care se află pe caliciu şi corolă şi în care se acumulează uleiul volatil. Perii secretori sunt predominant cu glanda uni- sau bicelulară, şi doar rar octocelulari, caracteristici labiatelor.

La nivelul inserţiei staminelor, tubul corolei are două inele de peri, unii tectori şi

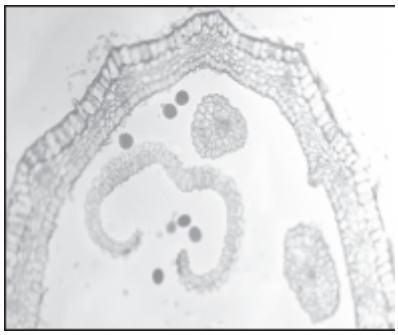

Fig. 9. Secţiune transversală prin floarea de *Lavandula angustifolia* ssp. *angustifolia :* corolă şi stamine

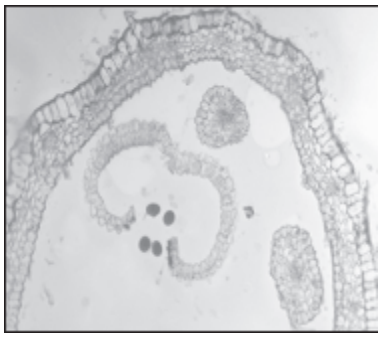

Fig. 10. Secţiune transversală prin floarea de *Lavandula angustifolia* ssp. *pyrenaica :* corolă şi stamine

alţii secretori, cu pedicel relativ lung (bi-sau tricelular) şi cu glanda măciucată, uni-sau bicelulară. Numărul perilor tectori şi secretori de pe caliciu şi corolă este mai mare la ssp. *pyrenaica* decât la ssp. *angustifolia*.

CONCLUZII

Studiul histo-anatomic a scos în evidenţă faptul că structura inflorescenţelor celor două subspecii investigate este similară cu cea a speciei originare *Lavandula angustifolia*, diferenţele la nivelul axei inflorescenţei fiind doar de ordin cantitativ şi se referă la gradul de sclerificare şi de lignificare a elementelor mecanice, mărimea fasciculelor conducătoare, grosimea cordoanelor de fibre perifloemice.

La nivelul florii, se observă caliciul cu coaste (nervuri) proeminente, corola cu celule epidermice de mărime diferită pe cele 2 feţe ale petalelor, gineceul cu 2 carpele unite, stil şi stigmat uşor bifid, precum şi androceul cu cele 4 antere (de contur circular fiecare în stadiu tânăr, cu şanţ ventral ce separă cele 2 loje în stadiul de maturitate deplină, înainte de deschiderea şi eliberarea granulelor de polen).

Deosebirile de structură între cele 2 subspecii sunt mici şi doar de ordin cantitativ. *Lavandula angustifolia* ssp. *pyrenaica* are :

- nervurile (coastele) caliciului mai puţin proeminente decât ale ssp. *angustifolia* ;
- corola cu petale mai subţiri, celulele ambelor epiderme fiind mult mai mari în comparaţie cu cele ale mezofilului ;
- numărul perilor tectori şi secretori de pe caliciu şi corolă, mai mare.

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STUDIU COMPARATIV PRIVIND STAREA DE SĂNĂTATE PARODONTALĂ PRIN PRISMA INDICILOR DE PLACĂ ŞI GINGIVALI

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COMPARATIVE STUDY ON THE PERIODONTAL HEALTH STATUS TROUGH PLAQUE AND GINGIVAL INDEX (Abstract): Nowadays, people are becoming more aware of the importance of oral hygiene in maintaining the health of the entire body. It is well known that plaque is the main etiological factor in producing caries disease and periodontal disease. The purpose of this study was to evaluate the comparative value indices of plaque and gingival inflammation in a group of young adults. **Material and method**: The study was conducted on a sample of 254 subjects. The study was clinical type. Data were collected after completion revelation plaque and clinical examination recorded the values calculated for each index in the observation sheet. The clinical evaluation of oral health status we used the API index (approximated Plaque Index) and BI indices for assessing periodontal status (bleeding index). All subjects included in the study were informed verbally about the purpose of the study noting the consent form. Patients were included in the database according to certain codes. Statistical data processing was done with the program for Windows SPSS14.00 setting a threshold of statistical significance of $p = 0.05$. **Results**: Comparative analysis of the presence of plaque and degree of gingival inflammation has revealed that there are differences statistically significant $p \leq 0.05$ for having a valoatre API index averaged 72.71 ($\pm$ 23.48) compared with gingival index = BI has an average of 20.05 ($\pm$ 18.32) and that influences the use of dental wire index periodontal inflammation. **Conclusions**: Gingival inflammation is present in those with plaque and is subject to use wire but not the frequency of tooth brushing which demonstrates that the use of adjuvant means brushing helps remove plaque but not entirely.
Key words: API, BI, GINGIVAL INDEX, YOUNG ADULTS

În prezent, populaţia este din ce în ce mai conştientă de importanţa igienei orale în menţinerea stării de sănătate a întregului organism. Este bine cunoscut, faptul că, placa bacteriană este factorul etiologic principal în producerea bolii carioase şi a afecţiunilor parodontale. Apariţia fenomenelor inflamatorii parodontale se observă cu predilecţie la nivelul spaţiilor aproximale, acolo unde îndepărtarea plăcii bacteriene se realizează cu dificultate. Astfel s-a introdus în practică cuantificarea plăcii bacteriene prin indicele API (Aproximal Plaque Index - Lange, 1981). Indicele de sângerare gingivală (BI – bleeding index) este un indice corelat cu API, care apreciază sângerarea la nivelul spaţiilor aproximale. Astfel se poate realiza corelarea dintre maturarea plăcii bacteriene de la nivelul situsurilor proximale şi fenomenele inflamatorii de la acest nivel.

Scopul acestui studiu a fost evaluarea comparativă a valorii indicilor de placă bacteriană şi a celor de inflamaţie gingivală la un lot de tineri.

MATERIAL ŞI METODĂ

În conceperea şi derularea studiului clinic s-a formulat ipoteza nulă că nu există diferenţe semnificative din punct de vedere statistic între prezenţa cantitativă şi calitativă a plăcii bacteriene pusă în evidenţă prin indicele API şi inflamaţia parodontală cuantificată cu ajutorul indicelui BI. Ipoteza testabilă constă în faptul că există diferenţe din punct de vedere statistic între valorile indicilor care cuantifică placa bacteriană şivalorile indicilor care cuantifică gradul de inflamaţie gingivală.

Studiul a fost realizat pe un lot de 122 de subiecţi, studenţi ai anului III, Facultatea de Medicină Dentară, UMF Gr.T.Popa Iaşi. Lotul a fost format din 78 de subiecţi de sex feminin şi 45 subiecţi de sex masculin, cu o medie de vârstă de 23,53 ani (vârste între 21-29 ani).

Criteriile de includere a subiecţilor au fost : prezenţa plăcii bacteriene după revelare, consimţământul pacienţilor de a participa la studiu. Criteriile de excludere au fost reprezentate de absenţa plăcii bacteriene, refuzul pacienţilor de a participa la studiu. Studiul a fost de tip clinic.

Datele au fost colectate în urma realizării revelării plăcii bacteriene şia examenului clinic consemnând valorile calculate pentru fiecare indice în fişa de observaţie. Pentru evaluarea clinică a stării de igienă orală am utilizat indicele API (Aproximal Plaque Index) iar pentru evaluarea statusului parodontal indicii BI (bleeding index).

Pentru determinarea prezenţei plăcii bacteriene în spaţiile aproximale prin indicele API s-a realizat revelarea plăcii bacteriene cu albastru de metil. API apreciază procentual acumularea plăcii bacteriene în spaţiile aproximale. Formula de calcul este : numărul de spaţii aproximale cu placă raportat la numărul de spaţii aproximale de înmulţit cu 100. Aprecierea stării de igienă orală în funcţie de valoarea API se face după următoarea scală de valori : API = 100-70% - igienă total nesatisfăcătoare ; API = 70-35% - igienă orală medie ; API = 35-25% - igienă orală relativ corectă ; API < 25% = igienă orală optimă.

Evaluarea statusului parodontal s-a realizat prin utilizarea indicelui gingival BI (Bleeding Index) care este definit astfel : 0- gingie fără sângerare la sondaj ; 1- sângerare la sondaj.

Indicele BI se determină cu ajutorul unei sonde parodontale, care se introduce la baza papilei, mezial şi distal, în unghi de 20-40^0 faţă de axul dintelui până la baza şanţului gingival. Scorurile utilizate pentru determinare sunt : 0- gingie fără sângerare la sondaj ; 1- sângerare la sondaj. BI se calculează împărţind suma valorilor înregistrate la numărul de papile interdentare examinate de înmulţit cu 100.

Toţi subiecţii incluşi în studiu au fost informaţi verbal cu privire la scopul studiului notându-se în fişă consimţământul acestora. Examinarea pacienţilor s-a realizat în cabinetul Policlinicii nr.1, Ambulatorul de Stomatologie. Pacienţii au fost introduşi în baza de date conform anumitor coduri.

Prelucrarea datelor statistice s-a realizat cu programul SPSS14.00 pentru Windows stabilindu-se un prag de semnificaţie statistică de $p \leq 0,05$.

REZULTATE ŞI DISCUŢII

Rezultatele analizei comparative a prezenţei plăcii bacteriene şia gradului de inflamaţie gingivală au pus în evidenţă faptul că există diferenţe semnificative din punct de vedere statistic $p \leq 0,05$ în favoarea indicelui API care are o valoatre medie de 72,71 ($\pm$23.48) în comparaţie cu indicile gingival BI care are o valoare medie de 20,05 ($\pm$ 18.32) (tabel I).

Din punct de vedere al corelaţiilor între acesti indici, analiza Pearson a datelor evidenţiază corelaţii între indicii API şi BI ($p \leq 0.05$) rezultate care susţin ipoteza acţiunii plăcii bacteriene asupra ţesutului gingival

TABELUL I
Analiza descriptiva a indicilor API şi BI

	N	Minimum	Maximum	Mean	Std. Deviation
API	253	,46	100,00	72,7111	23,48040
BI	253	,00	88,00	20,0533	18,32100
Valid N (listwise)	253				

TABELUL II
Corelaţii intre API şi BI

		API	BI
API	Pearson Correlation	1	,245(**)
	Sig. (2-tailed)		,007
	N	253	122
BI	Pearson Correlation	,245(**)	1
	Sig. (2-tailed)	,007	
	N	253	122

** Correlation is significant at the 0.01 level (2-tailed).

TABELUL III
Analiza statistică a mediilor indicelul API şi BI. Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	API	72,7111	253	23,48040	2,12582
	BI	20,0533	253	18,32100	1,65871

TABELUL IV
Analiza statistică a mediilor indicelul API şi BI. Paired Samples Correlation

		N	Correlation	Sig.
Pair 1	API & BI	253	,245	,007

TABELUL V
Analiza statistică a mediilor indicelul API şi BI. Paired Samples t-Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	API – BI	52,65787	26,00298	2,35420	47,99711	57,31863	22,368	121	,000

din spaţiile aproximale (tabel. II) manifestată prin modificare de aspect şitextură a gingiei dar şiprin apariţia sângerăriii la sondajul parodontal.

Analiza statistică realizată a permis identificarea unor diferenţe semnificative între nivelul mediei indicelui de placă bacteriană API şiindicele de inflamaţie parodontală BI; testul $t(121)=22,36$ pentru $p<0,001$ argumentând acest lucru (tabel III,IV,V). Astfel placa bacteriană este prezentă în mai multe cazuri decât inflamaţia gingivală tradusă prin sângerare ceea ce indică că la unii pacienţi placa bacteriană este nepatogenă în sensul afectării parodontale şi nevirulentă.

Corelarea prezenţei plăcii bacteriene în spaţiul aproximal cu utilizarea firului dentar indică că nu există diferenţă semnificativă între valorile API la cei care utilizează fir interdentar şi cei care nu utilizează (tabel VI).

În schimb la subiectii care utilizează fir interdentar nu apare inflamaţie gingivală $p\leq0,001$ (tabel VIII,IX). Acest lucru ne orientează asupra faptului că analiza printr-un indice calitativ de placă bacteriană care pune în evidenţă dacă este sau placă bacteriană nu ne dă întotdeauna rezultatele cele mai concludente.

Nu există diferenţe semnificative din punct de vedere statistic între frecvenţa periajului şi valoarea indicilor API şi SBI (X,XI) valorile fiind medii pentru cei care realizează periaj o dată pe zi şi pentru cei care realizează periaj de două ori pe zi.

TABELUL VI

Analiza statistică a mediilor indicelui API şi BI în comparaţie cu utilizarea firului interdentar Mann-Whitney Test

	utilizare fir dentar	N	Mean Rank	Sum of Ranks
API	da	164	61,78	3954,00
	nu	89	61,19	3549,00
	Total	253		

TABELUL VII

Teste statistice Mann-Whitnez si Wilcoxon

	API
Mann-Whitney U	1838,000
Wilcoxon W	3549,000
Z	-,092
Asymp. Sig. (2-tailed)	,926

a Grouping Variable: utilizare fir dentar

TABELUL VIII

Analiza statistică a mediilor indicelui API şi BI în comparaţie cu utilizarea firului interdentar. Statistica Chi-Square

	utilizare fir dentar	N	Mean Rank
API	da	164	61,78
	nu	89	61,19
	Total	253	
BI	da	164	50,87
	nu	89	73,23
	Total	253	

TABELUL IX

Teste statistice Chi-Square

	API	BI
Chi-Square	,009	12,277
df	1	1
Asymp. Sig.	,926	,000

a. Kruskal Wallis Test
b. Grouping Variable: utilizare fir dentar

TABELUL X

Analiza statistică a mediilor indicelui API şi BI în comparaţie cu frecvenţa periajului dentar. Statistica Chi-pătrat

	frecventa periaj	N	Mean Rank
API	o data/zi	163	64,83
	de doua ori/zi	90	54,42
	Total	253	
BI	o data/zi	163	62,13
	de doua ori/zi	90	60,17
	Total	253	

TABELUL XI

Testul statistic Chi-Square

	API	BI
Chi-Square	2,305	,082
df	1	1
Asymp. Sig.	,129	,774

a Kruskal Wallis Test
b Grouping Variable: frecventa periaj

CONCLUZII

Inflamatia gingivală este prezentă la cei care prezintă placă bacteriană şi este condiţionată de utilizarea firului dentar dar nu şide frecvenţa periajului ceea ce demonstrează că utilizarea mijloacelor adjuvante periajului dentar ajută la îndepărtarea plăcii bacteriene dar nu în totalitate. Deoarece nu toţi pacienţii care prezintă placă bacteriană în spaţiul aproximal prezintă şiinflamaţie gingivală se sugerează că în etiologia afectării parodonţiunui marginal este prezent şiun factor general care influenţează răspunsul imun local.

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CERCETĂRI PENTRU CUNOAŞTEREA INCIDENŢEI ŞI PREVALENŢEI ANOMALIILOR CONGENITALE, ÎN JUDEŢUL ŞI MUNICIPIUL IAŞI, ÎN PERIOADA 2001-2008

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RESEARCH ON THE INCIDENCE AND PREVALENCE OF CONGENITAL ABNORMALITIES IN IAŞI DISTRICT AND IAŞI CITY, FROM 2001 TO 2008 (Abstract): Congenital abnormalities (CA) are deviations from the normal embryonic development that appear antenatal and they are characterized by the alteration of the morphology and function of an organ, system of organs or even of the entire body. **Material and method**: The study, on a period of eight years, included 1685 children with CA, from which 58% were males and 50% were from the country-side. **Results**: It has been observed that 36% of the CA cases were premature births and 64% were normal term births. Also, 21% of the children with CA weighed less than 2700 grams at birth and 79% weighed more than 2700 grams at birth. The birth's APGAR score has been less than 7 in 30% of the cases and higher than 7 in 70% of the cases. 72% of the cases were natural births and 28% were caesarian births. 88% of the CA cases were singular congenital abnormalities and 12% were multiple congenital abnormalities. 24% of the CA were cardiac abnormalities and 21% were skeletal abnormalities. 3% of the subjects of the study have died, of which 69% died from cardiac abnormalities, 22% from hydrocephalus abnormalities, 7% from diaphragmatic hernia and 2% from renal congenital abnormalities. **Key words**: CONGENITAL ABNORMALITIES, PREVALENCE, EPIDEMIOLOGIC SURVELANCE, PREVENTION

Eficienţa redusă a metodelor de screening pentru depistarea precoce şi a unor programe terapeutice care să reducă impactul, de multe ori dezastruos, asupra bolnavului, familiei şi societăţii, situează anomaliile congenitale (AC) între maladiile care determină grave implicaţii sociale şi economice (4). Cercetările epidemiologice colaborative, multidisciplinare, realizate pe loturi reprezentative de bolnavi şi perioade multianuale, aduc informaţii valoroase pentru orientarea programelor de supraveghere epidemiologică, clinică şi de laborator a grupurilor cu risc şi a copiilor cu AC.

Datele cercetărilor noastre de factură clinico-epidemiologică pot constitui o contribuţie în direcţia ameliorării eficienţei prevenţional-terapeutice a asistenţei pentru pacienţii cu AC.

Prin anomalie congenitală se înţelege un defect morfologic, structural (macroscopic sau microscopic) al unui organ sau regiune anatomică, prezent la naştere, chiar dacă nu este evident la momentul ontogenetic

respectiv (1,5,7,8).

Din punct de vedere patogenic, anomaliile congenitale se împart în: malformaţii, disrupţii, deformaţii şi displazii. În raport cu manifestările clinice, anomaliile congenitale pot fi izolate şi multiple. Aceste două clasificări ale anomaliilor congenitale sunt cele mai folosite în practica medicală, deoarece permit stabilirea prognosticului, a conduitei practice şi, nu în ultimul rând, a riscului de recurenţă al afecţiunii (2,3,6).

MATERIAL ŞI METODE

Cercetările au inclus 1685 de copii diagnosticaţi cu AC în perioada 2001-2008, reprezentând o prevalenţă de 3,3% din totalul naşterilor raportate în Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi.

Culegerea informaţiilor s-a realizat prin colaborarea cu serviciile de statistică medicală ale clinicii şi prin studierea registrelor de evidenţă a copiilor cu AC, din cadrul secţiei de neonatologie. Au fost analizate cazurile înregistrate pe parcursul fiecăruia dintre cei 8 ani, în corelaţie cu sexul, mediul de provenienţă (rural, urban), felul naşterii (naturală, cezariană), greutatea la naştere (<2700 g, >2700 g), vârsta gestaţională (<37 săptămâni, >37 săptămâni) şi scorul APGAR (<7, >7).

Prelucrarea şi interpretarea datelor acumulate a fost realizată prin metode epidemiologice, statistico-matematice şi computerizate.

În realizarea diferitelor etape ale programului de cercetare au fost respectate: colaborarea etică cu ceilalţi medici implicaţi în managementul acestor cazuri, adoptarea unei atitudini nediscriminatorii, fără a emite judecăţi lipsite de profesionalism, confidenţialitatea actelor medicale şi a datelor personale, cu excepţia datelor statistice incluse în studiu.

REZULTATE ŞI DISCUŢII

1. Repartiţia pe sex şi mediul de provenienţă a 1685 de copii diagnosticaţi cu AC în secţia de neonatologie a Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă" Iaşi, în perioada 2001-2008. În cei 8 ani luaţi în studiu, din lotul de 1685 de copii cu AC, 710 (42%) au fost de sex feminin, 975 (58%) masculin; 843 (50%) au provenit din mediul urban şi 842 (50%) din rural.

S-a observat o afectare preponderentă a sexului masculin faţă de feminin ($p \leq 0,05$), fără diferenţe semnificative între rural şi

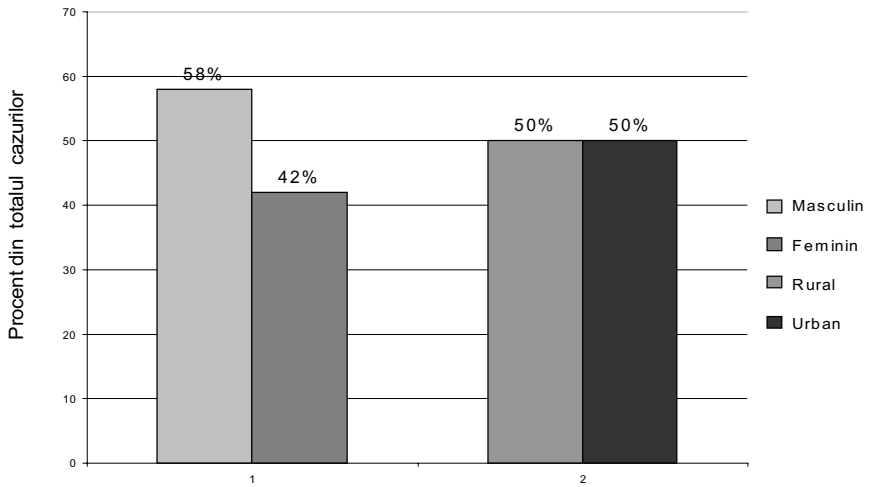

Fig. 1. Repartiţia pe sex şi mediul de provenienţă a cazurilor de anomalii congenitale, raportate la Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi, în perioada 2001-2008

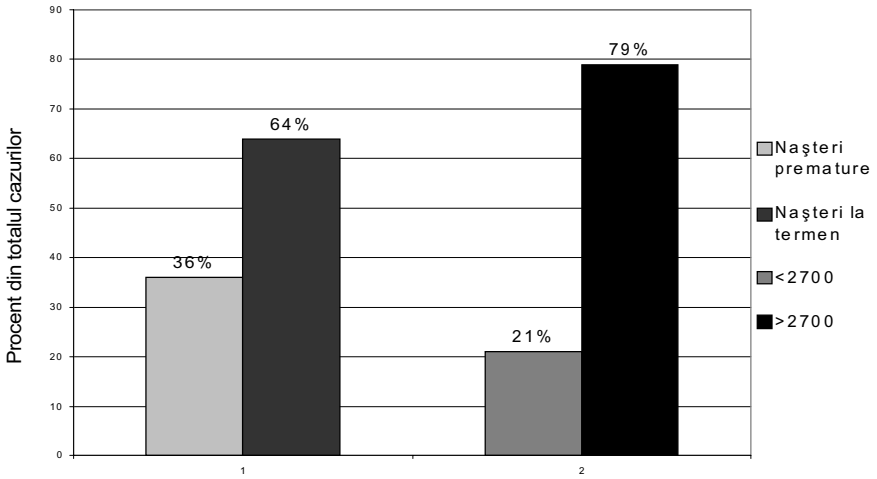

Fig. 2. Valorile prevalenţei cazurilor de anomaliilor congenitale, în raport cu vârsta gestaţională şi greutatea la naştere, raportate la Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi, în perioada 2001-2008

urban (p≥0,05). Estomparea diferenţei între mediul urban şi cel rural poate fi urmarea modificărilor care au loc între cele două medii, îndeosebi prin migrarea, în ultimele 3-4 decenii, din mediul rural spre cel urban, a unor importante categorii demografice. Acest fenomen a determinat un amestec şi o asociere puternică a unor factori care în mediul urban ar putea fi consideraţi cu risc pentru apariţia AC (fig.1).

2. Analiza repartiţiei, în relaţie cu vârsta gestaţională şi greutatea la naştere. În perioada luată în studiu, din cele 1685 de cazuri de AC înregistrate, 603 (36%) au provenit din sarcini care s-au încheiat prematur (înainte de 37 de săptămâni) iar restul de 1082 (64%) s-au născut la termen (depăşind 37 de săptămâni de sarcină). De asemenea, 351 (20%) de copii au avut o greutate la naştere mai mică de 2700 grame şi 1334 (80%) de copii au avut peste 2700 grame la naştere. Studiul asupra greutăţii înregistrate la naştere a evidenţiat pentu copiii cu greutate mică (<2700 grame) diferenţe anuale ale prevalenţei (9% în 2005 şi 41% în 2008)($p \leq 0,05$), comparativ cu cei cu greutate normală (91% în 2005 şi 59% în 2008)($p < 0,005$). În ceilalţi ani, diferenţele nu au avut o semnificaţie deosebită (fig. 2).

3. Repartiţia în relaţie cu felul naşterii şi scorul APGAR acordat la naştere. În perioada de 8 ani ce a făcut obiectul studiului, s-a constatat că prevalenţa suferinţei fetale (scor APGAR < 7) în rândul copiilor cu AC variază între 15% (în anul 2002) şi 34% (în anii 2004 şi 2008).

S-a mai constatat că prevalenţa naşterilor naturale, în cazul copiilor cu AC, variază între 59% (în anul 2004) şi 97% (în anul 2002), în timp ce prevalenţa naşterilor prin cezariană variază între 3% (în anul 2002) şi 41% (în 2004) (tab. I).

4. Distribuţia cazurilor în relaţie cu caracterul izolat sau multiplu, al prezenţei anomaliilor congenitale. Anomalii izolate s-au înregistrat la 88% din cazuri, cele mai frecvente fiind cele cardiace (24%) şi scheletice (21%)(tab. II).

Anomalii congenitale multiple. Prezenţa asocierii unor anomalii diferite la acelaşi caz s-a înregistrat într-un număr semnificativ scăzut ($p \leq 0,05$) faţă de cele izolate. În cei 8 ani de studiu au fost observate 196 de cazuri cu AC multiple (12% din totalul

TABELUL I

Distribuţia anuală a cazurilor de anomalii congenitale, în relaţie cu scorul APGAR şi felul naşterii, raportate la Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi, în perioada 2001-2008

Anul	Nr. Cazuri	SCOR APGAR				Felul naşterii (*)			
		< 7		> 7		Naturală		Cezariană	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
2001	122	26	21	96	79	95	78	27	22
2002	125	19	15	106	85	121	97	4	3
2003	168	56	33	112	67	160	95	8	5
2004	232	80	34	152	66	137	59	95	41
2005	281	82	29	199	71	182	65	99	35
2006	274	86	31	188	69	195	71	79	29
2007	323	102	32	221	68	218	67	105	33
2008	160	55	34	105	66	113	71	47	29
Total	1685	506	30	1179	70	1221	72	464	28

(*) Pentru valorile mai mici de 10, proporţiile sunt orientative.

TABELUL II

Distribuţia cazurilor de anomalii congenitale izolate, raportate la Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi, în perioada 2001-2008

Anul	Anomalii congenitale izolate						
	Nr. Cazuri	Cardiace		Scheletice		Altele	
		Nr.	%	Nr.	%	Nr.	%
2001	101	20	20	17	17	64	63
2002	112	31	28	27	24	54	48
2003	149	36	24	33	22	80	54
2004	215	45	21	56	26	114	53
2005	250	49	20	45	18	156	62
2006	234	55	24	38	16	141	60
2007	292	81	28	79	27	132	45
2008	136	49	36	13	10	74	54

(*) Pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL III

Prevalenţa cazurilor de anomalii congenitale multiple, în relaţie de numărul de anomalii asociate, raportate la Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă", Iaşi, depistate în perioada 2001-2008

Anul	Anomalii congenitale multiple (*)								
	Asociaţii de anomalii							Sindrom Down	
	Nr. Cazuri	2 anomalii		3 anomalii		4 anomalii			
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
2001	21	3	14	6	29	3	14	9	43
2002	13	7	54	0	0	2	15	4	31
2003	19	5	26	3	16	6	32	5	26
2004	17	4	24	4	24	1	6	8	46
2005	31	11	35	5	16	5	16	10	39
2006	40	18	45	5	13	6	15	11	31
2007	31	14	45	0	0	5	16	12	39
2008	24	6	25	3	13	6	25	9	37

(*) Pentru valorile mai mici de 10, proporţiile sunt orientative

cazurilor de AC). Dintre acestea, ponderea cea mai mare au avut-o cazurile de asociere a 2 anomalii (35%), urmate de cazurile de asociere a 4 anomalii (17%) şi cazurile de asociere a 3 anomalii (13%). Dintre sindroamele plurimalformative cel mai frecvent întâlnit a fost sindromul Down, determinat de trisomia 21 şi care se caracterizează printr-un model caracteristic de dezvoltare anormală (tab. III).

5. Decesele înregistrate la copiii cu anomalii congenitale. În perioada 2001-2008, au decedat 45 de copii (3%) din totalul de 1685 de cazuri cu AC. Dintre aceştia, 69% prezentau anomalii cardiace, 22% hidrocefalie, 7% hernie diafragmatică şi 2% anomalii renale.

CONCLUZII

Anomaliile congenitale (AC) reprezintă o cauză importantă a numeroase implicaţii nefavorabile, medicale şi socio-economice la nivel mondial.

Studiul, pe durata a 8 ani, a inclus 1685 de copii cu AC, dintre care 58% de sex masculin şi 42% de sex feminin. Proporţiile au fost egale pentru mediile urban şi rural. S-a evidenţiat o prevalenţă de 36% pentru naşterile premature şi 64% pentru naşterile la termen. 21% dintre copii au avut o greutate la naştere < 2700 grame şi 79% > 2700 grame. 30% dintre subiecţii luaţi în studiu au prezentat suferinţă fetală (scor APGAR < 7). În funcţie de caracterul naşterii, majoritatea (72%) au fost înregistrate naşteri naturale. Anomaliile congenitale izolate au reprezentat 88% şi cele multiple 12%. 24% dintre anomalii au fost de natură cardiacă, în timp ce 21% au fost de natură scheletică. 3% din numărul de copii diagnosticaţi cu AC au decedat, 69% dintre aceştia suferind de anomalii cardiace, 22% hidrocefalie, 7% hernie diafragmatică şi 2% anomalii congenitale renale.

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ASPECTE EPIDEMIOLOGICE ALE EDENTAŢIEI FRONTALE LA COPIL ŞI ADOLESCENT

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EPIDEMIOLOGICAL ASPECTS OF FRONTAL EDENTATION OF CHILD AND ADOLESCENT (Abstract): Increasingly frequency of frontal edentation of children and adolescents, mainly because of dental caries and its complications and other diseases requires closer analysis and a reconsideration of therapeutic attitudes that must be carefully adapted and refined. **Aim**: To study the establishment of prevalence and incidence of child and adolescent frontal edentation, identifying its etiology and prospects of restoration of morpho-functional parameters affected, anchored in temporary or permanent solutions, according to age and morpho-functional development of the patient. **Material and methods**: We studied a group composed of 450 patients, aged between 8 and 19 years of age, diagnosed with frontal edentation of different etiologies. **Results**: The two most common causes which are involved in producing edentation are odontal injuries and chronic periodontal disease. **Conclusion**: Etiology of frontal edentation has an esential part in the anatomoclinical shape of edentation, without evading the impact on bio-psycho-social dimension of this clinical entity. The size of the edentation, the emotional impact for the child, and the medical aspects are very important in the decision of the right treatment for long time. **Key words**: ADOLESCENT, CHILD, FRONTAL EDENTATION, STATISTIC

IN VITRO DISSOLUTION STUDIES OF AMIODARONE HYDROCHLORIDE FROM HYDROXY-PROPYL-β-CYCLODEXTRIN/AMIODARONE INCLUSION COMPLEX FORMULATED INTO MODIFIED-RELEASE TABLETS

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IN VITRO DISSOLUTION STUDIES OF AMIODARONE HYDROCHLORIDE FROM HYDROXY-PROPYL-β-CYCLODEXTRIN/AMIODARONE INCLUSION COMPLEX FORMULATED INTO MODIFIED-RELEASE TABLETS (Abstract) **Aim:** Drug release from modified-release matrix tablets made of Kollidon® SR and Chitosan is dependent on its degree of solubility in the dissolution medium as well as on the matrix forming polymer. By complexing hydrochloride amiodarone with hydroxypropyl-β-cyclodextrin, an inclusion complex was obtained, which showed an increase in solubility by more than 200%. The complex was used to obtain modified-release matrix tablets based on Kollidon® SR and Chitosan. **Materials and Methods**: Matrix tablets were obtained through direct compression method of non-complexed amiodarone and inclusion complex, and they were marked F1 and F10, respectively. The two formulations were studied comparatively in terms of release kinetics of the active substance through in vitro drug release tests. Those tests were conducted using a paddle apparatus II for 12 hours and two gastrointestinal simulation liquids with different pH values relevant for oral administration - 2 hours at pH 1.2 and 10 hours at pH 6.8. The release of hydrochloride amiodarone was quantified using a validated HPLC method. Two factors were calculated to assess the release profile of amiodarone: the similarity factor f1 and difference factor f2. **Results:** The increase in Kollidon® SR concentration resulted in a slower release of amiodarone at both pH values. The use of Chitosan resulted in a decrease of AMD release only at pH 6.8. **Conclusions:** The similarities between the two release profiles of AMD were confirmed by the values of the similarity factor (f1 = 43.697) and difference factor f2 (f2 = 68.263). **Keywords:** AMIODARONE, INCLUSION COMPLEXE, MATRIX TABLETS, SUSTAINED RELEASE, KOLLIDON® SR, CHITOSAN.

Amiodarone [2-butyl-3-(3,5-diiodo-4-β-diethylaminoetoxybenzoyl)benzofuran] (AMD) originally used as an antianginal, is currently administered as cardiovascular medication in the treatment of severe supraventricular and ventricular arrhythmias (1). AMD is a Class III antiarrhythmic substance which prolongs the duration of action potential and effective refractory period in both the atria and ventricles. The absorption of

amiodarone in the gastro-intestinal tract is variable and hard to predict. The oral bioavailability ranges from 22% to 86%. Large individual variations were attributed to the dealkylation of the molecule to its primary metabolite desethylamiodarone (2,3). This unpredictable absorption may be explained by the low solubility of amiodarone in aqueous solutions and by the hepatic first-pass effect, which has been not clearly defined (4).

In terms of pharmacokinetics, amiodarone is included in the II BCS class (biopharmaceutical classification system), as it is characterized by increased membrane permeability and a low rate of dissolution, due to the low solubility in water (5). The formulation of AMD-based polymer matrix tablets can alter the dissolution rate and oral bioavailability of this active substance.

Currently, AMD is used in therapy as conventional release pharmaceutical products - 200 mg AMD oral tablets, under the trade name Cordarone®.

The aim of this study was to improve the biopharmaceutical properties of AMD by various pharmacotechnical methods. In the first stage AMD was included in hydroxypropyl-β-cyclodextrin (HP-β-CD) which resulted in the HP-β-CD/AMD inclusion complex that has been physicochemical and pharmacokinetic characterized (6-9).

In the second phase modified release matrix tablets were formulated based on Kollidon® SR (KOL) and Chitosan (CHT) in which AMD was used as non-complexed active substance and as HP-β-CD/AMD inclusion complex. Two matrix tablet formulations were comparatively studied in terms of AMD release profile.

MATERIAL AND METHODS

The raw materials used were 100.2% pure Amiodarone hydrochloride (Zhejlang Sanmen Hengkang Pharmaceutical Co. Ltd., China,), Kollidon® SR and practical grade Chitosan (BASF, Germania), Aerosil 200 (Degussa, Germania) and Magnesium stearate (Union Derivan S.A., Spania), and HP-β-CD/AMD inclusion complex ("Petru Poni" Institute of Macromolecular Chemistry Iaşi, România).

The preparation of matrix tablets: In a previous study we analyzed the concentration of polymer matrix for tablets with optimal technical and pharmacokinetic properties. Based on the results of that study two formulations of AMD modified-release matrix tablets were formulated and their pharmaceutical formulation is presented in Table I. AMD matrix tablets were obtained by direct compression method using a Korsh EK0 machine with 9 mm ponson diameter and 8-10kN compression force.

TABLE I
Formulation of matrix tablets

Matrix components (% w/w)	Formula	
	F1	F10
AMD·HCl	33.33	-
HP-β-CD/AMD	-	33.33
KOL	40	40
CHT	3	3
Aerosil	1	1
Magnesium stearate	0.5	0.5
Avicel	up to 100%	

In vitro dissolution studies were performed according to the specifications of "Dissolution test for solid pharmaceutical forms" monograph of the European Pharmacopoeia 8th Ed. (10) according to the following experimental protocol: pH 1.2 dissolution medium (0.1N HCl solution) for the first 2 hours (simulation medium for gastric fluids) and pH 6.8 dissolution medium (phosphate buffer) for the next 10 hours (simulation medium for intestinal fluids); SR 8 Plus Series II Apparatus with

paddle (ABL&E-JASCO); 37±0.5°C bath temperature; 50 rpm rotation speed. Sampling interval was set to every hour during the 12 hours of the test (7 mL of sample were replaced at each harvest with the same volume of medium).

AMD quantitative determination was performed by high performance liquid chromatography (HPLC), using an octyldodeculsilyl solid phase and a 0.5% formic acid and methanol mixture as mobile phase in a 25:75 ratio. The temperature of the column was 45±0.2°C. The HPLC method has been validated (11).

The tests were carried out on three tablets, and the results are the mean of determinations.

Analysis of difference factor f1 and similarity factor f2: According to pharmacotechnical specifications for the preparation of modified release tablets, the release profile of the active substance in that type of tablet was analyzed by determining the difference factor f1 and similarity factor f2 between two or more formulations.

These two factors for assessing the AMD release profile from the studied formulations were calculated according to the following equations:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100$$

$$f_2 = 50 \log_{10} \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where: n = the number of samplings, R_t = the amount of dissolved drug substance of reference formula at t moment, T_t = the amount of dissolved drug substance of tested formula at t moment.

RESULTS AND DISCUSSION

The results showed that the two tablet formulations achieved a sustained AMD release. It was noted that F10 formula containing HP-β-CD/AMD inclusion complex generated a faster release of AMD compared to F1, but the release kinetic profile did not change. This finding was consistent with the results of previous studies which had shown that the solubility of AMD had increased considerably by complexation with HP-β-CD (7,12).

The studied formulations achieved a prolonged release of AMD for 6 to 8 hours. Within six hours F1 formula has released 80.95% of AMD while F10 formula 86.59% of the active substance (fig. 1).

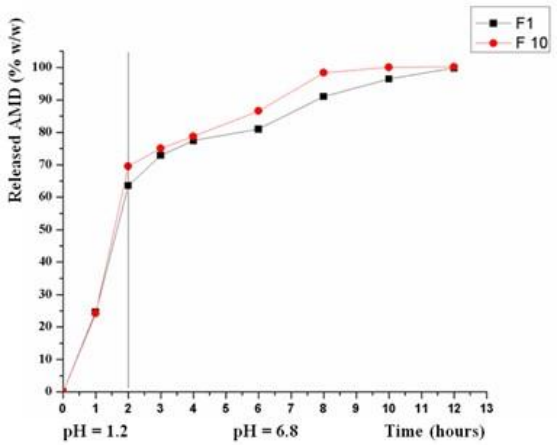

Fig. 1. The *in vitro* dissolution profile of AMD·HCl from F1 and F10

The influence of matrix forming agents on the release of AMD from the inclusion complex formulated as modified-release tablets was confirmed by the values of f1 and f2 factors shown in tab. II.

TABLE II

Values of difference and similarity factors for F1 and F10 formulations

Reference formulation F1	Determined factor	F10
	f1	43.697
	f2	68.263

The results obtained in that step revealed that the two formulations were the same in terms of the release profile of the active substance. It appeared that improving the solubility of AMD in the HP-β-CD/AMD inclusion complex was reflected to a lesser extent in AMD release. Thus, we believe that AMD release from modified-release tablets based on KOL and CHT is controlled only by those two matrix formers and that the low solubility of the substance had a low impact and it can be overcome by extended release formulation. Similarities between the two release profiles of AMD were confirmed by the values of difference factor (f1 = 43 697) and of similarity factor (f2 = 68.263).

CONCLUSIONS

During our conducted research, we assessed the *in vitro* release profile of AMD when formulated in a tablet matrix in two different states: as hydrochloride, and as HP-β-CD/AMD inclusion complex. Our results showed that AMD release in both studied formulas was similar and it was controlled only by the two matrix formers. The values of difference factor and similarity factor led us to confirm the similarities between the two release profiles of AMD. The studied tablets were obtained through direct compression and they contain low cost excipients, which will facilitate the transfer of optimal formulation to industrial production.

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NEWS

GENETIC SUSCEPTIBILITY TO INSULIN RESISTANCE ASSOCIATED WITH LOWER ADIPOSITY

The researchers tried to demonstrate the relationship between adiposity, genetic susceptibility to insulin resistance or insulin secretion and the response to GH treatment in short children born small for gestational age(SGA).The study was realised on 89 short prepubertal SGA children treated with GH for one year in a multicentre study. Combined multiallele gene scores based on single nucleotide polymorphisms with known associations with lower insulin sensitivity and insulin secretion were analysed. The results showed that during GH treatment, GS-In Res was related to a lesser decline in trunk fat and a higher trunk- limb fat ratio at 1 –year. GS-In Sec was positively associated with truncal fat.(Thankamony A[1,2], Jensen RB[1], O'Connell SM[3] et al. Adiposity in children born small for gestational age is associated with β-cell function, genetic variants for insulin resistance and response to growth hormone treatment. *J Clin Endocrinol Metab.* 2015 Nov 20:jc20153019**).**

Diana Tatarciuc

AGGRESSIVE PERIODONTITIS: THE SUBGINGIVAL MICROBIAL PROFILE OF AN AFRICAN POPULATION

The aim of this study was to determine the subgingival microbial profile of African individuals from Sudan.The study was conducted on 34 patients from Kartoum, 19 of wich presented a form of aggressive periodontitis (AgP). The other 15 patients were periodontally healthy and considered as controls. For the determination of the subgingival microbial profile the following tests were used: real time PCR and DNA-DNA hybridization checkerboard. *Porphyromonas gingivalis* was absent from the samples in the AgP group, but present in two control patients. On the other hand, *Aggreggatibacter actinomycetemcomitans* was present in 6 of the 19 patients from the AgP group. The two periodontal „classic" pathogens were not significantly present in the AgP gruop, compared with the control group. More studies are needed in order to fully understand the subgingival microbial profile of aggressive periodontitis (Elabdeen HRZ, Mustafa M, Hasturk H et al. Subgingival microbial profiles of Sudanese patients with aggressive periodontitis. *J Periodont Res* 2016; 50: 674–682).

Alexandra Cornelia Oanță

INFLUENCE OF CARBOXYMETHYLCELLULOSE SODIUM AND LUTROL ON THE SWELLING INDEX AND DISINTEGRATION TIME OF BIOMUCOADHESIVE TABLETS WITH MICONAZOLE NITRATE

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INFLUENCE OF CARBOXYMETHYLCELLULOSE SODIUM AND LUTROL ON THE SWELLING INDEX AND DISINTEGRATION TIME OF BIOMUCOADHESIVE TABLETS WITH MICONAZOLE NITRATE (Abstract): **Purpose.** To develop original pharmaceutical formulation with miconazole nitrate, biomucoadhesive tablets, used in antifungal medication. **Material and Methods:** The oral biomucoadhesive tablets with miconazole nitrate were developed by direct compression of the excipient mixture: carboxymethylcellulose sodium and lutrol 6000, excipients used for bioadhesivity, mannitol as a sugar substitute and aerosil as a lubricant. The main goal of the study is to determine the disintegration time and the swelling index of biomucoadhesive tablets with miconazole nitrate in order to estimate the time of contact with mucosa, respectively the prolongation of drug substance release. **Results:** The swelling index was calculated depending on time in all the 5 formulations that included the carboxymethylcellulose sodium and Lutrol 6000 as matrix-forming, and the studied were time and association ratio between polymers. **Conclusions:** Analysing the results, we noticed that out of the four excipients we used, carboxymethylcellulose sodium had the higher influence on the swelling index and disintegration time. **Keywords:** CARBOXYMETHYLCELLULOSE SODIUM, POLYETHYLENE GLYCOL 6000, SWELLING INDEX, MUCOBIO-ADHESIVE TABLETS.

To improve the bioavailability of the oral drugs, bioadhesive tablets have been developed in recent years (1). Oral adhesive tablets (2) can be applied in various areas of oral cavity, such as: palate, mucosa of the cheek, and between the gingiva and the upper lip. The tablet soaks, adheres to the substrate and is retained in its position until dissolution or its complete release. After a while, the tablet is no longer felt by the patient. Once positioned, the pharmaceutical formulation should not be moved in the mouth, since it causes an accelerated release of the product (3).

The excipients used in formulation of these pharmaceutical forms can influence the pharmaceutical – technological features and implicitly the swelling index (SI), an important parameter in setting the time of contact with mucosa, prolongation of the action and modified release.

SI is a generally studied parameter in

tablets with matrix-forming polymers as bioadhesion excipients. The polymers we used in preparing new bioadhesive formulations with miconazole nitrate and which offer bioadhesive capacity are PEG 6000 and carboxymethylcellulose sodium. This study analyses the values of SI, depending on quantity and type of the polymer.

The bioadhesive pharmaceutical formulations use adhesion as an including process, in which the adhesive agents penetrate the irregularities of the surface of the substrate and finally harden, producing many adhesive anchors. The process of establishing a continuous contact between an adhesive and a substrate is known as humectation. Humectation is closely related to the SI of the biomucoadhesive tablets.

The type of the polymer and its features are very important, because of the fact that bioavailability and efficiency of the pharmaceutical formulation depend on them (4, 5).

MATERIAL AND METHODS

Miconazole nitrate was used in the oral mucobioadhesive tablets as an antifungal active substance with 99% purity (14).

TABLE I

Mucobioadhesive tablet formulations with miconazole nitrate

Pharmaceutical substances mg/tablet	I	II	III	IV	V
Miconazole nitrate	25	25	25	25	25
Carboxymethylcellulose sodium (CMC-Na)	20	60	100	100	140
Mannitol	90	90	90	50	30
PEG 6000(Lutrol)	80	40	-	40	20
Aerosil	4	4	4	4	4
Total (mg)	219	219	219	219	219

Miconazole nitrate (Sigma Aldrich, Germany); *Carboxymethylcellulose sodium (*China Aroma Technology Co., Ltd. Zhejiang, China); *Mannitol (East Chemical, Weifang City, China) Polyethylene glycol - PEG 6000 microcrystalline powder (Lutrol, BASF Pharma, Germany)*; Aerosil (Degussa, Germany).

The working methods aimed at determining the swelling index (SI) of a mucoadhesive preparation, an important parameter for prolonged drug release and mucoadhesive action.

SI of the mucoadhesive tablets was determined by weighting a tablet of each formulation and recording the weights before placing them separately in a Petri dish. The individual initial weight is marked as (W_1).

15 ml of phosphate buffer (pH = 6.8) is added in each dish and the temperature is set for 37 °± 0.5°C. The tablets are taken out at regular period of times (60s, 120 minutes), are dried on a filter paper and weighted again (W_2).

The SI is calculated for each formulation using the formula: $SI = (W_2-W_1)/W_1$; where W_1 – the initial weight of the tablet; W_2 – the weight of the tablet after 60, respectively 120 minutes at 37ºC, in phosphate buffer solution.

The disintegration time was determined

according to USP 32, method A requested by FRX, on 6 tablets of each formulation (14). Through disintegration test, one can determine the time needed for transforming the tablets in fine particles when inserted in a liquid environment in specific working conditions. The data analysis was made in IBM *SPSS 20.*

RESULTS AND DISCUSSION

The specialised literature mentions several necessary features: rapid adherence to mucosa (6); strong interaction with mucin, respectively epithelial tissue (7); good capacity to stretch, moisten, swell, become soluble and biodegradable (8); keep its properties regardless of pH (9); not to influence the quantitative determination of the active substance (10).

Carboxymethylcellulose sodium (CMC-Na) is an anionic polymer, a cellulose derivative, used as a matrix system, due to its strong jellifying capacity (fig.1).

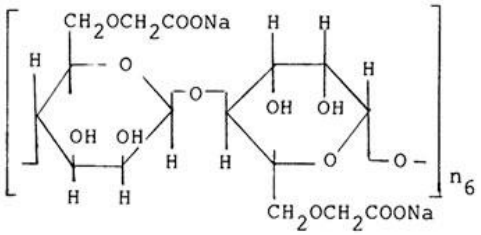

Fig. 1. Carboxymethylcellulose sodium

Carboxymethylcellulose sodium, also named croscarmellose, is a reticulated polymer, one of the hydrophilic materials, highly absorbing, with excellent swelling properties due to their fibrous nature, water soaking. Used for formulating bioadhesive tablets, croscarmellose offers good dissolution and disintegration, improving thus the bioavailability of the drug (11).

Considering its stronger hydrating properties, in comparison with hydroxypropyl methylcellulose, CMC-Na regulates the dissolution speed of the drug substances with low solubility (12).

PEG 6000 (Lutrol) is a last generation polymer, used in preparing bioadhesive pharmaceutical formulations, due to adhesive and moisture capacity (fig.2).

Fig. 2 PEG 6000

The weight of the tablets that were subject to the swelling test changed once with the swelling of the polymers in watery solution. The SI increased due to the fact that the weight of the tablets increased in direct relationship with the hydration speed up to 2 hours, and after that it decreased as a result of disintegration in dissolution environment. We noticed total disintegration when we added a smaller concentration of polymer (FI) in the formulation. This happened because of the erosion of the layer of gel in the tablets with smaller quantities of polymer (Table II).

The higher the value of SI is, the better the adherence of the tablet on the buccal mucosa will be, gradually releasing the antifungal. The highest SI is shown in formula FIII and formula FV, explained by the high quantity of added CMC-Na (fig. 3). We notice that the original formulations with miconazole nitrate have a good SI,

above 1.5, except for FI with a 0.86 SI, due to the small quantity of CMC-Na, which doesn't have the layer of gel around the tablet.

TABLE II

The swelling index of the bio adhesive tablets

Parameter	W_1 initial	W_2 at 60 minutes	W_2 at 120 minutes
Formula I	0.2185 g	0.4106 g	Tablet disintegrated
S.I.		**0.879**	-
Formula II	0.2210 g	0.5907 g	0.6369 g
S.I.		**1.67**	**1.88**
Formula III	0.2160 g	0.6680 g	0.7241 g
S.I.		**2.09**	**2.35**
Formula IV	0.2232 g	0.5231g	0.7322 g
S.I.		**1.34**	**2.28**
Formula V	0.2110 g	0.6500 g	0.7051 g
S.I.		**2.08**	**2.34**

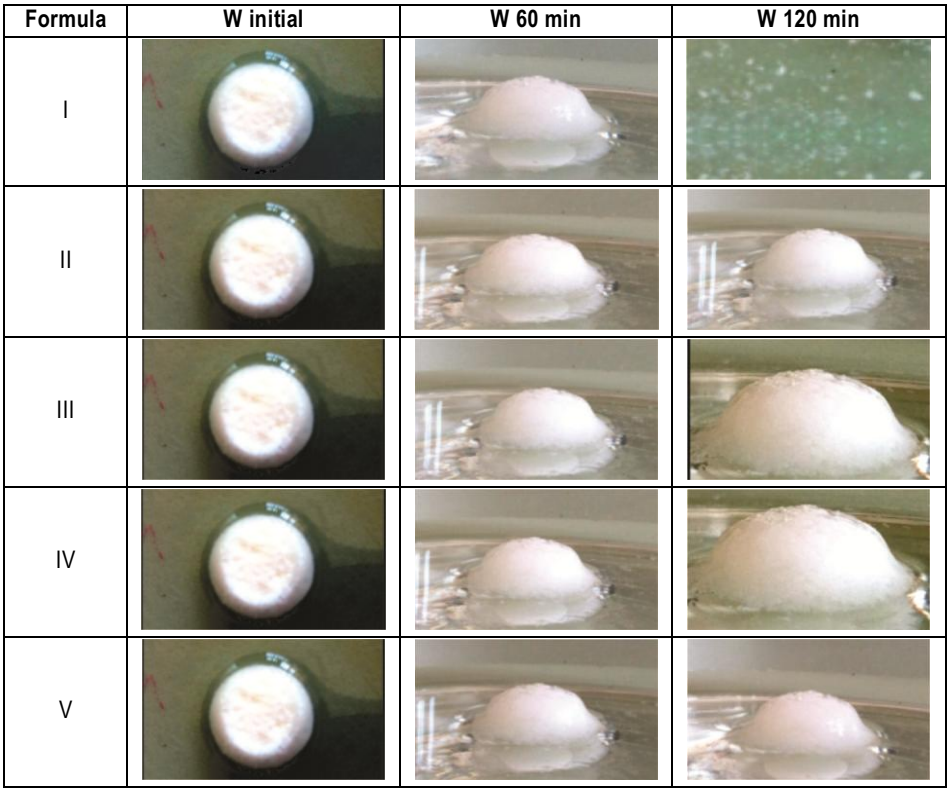

Fig. 3. The images of biomucoadhesive tablets with miconazole nitrate while determining the swelling index

Formula II has a 1.88 SI, a value explained by the quantity of used CMC-Na, 60 mg. Tripling the quantity of CMC-Na increased the value of SI, even if the value of PEG halved.

Composition of F III and F IV offers useful information about the capacity of the two polymers to influence the value of the SI.

In formula FIII, we only used CMC-Na as a matrix-forming polymer, obtaining a SI higher than 2, for the two time frames we included in the study.

Formula IV uses the same quantity of CMC-Na as formula III, but is also contains 40 mg of PEG as bioadhesive polymer. The value of SI at 60 minutes is smaller, 1.34, but it increases at 120 minutes at over 2, reaching a closer value to formula III, which proves that PEG 6000 does not influence directly the value of this parameter.

Formula V contains the biggest quantity of CMC-Na, 140 mg, together with a quantity of 20 mg Lutrol, which led to a value of 2.34 of the IU.

Figure 4 shows the necessary information for the high negative correlation with the additional marking of the point where SW = 2 and the corresponding concentration of Lutrol.

Hence, the value of SI also depends on quantity of PEG in the formulations we studied. After performing the Spearman test, we noticed in the above presented correlation scatter-plot that the ideal value of PEG is between 15 and 20 mg, a quantity we found in FV.

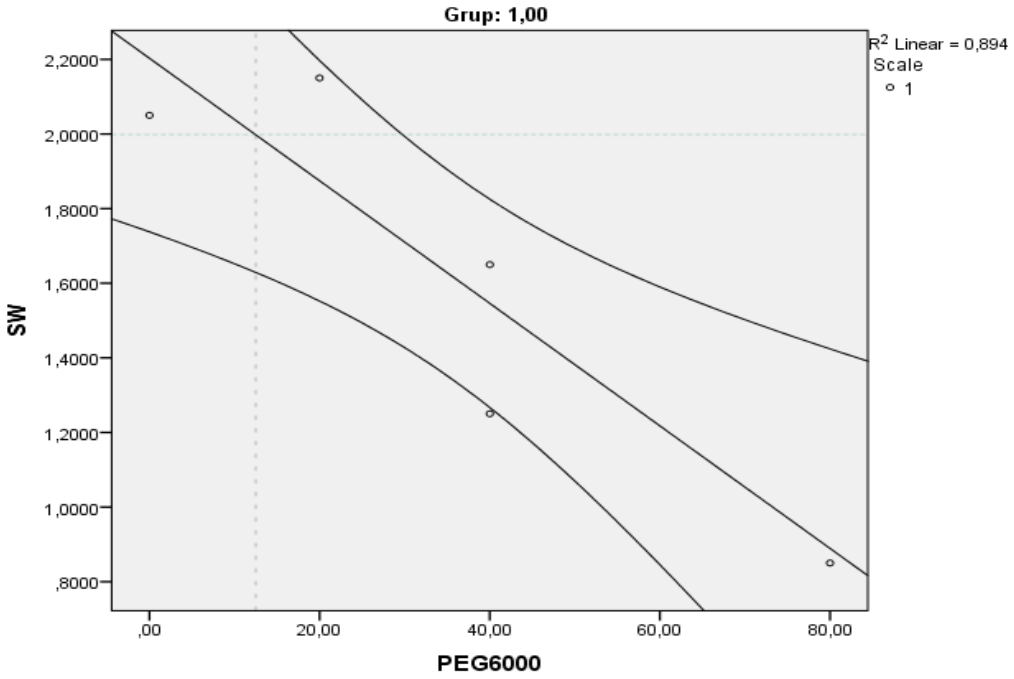

Fig. 4 Correlation Scatter plot between the swelling index and polymer PEG 6000

TABLE III

Descriptive statistics of the swelling index for formulations FI-FV of biomucoadhesive tablets with miconazole nitrate

	N	Minimum	Maximum	Mean	Std. Deviation
SW	5	.8790	2.0900	1.611800	.5153020
Valid N (listwise)	5				

The value of the SI for the biomucoadhesive tablets containing CMC-Na and Lutrol as matrix-forming excipients is between 0.88 and 2.09.

The polymers used for formulating the 5 types of biomucoadhesive tablets have a great influence on the disintegration time. The increase of the disintegration time can be highlighted by increasing the quantity of polymers. Carboxymethylcellulose sodium significantly influences the disintegration time. Formulations III, IV and V contain the biggest quantity of carboxymethylcellulose, therefore the longest disintegration time. This influence is confirmed by formula III and IV, which have the closest disintegration time. Formula IV disintegrates in 65 minutes, compared to formula III (60 minutes), due to the association of 100 mg of carboxymethylcellulose sodium with 40 mg of PEG, an excipient missing from formula IV. The lack of PEG does not significantly influence the disintegration time. Formula V shows the longest disintegration time, 90 minutes, due to the biggest quantity of carboxymethylcellulose sodium, 140 mg (fig. 5).

To conclude, PEG 6000, even if under 15% of the weight of the tablet, does not significantly influence the disintegration time, compared to CMC-Na, which, when over 5%, significantly influences the disintegration time.

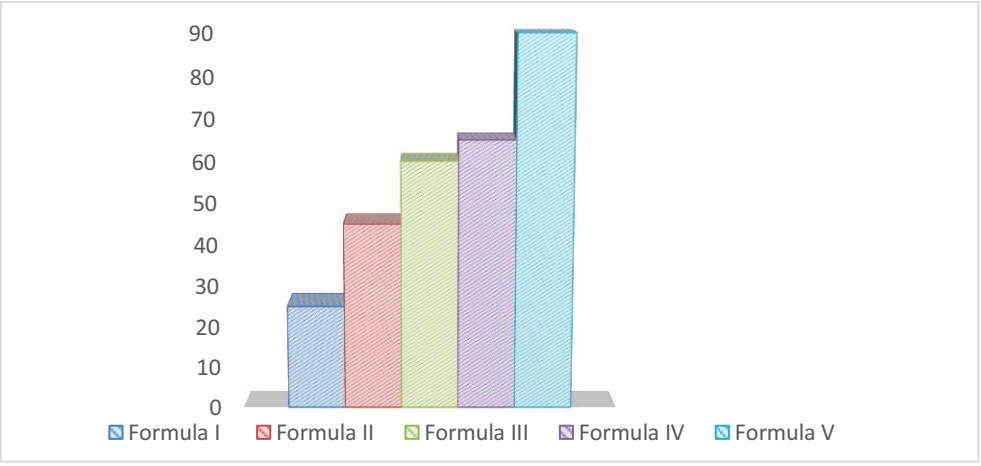

Fig. 5. Values of disintegration time, expressed in minutes

CONCLUSIONS

Our research showed that the ideal value of SI for biomucoadhesive tablets is 2. This value can be reached only if certain biomucoadhesive polymers are used, with the right concentration and the right ratio.

Our study shows the direct relationship between the quantity of carboxymethylcellulose sodium and the value of SI.

The disintegration time of biomucoadhesive tablets is influenced by the type and quantity of the polymers.

The disintegration time of over 60 minutes can be achieved with minimum 100 mg of carboxymethylcellulose sodium. The quantity of PEG in the proposed formulations does not significantly influence the value of the parameters we included in the study.

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PHARMACISTS' PERCEPTION OF DOCTORS' PRACTICES OF DRUG PRESCRIPTION

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PHARMACISTS' PERCEPTION OF DOCTORS' PRACTICES OF DRUG PRESCRIPTION (Abstract). The promotion, prescription and dispensing of drugs are influenced by a series of factors and attempts to face dilemmas often distance companies, physicians or pharmacists from patients, those who are supposed to be the main beneficiaries of healthcare services. **Purpose of the study**: identifying pharmacists' perception of doctor's practices regarding drug prescription. **Material and methods**: A number of 72 pharmacists have answered a survey about doctors' practices of drug prescription to patients. Variables like age, work environment (urban, rural), length of employment have been taken into consideration. **Results**: in pharmacists' view, doctors take into consideration the protocol about 40% while they are prescribing; in their opinion, over 95% of doctors respect the budgetary limitations from the National Health Insurance House. They consider that 65% of doctors are influenced by their relationship with the medical representative. **Conclusions**: Pharmacists' view on doctors' prescribing is influenced by a number of factors. Few unethical practices of doctors are negatively appreciated by them. **Keywords**: PRESCRIPTION, ETHICS, PHARMACIST, DOCTOR, DRUG.

The promotion, prescription and dispensing of drugs are influenced by a series of factors and attempts to face dilemmas often distance companies, physicians or pharmacists from patients, those who are supposed to be the main beneficiaries of healthcare services. Prescribing drugs by doctors is both directly and indirectly. Direct methods include the various mechanisms within law designed to limit the prescription of pharmaceutical products. Indirect methods are those of persuasion, like medical education and techniques used by pharmaceutical representatives referring to aspects of the message, the source and the receiver (1, 2). Prescribing practices are often irrational and, by consequence, dangerous. There are a lot of studies that are shown that prescribing drugs by doctors are influenced by a lot of factors like physician's age, background education, and primary care patient load (3, 4).

Others studies put in evidence factors that influence prescribing include generic prescribing and knowledge of drug con-

tents, provincial drug programs, physician and patient characteristics, type of practice, source of information about drugs, and rapidity of adoption of new drugs (5).

Doctors' exposure to promotional campaigns leads to a rise in prescription rates (6). With very rare exceptions, direct exposure to campaigns conducted by pharmaceutical companies' results in: the frequent prescription of promoted medicine, price increase and a decrease in the quality of prescriptions. Many studies identify a risk in what concerns the quality of prescriptions per se, and raise a question about the veracity of drug prescriptions (7, 8).

Patients receive information about medicine through several channels, and they are under the pressure of advertising in pharmacies; however, in their turn, they put certain pressure on doctors and pharmacists (9).

MATERIAL AND METHODS

From January to May 2015, a number of 72 pharmacists answered a survey regarding their perception of drug dispensing by doctors. Variables such as age, work environment (urban, rural), length of employment was taken into consideration; the following items were defined in order to identify pharmacists' perception of the following behaviors exhibited by doctors:

1. criteria the doctor takes into account when prescribing a treatment (his personal experience with a certain molecule; meta-analyses related to the drug under discussion, published in specialized journals; the innovative character of the drug under discussion; the standard treatment protocol for the given diagnosis);

2. means by which the doctor covers the cost of his continuing education and training (sponsorships from the pharmaceutical industry; sponsorships from his employing institution; national and international projects/grants; personal resources);

3. criteria used by the doctor when prescribing a treatment (the patient's financial capacity; budgetary limitations from the *National Health Insurance House* (the possibility of compensation); the drug's availability in local pharmacies);

4. the importance of the doctor's relationship with the medical representative for the doctor's prescription of a certain drug;

5. doctors' recommendation of OTC products and supplements in addition to the treatment of the given pathology by RX medication;

6. prescribing drugs with international nonproprietary names (INNs);

7. prescribing INNs and, at the same time, recommending the brand name product.

The objectives of the research are the following:

1. qualitative analysis of pharmacists' answers to the 8 items listed above;

2. comparative analysis of results, depending on the following demographic variables of the batch of subjects: gender, background, education, geographic region of employment;

3. setting correlations between the variables of the survey and subjects' length of employment, age and experience in the field.

The survey was applied to 72 pharmacists aged from 24 to 68 (with an average age of 38 and a 10.57991 standard deviation), with 1-48 years of experience working in the pharmaceutical field (14.4265± 11.30489). 91.67% of the surveyed pharmacists are female and 90.28% work in pharmacies located in urban areas.

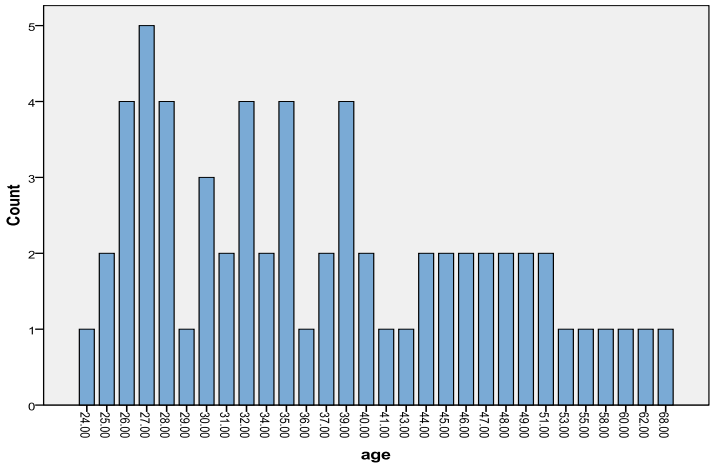

Fig 1. Distribution of pharmacists according to the age

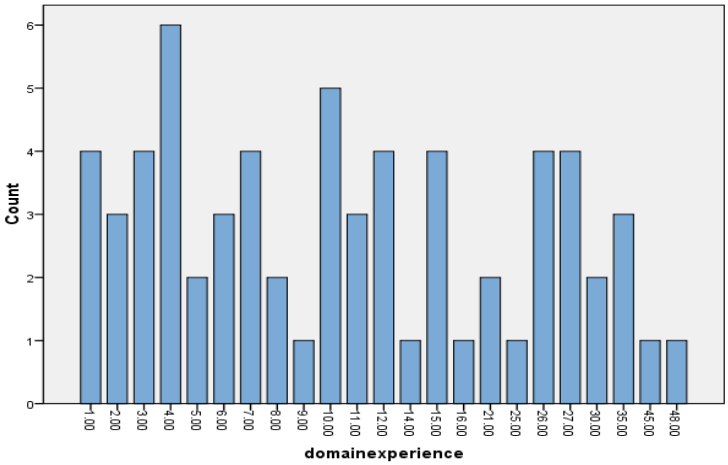

Fig. 2. Distribution of pharmacists according to the years of experience in the field

RESULTS AND DISCUSSION

In pharmacists' opinion, the criteria taken into account by the doctor when prescribing a treatment are:

a. personal experience with a certain molecule (average = 3.2154), for which never = 3.1%; rarely = 13.8%; often =41.5%; always = 41.5%;

b. meta-analyses related to the given drug, published in specialized journals (average = 2.5873), for which never = 12.7%; rarely = 33.3%; often = 36.5%; always = 17.5%;

c. the innovative character of the given drug (average = 2.5079), for which never = 7.9%; rarely = 44.4%; often = 36.5%; always = 11.1%;

d. the standard treatment protocol for the given diagnosis (average = 3.2097), for which never = 1.6%; rarely = 12.9%; often = 48.4%; always = 37.1%.

Regarding the means by which the doctor covers the cost of this continuing education and training, the pharmacists consider

that they are coming from:

a. sponsorships from the pharmaceutical industry (average = 2.7833), for which never = 6.7%; rarely = 30.0%; often = 41.7%; always = 21.7%;

b. sponsorships from the employing institution (average = 2.3333), for which never = 22.8%; rarely = 33.3%; often = 31.6%; always = 12.3%;

c. national and international projects/grants (average = 2.4754), for which never = 16.4%; rarely = 31.1%; often = 41.0%; always = 11.5%;

d. personal resources (average = 2.7833), for which never = 18.3%; rarely = 15.0%; often = 36.7%; always = 30.0%.

The criteria used by the doctor when prescribing a treatment are:

a. the patient's financial capacity (average = 3.0323), for which never = 3.2%; rarely = 24.2%; often = 38.7%; always = 33.9%;

b. budgetary limitations from the *National Health Insurance House* (the possibility of compensation) (average = 3.2807), for which never = 3.5%; rarely = 10.5%; often = 40.4%; always = 45.6%;

c. the drug's availability in local pharmacies (average = 2.7018), for which never = 7.0%; rarely = 31.6%; often = 45.6%; always = 15.8%.

Regarding the importance of the doctor's relationship with the medical representative for the doctor's prescription of a certain drug (average = 2.8475), for which never = 8.5%; rarely = 27.1%; often = 35.6%; always = 28.8%.

Regarding the recommendation of OTC products and supplements in addition to the treatment of the given pathology by RX medication (average = 2.9672), for which never = 0%; rarely = 27.9%; often = 47.5%; always = 24.6%.

Regarding the prescribing drugs with international nonproprietary names (inns) (average = 3.1746), for which never = 1.6%; rarely = 14.3%; often = 49.2%; always = 34.9%.

Regarding the prescribing international nonproprietary names (INNs) and, at the same time, recommending the brand name product (average = 2.7619), for which never =0%; rarely = 41.3%; often = 41.3%; always = 17.5%.

A comparative analysis of results was done, depending on the following demographic variables of the lot of subjects: gender, background, education, geographic region of employment. In order to highlight the difference between averages for the results obtained by our subjects depending on the considered demographic variables, we used the *t test* for independent samples.

The research obtained the following results:

a. According to the gender variable, there are significant differences between female and male subjects in what concerns the following variables:

- the patient's financial capacity as a criterion used in prescribing treatment by the doctor (3b) (t=2,051, p=.045), in that female subjects (M=4.00) consider that doctors use this criterion more frequently than male subjects (M=3.00);
- the importance of the doctor's relationship with the medical representative for the former's prescription of a certain drug (4) (p=-2,094, t=.041), in that female subjects (M=2.8750) consider the doctor's relationship with the medical representative more important for his prescription of a certain drug than male subjects (M=1.500);
- the recommendation of OTC products and supplements in addition to the treatment of the given pathology by Rx medication (5) (p=-2,094, t=.041), in that female subjects (M=2.8947) consider doctors' recommendation of OTC products and

supplements in addition to the treatment of the given pathology by RX medication less frequent than male subjects (M=4.00).

b. According to the (medical and non-medical) education variable, we obtained no statistically significant differences;

c. According to the background variable, there are statistically significant differences between subjects coming from a rural background and those from an urban background in what concerns the following variables:

- means by which the doctor covers the cost of his continuing education and training by sponsorships from the employing institution (2b) (t=-2,534, p=.014). Thus, subjects from urban backgrounds (M=2.2642) consider that doctors are less supported in their continuing education and training by sponsorships from their employing institutions than considered by subjects from rural backgrounds (M=3.6667);
- the importance of the doctor's relationship with the medical representative for the former's prescription of a certain drug (4) (t=-2,303, p=.025). Subjects from urban backgrounds (M=2.7636) consider that the doctor's relationship with the medical representative for his prescription of a certain drug is less important than considered by subjects from rural backgrounds (M=4.00).

d. According to the geographic region variable, there are statistically significant differences between subjects from regions Bacău and Neamț in what concerns the following variables:

- the criteria taken into account by the doctor when prescribing a drug, that is, meta-analyses related to that drug, published in specialized journals (1b) (t=-3,865, p=.004), in that subjects from Bacău (M=1.8333) consider that doctors use meta-analyses related to a drug published in specialized journals as a criterion for prescribing it less frequently than subjects from Neamț (M=3.4000);
- means by which the doctor covers the cost of his continuing education and training by sponsorships from the pharmaceutical industry (2a) (t=4.692, p=.001), in that subjects from Bacău (M=3.50) consider that doctors use sponsorships from the pharmaceutical industry as a means of covering the cost of their continuing education and training more frequently than those in Neamț (M=2.42);
- recommending OTC products and supplements in addition to the treatment of the given pathology by Rx medication (5) (t=3,029, p=.011), in that subjects from Bacău (M=3.8333) consider that doctors recommend OTC products and supplements in addition to the treatment of the given pathology by Rx medication more frequently than subjects from Neamț (M=2.8571);
- prescribing drugs with international nonproprietary names (INNs) (6) (t=2,171, p=.05), in that subjects from Bacău (M=3.8333) consider that doctors prescribe drugs with international nonproprietary names (INNs) more frequently than subjects from Neamț (M=3.2871).

In order to highlight the existing correlations between survey variables and subjects' length of employment, age and experience in the field, we carried out Spearman correlations.

Remotely significant statistically positive correlations are:

a. subjects' age and the importance of the doctor's relationship with the medical representative for the former's prescription of a certain drug (r= 340*, p=,015);

b. age and doctor's recommendation of OTC products and supplements in addition to the treatment of the given pathology by Rx medication (r=.,339*, p=.013);

c. work experience and sponsorships from the pharmaceutical industry as a

means by which the doctor covers the cost of his continuing education and training (r=,265*, p=.046);

d. work experience and sponsorships from the employing institution as a means by which the doctor covers the cost of his continuing education and training (r=,314*, p=.021);

e. work experience and the perception of the doctor's prescription of drugs with international nonproprietary names (INNs) (r=,295*p=.022);

f. experience in the field and the perception of the doctor's prescription of drugs with international nonproprietary names (INNs) (r=,316*, p=.018).

Strongly significant statistically positive correlations were pointed:

a. age and perception of the doctor's prescription of drugs with international nonproprietary names (INNs) (r=,416**, p=.002);

b. work experience and the importance of the doctor's relationship with the medical representative for the former's prescription of a certain drug (r=,394**, p=.003);

c. experience in the field and the importance of the doctor's relationship with the medical representative for the former's prescription of a certain drug (r=,462**, p=.001).

CONCLUSIONS

Our study proves that pharmacists' view on doctor's practices of prescribing drugs is influenced by a larger number of factors. Few practices of doctors are negatively appreciated by them. In their opinion, more than 95% are limited by the *National Health Insurance House* and over 64% are perceived like being influenced by the medical representative when they are prescribing drug to patients.

ACKNOWLEDGEMENTS

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RISK FACTORS AND PREDICTORS OF CROSSBITE AT CHILDREN

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RISK FACTORS AND PREDICTORS OF CROSSBITE AT CHILDREN (Abstract). **Aim:** was to determine the etiological risk factors and predictors of crossbite' treatment at children. **Material and methods:** 270 cases with crossbites and 255 controls with other malocclusions were uniformly evaluated. The crossbites cases were divided in group I (anterior crossbite), group II (posterior crossbite) and group III (anterior and posterior crossbites) and were compared between them per Angle Class malocclusions, dysfunctional and dental causes, associations with other discrepancies, as well by need and types of treatment. Binary logistic regression was used to find risk factors and predictors. **Results:** The mean age of cases was 10.25±2.770 years. There were found significant differences between the groups of crossbites cases ($p<0.05$). There were identified risk factors (non-attrition of deciduous teeth, Angle Class II malocclusion and permanent teeth extraction) and protective factors of the crossbite (the tooth decay of deciduous teeth, eruption anomalies and tongue-thrust swallowing). There were found predictors for early corrective treatment (sucking habit, non-attrition and buds crowding) with a precision of 78.5%; for normal treatment (mouth breathing and buds crowding) with a precision of 71.5%; for late treatment (Angle Class III malocclusion, dental crowding and TMJ disorders) with a precision of 83.0%; for maxillary expansion (eruption anomalies and buds crowding) with a precision of 77.0%; for oral associated surgery (macrodontia and TMJ disorders) with a precision of 75.9% (HL test, $p>0.05$). **Conclusion:** The risk factors were dental furthermore; the predictors of treatment were adequate with dentition, etiology and disorders at age identification, suggesting the contribution of practitioners in intercepting this orthodontic emergency. **Keywords:** CROSSBITE, RISK FACTORS, TREATMENT, PREDICTOR FACTORS, CHILDREN.

Crossbite (CB) is the only malocclusion that requires emergency treatment as soon as possible, before maxillary discrepancies occur. The maxillary teeth positions are lingual to the mandibular teeth in centric occlusion. It can vary in clinical forms from one single tooth to all, and can be anterior or/and posterior and unilateral or/and bilateral (1, 2). Abnormal labiolingual relationship between upper and lower incisors is called anterior CB and between maxillary and mandibular canines, bicuspids or molars it is defined as posterior CB. The frequency found in population varies between 2.2-23 %, depending on the age, the racial group and types of CB studied (3, 4, 5).

CB can be a feature of some genetic and endocrine syndromes, but, usually it ap-

pears isolated (non-syndromic). CB etiopathogenesis is still unknown. Several forms of CB were associated with muscle and mandibular functional changes (6, 7), with delayed dental maturation (8), with temporomandibular joint (TMJ) disorders (9, 10), with craniomandibular asymmetry (11, 12, 13), or with leg length inequality (14). Previous study has not been reached a unanimous conclusion on the cause and effect relationship of CB. Many studies have focused on the CB treatment with various orthodontic appliances, but authors was not specified a causal therapy (3, 5). Also, predict factors CB' treatments are almost nonexistent in previous study.

Our study is based on the hypothesis that CB may occur due to determinants cause factors. The detection and early removal can be prevented future severe skeletal discrepancies. Correctives treatment options depend by development stage and of child at the moment of orthodontic intervention. Objects of our study were be to compare two samples of children and adolescents with same characteristics, with/without CB, with orthodontic treatment, to assess the mean age for detecting of malocclusions, etiological causes, oral status, need and types of CB' treatment. The aim of this study was to establish the possible risk factors for etiological causes of CB types and the accuracy of predictive factors for treatment options.

MATERIAL AND METHODS

Samples

This retrospective and case-control study was conducted for 525 orthodontic patients (217 - 41.3% males and 308 - 58.7% females) aged between 6-18 years old, at the Orthodontics Clinic from "St. Spiridon" University Emergency Hospital Iasi, Romania, between January 2009 and April 2013. The ethical approval was attained from the institute's ethical committee. 270 (51.4%) patients with CB were included as case samples and were divided in three groups, to the anomaly's location by group I with anterior CB (n=175 - 33.3%), group II with posterior CB (n=46 - 8.8%) and group III with anterior with posterior CB (n=49 - 9.3%). Patients with endocrine and genetic syndromes or clefts palate were excluded. The diagnosis was established by clinical and complementary exams (plaster cast, panoramic radiographs and lateral cephalometric radiography). All cases received orthodontic treatment. The control sample consisted in 255 (48.6%) patients without CB, being diagnosed with other malocclusions throughout the visit at the orthodontic office, in order to be compared with the case samples. The informed consent was acquired from all of the patients' parents.

Studied parameters

The data were collected from orthodontic records for both samples were uniformly evaluated per socially (age and gender) and oral status parameters (dentition stages and Angle Class malocclusion). The groups of CB cases were compared between them, introducing etiological parameters (dysfunctional and dental), associations with crowding, overbite, mandibular lateral deviation, jaw discrepancies and TMJ disorders, as well by need and types of treatment.

The skeletal discrepancies were established on lateral cephalometric radiography by measuring the angles between the points A Down, Nasion, B Down (ANB) in: Class I=ANB 2-4°, Class II=ANB>4° and Class III=ANB<2° (15). The treatment need was defined by the discrepancy of the teeth between retruded contact position and intercuspal position, using guidelines of Dental Health Components of the Index of

Orthodontic Treatment Need classifying them in moderate need (≤1 mm discrepancy), urgent need (with >1 mm but ≤ 2 mm discrepancy) and very urgent need (>2 mm discrepancy) (16).

Statistical methods

Statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. The data were characterized through descriptive statistics and cross tables; we used the chi-squared test to compare the variables between cases-controls and between the three groups of CB cases, in order to find the significant differences. The risk and prediction factors that were significant on binary analysis were brought into a forward stepwise binary logistic regression model (B) and the independent factors were determined. In order to select the most suitable model, the Hosmer-Lemeshow goodness-of-fit test (HL test) was used and a p value >0.05 was considered as good fit. Any association between the factors was tested using correlation, to eliminate the multicollinearity problems. The entire statistical analyses were conducted at 5% level of significance and p value <0.05 was regarded as significant.

RESULTS

The study included 525 orthodontic patients, 270 (51.4%) cases with a mean age of 10.96±3.225 years (males 10.82±3.079 years and females 11.06±3.331 years) and 255 (48.6%) controls with a mean age of 10.25±2.770 years (males 10.08±2.818 years and females 10.36±2.739 years). Their baseline characters were similar for both samples (tab. I). There were no significant differences between cases and controls in gender, dentition stages and Angle Class malocclusions (p>0.05).

TABLE I

Baseline characteristics of cases and controls

Characteristics	**Cases** (n=270)		**Controls** (n=255)		Chi-square	p value
	n	%	n	%		
Gender					0.005	0.943
Males	112	41.5	105	41.2		
Females	158	58.5	150	58.8		
Dentition stages					3.744	0.154
Early mixed dentition	66	24.4	78	30.6		
Late mixed dentition	107	39.6	103	40.4		
Young dentition	97	36.0	74	29.0		
Angle malocclusions					0.262	0.877
Class I	190	70.4	184	72.2		
Class II	39	14.4	36	14.1		
Class III	41	15.2	35	13.7		

Group I of CB cases consisted of 175 (64.8%) patients with a mean age of 10.71±2.926 years (males 10.53±2.864 years and females 10.85±2.984 years), group II of 46 (17.0%) patients with a mean age of 10.91±3.693 years (males 11.15±3.760 years and females 10.73± 3.705 years) furthermore, group III of 49 (18.1%) patients with a mean age of 11.90±3.653 years (males 12.08±3.095 years and females 11.83±3.873 years). The groups of CB cases were compared using multiple parameters (tab. II).

TABLE II

Statistically significant differences between groups of cases

Parameters	Cases (n=270)		Group I (n=175)		Group II (n=46)		Group III (n=49)		p value
	n	%	n	%	n	%	n	%	
Angle malocclusions									
Class I	190	70.4	138	78.9	30	65.2	22	44.9	0.000
Class II	39	14.4	18	10.3	15	32.6	6	12.2	
Class III	41	15.2	19	10.9	1	2.2	21	42.9	
Dysfunctional causes									
Vicious oral habits	10	3.8	6	3.4	3	6.6	1	2	0.022
Tongue-thrust swallowing	10	3.7	1	0.6	5	10.9	4	8.2	
Mouth breathing	34	12.6	20	11.4	8	17.4	6	12.2	
Dental causes									
Changes of the tooth axle	129	47.8	94	53.7	14	30.4	21	42.9	0.014
Permanent teeth extraction	3	1.1	0	0.0	1	2.2	2	4.1	0.041
Buds crowding									
Canine	106	39.3	67	38.3	22	47.8	17	34.7	0.000
Three molars	38	14.0	16	9.1	4	8.7	18	36.8	
Overbite									
Reduced (<2 mm)	28	10.4	11	6.3	10	21.7	7	14.3	0.000
Excessive (>2 mm)	88	32.6	48	27.4	22	47.8	18	36.7	
Mandibular deviation	122	45.2	56	32.0	25	54.3	41	83.7	0.000
Skeletal discrepancy									
Class I	129	47.8	105	60.0	18	39.1	6	12.2	0.000
Class II	55	20.4	27	15.4	21	45.7	7	14.3	
Class III	86	31.9	43	24.6	7	15.2	36	73.5	
TMJ disorders	87	32.2	33	18.9	24	52.2	30	61.2	0.000
Treatment need									
Moderate	50	18.5	35	20.0	13	28.3	2	4.1	0.026
Urgent	175	64.8	114	65.1	24	52.2	37	75.5	
Very urgent	45	16.7	26	14.9	9	19.6	10	20.4	
Treatment types									
Expansion	169	62.6	95	54.3	32	69.6	42	85.7	0.000
Oral surgical	122	45.2	72	41.1	21	45.7	29	59.2	

Through the binary logistic regression model, we found statistically significant etiological risk factors, with an internal precision accuracy of 66.3% for all CB (HL test, p=0.890), of 66.5% for anterior CB (HL test, p=0.811), of 85.7% for posterior CB (HL test, p=0.994) and of 83.2% (HL test, p=0.449) for anterior with posterior CB. Value odds ratio (OR) <1.00 corresponds to protective factors (tab. III).

We found significant predictors for the precocious corrective treatment with a precision of 78.5% (HL test, p=0.708), for normal corrective treatment with a precision of 71.5% (HL test, p=0.821) and for late corrective treatment with a precision

of 83.0% (HL test, p=0.485), as well as for alternative methods of correction (overall percentage 77.0%, HL test, p=0.992 for maxillary expansion and 75.9%, HL test, p=0.102 for oral surgical) (tab. IV).

TABLE III

Binary logistic regression analysis of risk factors

Parameters	B	Wald statistic	p value	OR	95% Confidence Interval for OR	
					Lower bound	Upper bound
All CB						
Tooth decay	-2.691	12.698	0.000	0.068	0.015	0.298
Eruption anomalies	-1.183	18.674	0.000	0.306	0.179	0.524
Non-attrition	1.002	4.175	0.041	2.725	1.042	7.128
Anterior CB						
Tongue-thrust swallowing	-2.821	7.305	0.007	0.060	0.008	0.461
Tooth decay	-2.460	10.644	0.001	0.085	0.019	0.374
Eruption anomalies	-1.365	17.866	0.000	0.256	0.136	0.481
Posterior CB						
Angle Class II	1.146	8.920	0.003	3.147	1.483	6.678
Non-attrition	1.847	7.912	0.005	6.341	1.751	22.968
Anterior with posterior CB						
Non-attrition	1.866	8.150	0.004	6.461	1.795	23.259
Permanent teeth extraction	2.997	5.110	0.024	20.033	1.490	269.371

TABLE IV

Binary logistic regression analysis of predictor factors

Parameters	B	Wald statistic	p value	OR	95% Confidence Interval for OR	
					Lower bound	Upper bound
Precocious treatment						
Sucking habit	2.342	3.723	0.054	10.397	0.964	112.168
Non-attrition	1.689	10.259	0.001	5.416	1.926	15.228
Buds crowding	0.904	5.671	0.017	2.469	1.173	5.193
Normal treatment						
Mouth breathing	0.881	4.451	0.035	2.413	1.065	5.469
Buds crowding	2.069	32.417	0.000	7.918	3.884	16.143
Late treatment						
Angle Class III	1.998	16.779	0.000	7.376	2.835	19.191
Dental crowding	1.572	11.922	0.001	4.818	1.974	11.764
TMJ disorders	2.288	30.224	0.000	9.855	4.359	22.280
Maxillary expansion						
Eruption anomalies	1.337	4.867	0.027	3.809	1.161	12.497
Buds crowding	2.335	52.212	0.000	10.329	5.483	19.459
Oral surgical associate						
Macrodontia	2.870	30.782	0.000	17.639	6.399	48.618
TMJ disorders	1.374	18.962	0.000	3.951	2.129	7.332

DISCUSSION

Early detection of a malocclusion and finding the cause-treatment relationship represents a necessity for practitioners, especially in emergency situations.

Complex analysis of types of CB in children compared with other malocclusions in order to establish the etiological risk factors and predictors of orthodontic treatment was investigated in this study. There were selected samples with same characteristics to avoid statistical errors. Patients without syndromes and clefts palate were selected, in order to avoid introducing additional variables, such as such as growth deficiencies, general skeletal and jaw development. The CB cases were divided into three groups and studied by comparisons. Types of CB presented highly significant differences, which explain the separate study of the clinical forms of CB by other authors (3, 5). We used multiple variables from clinical and complementary exams, which is an enhanced method against previous studies (17, 18).

The effects we pursued were focused on the relationship between age detection of malocclusions, cause, effect and therapy. According to the average age of the studied patients, the detection of CB was done in the period of late mixed and permanent dentitions, for both sexes. Identified at the early mixed dentition, CB benefit from a technique, which is simple, non-invasive, quick (3-6 months), and with minimal cost (19, 20, 21) Detected at the adolescent patient, the nature of intervention must be different, as a complex orthodontic and surgical treatment, for a longer time (2-3 years) and with a maximal cost (22, 23, 24).

Our binary logistic regression model found only dental causes as risk factors for the CB occurrence (non-attrition of deciduous teeth, Angle Class II malocclusion and permanent teeth extraction), which may be easily intercepted by practitioners. In addition, there were identified protective factors of the CB' (the tooth decay of deciduous teeth, eruption anomalies and tongue-thrust swallowing), which were not investigated by previous studies.

Predictors for corrective treatment options of CB were different according to the nature of the intervention. The buds teeth crowding and eruption anomalies were indicated for maxillary expansion in precocious and normal treatment. Macrodontia and TMJ disorders were indicated for oral surgical associated procedures in late treatments. In addition, sucking habit and mouth breathing were predictors for treatment in mixed dentition as well as dental crowding and Angle Class III malocclusion for treatment in young permanent dentition. These predictors were not identified in previous studies, dealing with predict factors for future mandibular growth of children with anterior CB (25).

CONCLUSIONS

Our case-control study revealed the following results: the mean age of malocclusions' detection was late; the groups of CB cases were highly significant different; etiological risk factors were only dental; protective factors of CB were identified; predictors of treatment were adequate to the dentition, etiology and disorders.

The study suggests the necessity of dental practitioners' involvement in intercepting this occlusal disorder. Similar further studies can be extended on other malocclusions that affect the young population's dental and facial esthetics.

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NEWS

PROTECTIVE EFFECTS OF PIOGLITAZONE AND/OR LIRAGLUTIDE ON PANCREATIC B-CELLS IN DB/DB MICE: COMPARISON OF THEIR EFFECTS BETWEEN IN AN EARLY AND ADVANCED STAGE OF DIABETES

Diabetes is a major health problem for developed and for developing countries and is considered the 21st century epidemic. The global prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000 and is projected to rise to 4.4% in 2030. Diabetes mellitus is a chronic disease caused by inherited and/ or acquired deficiency in production of insulin by pancreas, or by ineffectiveness of the insulin produced. Failure to maintain a normal level of glucose concentration in the blood can result in micro- and macrovascular complications. In type II diabetes insulin secretion is decrease due to pancreatic damage by "β-cell glucose toxicity" with beta cells mass affection and decrease proliferation of these. It is know that there is a statistically significant relationship between appropriate long-term blood glucose control and reduction of diabetic complications, so maintenance of strict glycemic control is necessary but this requires the preservation of β-cell function. Some anti-diabetic drugs, like thiazolidines and glucagon like peptide-1 receptor agonist possess a protective effect upon β-cells. The researchers was revealed that in obese type 2 diabetes model mice exhibiting significant hyperglycemia, compensatory hypertrophy of the islets can be seen in the early stage of diabetes but the capacity of such compensation is markedly attenuated in the advanced stage. The aim of this study was to compare the protective effects of pioglitazone and/or liraglutide on pancreatic β-cells between in an early and advanced stage of diabetes. The experiment was carried out for a period of 2 weeks with 20 diabetic rats that were divided into four groups of five rats each (group 1-4). Group 1 received Pioglitazone, group 2 – Liraglutide, group 3 - Pioglitazone and Liraglutide combination, group 4 – vehicle. During the entire period of experiment was determinate and monitored the body weight, food intake, insulin plasma concentration, cell apoptosis and proliferation in pancreatic islets. The results of this study showed that the food intake was significantly decreased just in an early stage, after 1 week of treatment, by Liraglutide and Liraglutide-Pioglitazone treatment and body weight gain was suppressed by Liraglutide and was significantly accelerated by Pioglitazone. Fasting blood glucose levels were significantly lower after Liraglutide and Pioglitazone treatment in early stage and it was enhanced by combination treatment. Plasma insulin levels in an advanced stage was lower than those in early stage. Liraglutide and Liraglutide-Pioglitazone treatments significantly reduced plasma glucagon concentrations compared with other groups in an early stage, but not in an advanced stage. After the evaluation of β-cell ratio and mass for all 4 groups in early and advanced stage it was showed that in an early stage, β-cell mass was increased in Pioglitazone and Liraglutide-treated mice, and the most significant effect was observed in the associated drugs group. In an advanced stage, on the other hand, the effect of these drugs on β-cell mass have not significant changes. In conclusion, protective effects of pioglitazone and/or liraglutide on pancreatic β-cells were more powerful in an early stage of diabetes compared to those in an advanced stage. (Kimura T, Kaneto H, Shimoda M et al. Protective effects of pioglitazone and/or liraglutide on pancreatic β-cells in db/db mice: Comparison of their effects between in an early and advanced stage of diabetes. *Mol Cell Endocrinol:* 2015; 400: 78-79).

Florentina Lupaşcu

RELIABILITY AND ACCURACY OF THREE DIFFERENT COMPUTERIZED CEPHALOMETRIC ANALYSIS SOFTWARE

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RELIABILITY AND ACCURACY OF THREE DIFFERENT COMPUTERIZED CEPHALOMETRIC ANALYSIS SOFTWARE (Abstract): The aim of this investigation was to determine, compare and evaluate three different computerized tracing programs, where the lateral cephalograms were digitized on the screen. 39 randomly selected cephalometric radiographs were used in the present study. Three programs Planmeca Romexis® (Romexis 3.2.0., Helsinki, Finland), Orthalis (France) and AxCeph (A.C 2.3.0.74, Ljubljana, Slovenia) were evaluated. 12 skeletal, 9 dental and 3 soft tissue parameters were measured that consisted of 11 linear and 13 angular measurements. Statistical analysis was carried out using multivariate analysis of variance (MANOVA), Levene test, Tukey Honestly Significant Difference (HSD) test and Kruskal-Wallis test. The measurements obtained with the cephalometric analyses programs used in the study were reliable. **Keywords:** CEPHALOMETRIC ANALYSIS, COMPUTERIZED, LANDMARKS

Since Broadbent and Hoffrat has been developed cephalometrics (1) in 1931, this method it has become, especially in orthodontics field, essential in the diagnosis, treatment planning (2), result evaluation of cases treated with orthodontics, for analyzing both maxillofacial and orthodontic deformities, especially to evaluate growth and/or treatment changes (3, 4) and more recently, for evolution of orthognathic surgery.

Computers in assisting cephalometric analysis gained popularity and many authors highlighted that the linear and angular measurements were more precise in the digitally obtained radiographs, emphasizing that the quality of digital radiographs facilitates cephalometric measurements (5).

The aims and objectives of this retrospective study are to investigate the reliability between three computerized cephalometric programs to each other and to compare Steiner and Tweed analyses performed to the same patients with all three software.

MATERIAL AND METHODS

39 retrospective lateral cephalograms were taken from consecutively attending patients to radiographic clinic using a Planmeca ProMax 3D Mid (Planmeca,

Helsinki, Finland) device. 11 subjects were males and 28 were female, with age ranged between 7–33 years.

The inclusion criteria for the sample selection were as follows: the film with good quality and no unerupted or partially erupted teeth that will hinder landmark identification.

The exclusion criteria for the sample selection were as follows: history of previous orthodontic treatment, developmental abnormality such as cleft palate, cleft lip, and presence of gross facial asymmetry or deformity.

A total of 24 cephalometric measurements, 13 angular and 11 linear, commonly used to assess dentofacial relationships were selected. All measurements were performed twice by the same observer, with 14 days distance between them.

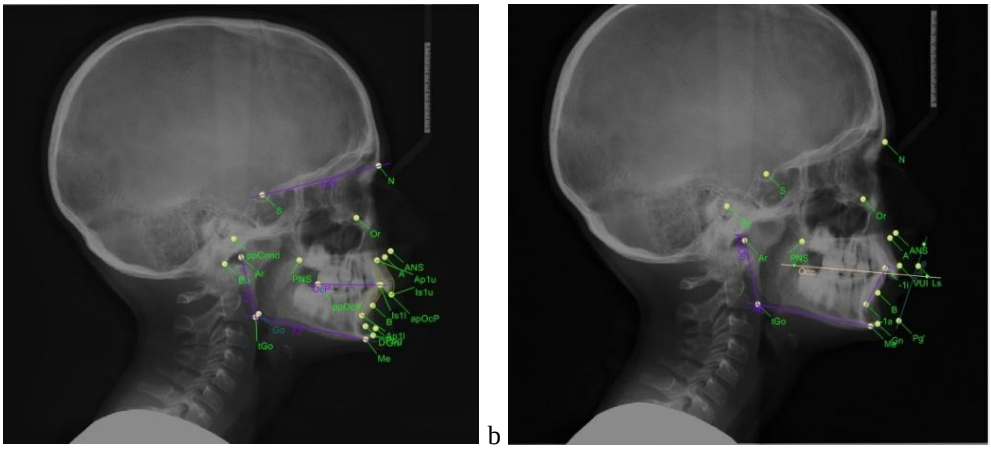

Fig. 1. a– Steiner Analysis; b – Tweed Analysis
(AX Ceph and Romexis Planmeca softwares)

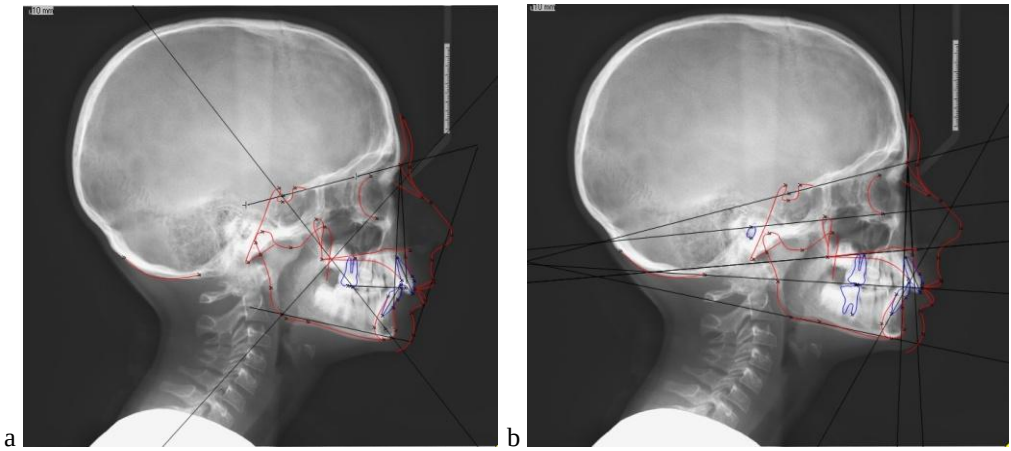

Fig. 2. a – Steiner Analysis; b – Tweed Analysis
(Orthalis software)

For all cephalometric radiographs evaluated in this study, the linear measurements were AO-BO (mm), Upper lip thickness (mm), Total chin thickness (mm), Anterior facial height (mm), Posterior facial height (mm), 1u-NA (mm), 1l-NB (mm), Pog-NB (mm), Holdaway Ratio (PO AND I TO NB) (mm), S-L (mm), S-E (mm), and the angular measurements were Frankfort-mandibular incisor angle (U1 to SN), Frankfort-mandibular plane angle, Incisor-mandibular plane angle, Occlusal plane, Z-Angle, SNA, SNB, ANB, SND angle, Inter-incisal angle, SN-OcP, SN-GoGn, Max1-NA (angle incisive superior), Mand-NB (angle incisive inferior), whence 12 skeletal parameters, 9 dentoalveolar parameters and 3 soft tissue parameters.

All the tracings were subjected to Steiner and Tweed cephalometric analysis (fig. 1 a, 2 a – Steiner Analysis and fig. 1 b, 2 b – Tweed Analysis)

RESULTS

The analysis was carried out for a confidence interval of 95%, research based on multivariate analysis of variance (MANOVA). The parameter p was our reference, which was calculated in all the tests, and the meaning of this parameter is the significance level of the test (p = 0.05). Results were presented as mean ± SD.

All the results from the study were summarized in table I and table II.

TABLE I

Mean value, standard deviation (SD), pair wise comparison based on the average estimate grouped based on the cephalometric analysis programs

	Mean±SD*			p-value (95%CI)	Multiple Comparisons - p values		
	ROMEXIS	A.C.CEPH	ORTHALIS		ROMEXIS/ ACC	ACC/ ORTHALIS	ROMEXIS/ ORTHALIS
TWEED ANALYSIS							
Frankfort-mandibular incisor angle	63.02±8.68	63.78±9.64	64.45±9.69	**0.801964**	0.933065	0.948650	0.784595
Frankfort-mandibular plane angle	21.97±6.89	20.82±5.81	21.84±6.58	**0.693646**	0.714918	0.767495	0.995834
Incisor-mandibular plane angle	95.01±10.18	95.04±10.3	91.26±18.02	**0.332414**	0.990953	0.370137	0.442612
SNA	80.59±3.35	80.01±3.12	78.97±4.17	**0.142186**	0.761917	0.419340	0.125512
SNB	77.55±4.24	77.81±4.33	77.24±4.52	**0.848600**	0.963206	0.835005	0.948017
ANB	3.04±4.05	2.20±3.88	1.74±5.05	**0.421361**	0.679902	0.888662	0.397201
AO-BO	-0.71±5.07	-0.91±4.96	-0.29±5.20	**0.862660**	0.984799	0.855938	0.929439
Occlusal plane	8.09±4.10	6.60±3.47	7.18±4.18	**0.257661**	0.231726	0.796954	0.577669
Z-Angle	79.14±7.9	76.38±7.89	76.50±9.05	**0.265177**	0.318596	0.997695	0.351996
Upper lip thickness	10.42±2.30	11.10±1.88	10.24±1.97	**0.163460**	0.328605	0.167036	0.919140
Total chin thickness	11.31±2.37	12.41±1.94	11.58±2.42	**0.090153**	0.088948	0.247834	0.860478
Posterior facial height	37.87±6.91	38.44±5.77	36.18±5.17	**0.238839**	0.911131	0.234555	0.440606
Anterior facial height	55.85±9.63	57.23±6.14	56.89±6.56	**0.711083**	0.707889	0.979417	0.821110
Facial height index	68.32±8.71	67.37±8.97	-	**0.639644**	-	-	-

	Mean±SD*			p-value (95%CI)	Multiple Comparisons - p values		
	ROMEXIS	A.C.CEPH	ORTHALIS		ROMEXIS/ ACC	ACC/ ORTHALIS	ROMEXIS/ ORTHALIS
STEINER ANALYSIS							
SNA	80.77±3.16	80.09±3.25	79.58±3.01	**0.259382**	0.613530	0.762549	0.230531
SNB	77.81±4.36	77.82±4.39	77.42±4.65	**0.906228**	0.999999	0.921643	0.922126
ANB	2.95±4.25	2.27±3.85	2.16±3.98	**0.650767**	0.741940	0.992128	0.668047
SND angle	75.08±4.53	75.01±4.58	74.63±4.89	**0.902673**	0.997617	0.933226	0.907008
Interincisal angle	128.4±12.2	128.2±12.3	129.1±12.3	**0.933897**	0.997431	0.935075	0.957776
SN-OcP	16.06±4.57	15.96±4.17	16.39±4.23	**0.901322**	0.994737	0.901161	0.939679
SN-GoGn	30.93±5.8	30.99±5.76	32.74±5.58	**0.297119**	0.998605	0.381700	0.353999
Max1-NA (ANGLE INC SUP)	24.22±8.94	24.56±8.91	25.13±9.81	**0.909254**	0.985792	0.960628	0.902478
Mand-NB(ANGLE INC INF)	24.4±7.42	24.95±8.03	24.05±7.33	**0.874138**	0.947181	0.864332	0.978120
1u-NA	3.67±3.36	4.58±2.94	5.87±2.17	**0.004518**	0.352039	0.125689	0.003235
1l-NB	3.97±2.32	3.99±2.06	4.74±1.94	**0.203957**	0.999146	0.279053	0.260483
Pog-NB	1.40±1.8	1.14±2.24	1.951.47±	**0.157296**	0.817027	0.143941	0.402605
Holdaway Ratio(PO AND I TO NB)	2.58±3.55	2.86±3.82	0.61±1	**0.0007**	1.000	0.001939	0.004435
S-L	43.88±10.1	46.26±8.47	44.87±8.47	**0.514740**	0.485346	0.780304	0.881659
S-E	17.37±4.02	18.53±3.18	18.39±3.07	**0.282216**	0.313534	0.984781	0.401513

TABLE II

Difference mean, the range of difference, pair wise comparison based on estimating the average difference grouped based on the cephalometric analysis programs and reported to Romexis Software.

	ROMEXIS / ACC			ROMEXIS / ORTHALIS		
	Difference mean (95%CI for difference*)	p-value (95%CI)	r*	Difference mean (95%CI for difference**)	p-value (95%CI)	r
TWEED ANALYSIS						
Frankfort-mandibular incisor angle	-0.761 (-1.98÷0.457)	**0.213**	0.9235	-1.426 (-2.91÷0.06)	**0.06**	0.8847
Frankfort-mandibular plane angle	1.156 (0.56÷2.257)	**0.040**	0.87	0.13 (-0.814÷1.074)	**0.782**	0.91
Incisor-mandibular plane angle	-0.395 (-1.447÷0.657)	**0.452**	0.9512	3.743 (-1.215÷8.701)	**0.135**	0.5471
SNA	0.577 (-0.042÷1.197)	**0.067**	0.8329	1.614 (0.408÷2.820)	**0.010**	0.5423
SNB	-0.261 (-1.009÷0.486)	**0.483**	0.8591	0.312 (-0.137÷0.76)	**0.167**	0.9534
ANB	0.839 (0.339÷1.333)	**0.002**	0.9276	1.302 (0.260÷2.344)	**0.016**	0.7789
AO-BO	0.195 (-0.496÷0.885)	**0.571**	0.9125	-0.425 (-1.011÷0.161)	**0.150**	0.9401

	ROMEXIS / ACC			ROMEXIS / ORTHALIS		
	Difference mean (95%CI for difference*)	**p-value (95%CI)**	**r***	**Difference mean (95%CI for difference**)**	**p-value (95%CI)**	**r**
Occlusal plane	1.482 (0.388÷2.576)	**0.009**	0.6244	0.903 (-0.722÷2.528)	**0.268**	0.2871
Z-Angle	2.763 (0.576÷4.95)	**0.015**	0.6453	2.638 (0.386÷4.890)	**0.023**	0.6809
Upper lip thickness	-0.675 (-1.199÷-0.152)	**0.013**	0.7275	0.185 (-0.387÷0.756)	**0.517**	0.6783
Total chin thickness	-1.101 (-1.716÷-0.486)	**0.001**	0.6393	-0.270 (-1.154÷0.613)	**0.539**	0.3710
Posterior facial height	-0.566 (-2.462÷1.331)	**0.549**	0.5993	1.686 (-0.298÷3.669)	**0.093**	0.5330
Anterior facial height	-1.384 (-3.779÷1.010)	**0.249**	0.6538	-1.045 (-3.379÷1.290)	**0.370**	0.6748
Facial height index	-	-	0.8498	÷	-	-
* Lower Bound- Upper Bound (95% Confidence Interval for Difference)						
STEINER ANALYSIS						
SNA	0.681 (-0.065÷1.1426)	**0.072**	0.7498	1.187 (0.601÷1.773)	**0.000**	0.8339
SNB	-0.001 (-0.789÷0.787)	**0.997**	0.8500	0.394 (-0.013÷0.800)	**0.057**	0.9644
ANB	0.682 (0.184÷1.180)	**0.009**	0.9346	0.793 (0.371÷1.215)	**0.001**	0.9533
SND angle	0.071 (-0.672÷0.814)	**0.847**	0.8768	0.451 (0.024÷0.873)	**0.039**	0.9649
Interincisal angle	0.196 (-1.112÷1.504)	**0.763**	0.9479	-0.793 (-2.678÷1.092)	**0.399**	0.8918
SN-OcP	0.098 (-0.516÷0.712)	**0.749**	0.9124	-0.334 (-1.428÷0.761)	**0.541**	0.7159
SN-GoGn	-0.068 (-0.528÷0.393)	**0.767**	0.9706	-1.811 (-2.343÷-1.273)	**0.000**	0.9601
Max1-NA (ANGLE INC SUP)	-0.342 (-1.414÷ 0.730)	**0.522**	0.9333	-0.914 (-2.166÷0.337)	**0.147**	0.9217
Mand-NB(ANGLE INC INF)	-0.547 (-1.478÷0.383)	**0.241**	0.9359	0.350 (-0.357÷1.055)	**0.322**	0.9676
1u-NA	-0.911 (-1.636÷0-0.185)	**0.015**	0.7622	-2.203 (-3.177÷-1.229)	**0.000**	0.4950
1l-NB	-0.020 (-0.363÷0.322)	**0.906**	0.8935	-0.764 (-1.056÷-0.472)	**0.000**	0.9286
Pog-NB	0.259 (-0.064÷0.582)	**0.112**	0.9036	-0.552 (-1.051÷-0.054)	**0.031**	0.5864
Holdaway Ratio(PO AND I TO NB)	-0.279 (-0.690÷0.131)	**0.176**	0.9451	1.972 (0.547÷3.393)	**0.008**	**-0.734**
S-L	-2.387 (-5.071÷0.295)	**0.080**	0.6258	-0.993 (-3.343÷1.357)	**0.397**	0.7166
S-E	-1.156 (-2.153÷-0.159)	**0.024**	0.6672	-1.024 (-2.01÷-0.033)	**0.042**	0.6716
* r – Pearson correlation coefficient ** Lower Bound- Upper Bound (95% Confidence Interval for Difference)						

Pearson correlation coefficient allows assessment of the degree of concordance between the values measured by Ax. CEPH and Orthalis relative to the values measured with Romexis, which is considerate, the gold standard for this research.

Graphic illustration of the degree of concordance between the values provided by the three programs is shown in the following graphs (fig. 3 and fig. 4). Parameters situated under the red line show moderate or weak correlation.

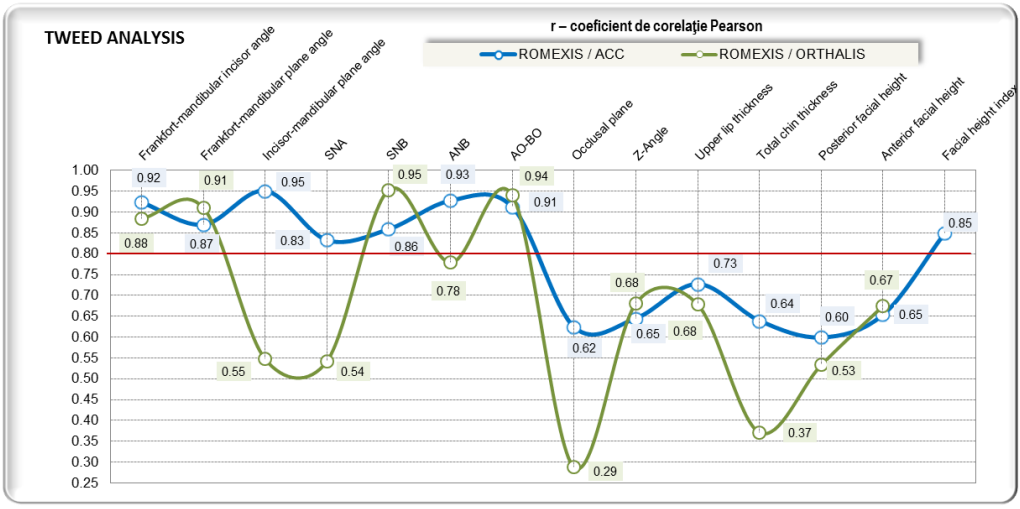

Fig. 3. Pearson correlation for values from Tweed Analysis, measured with Ax. CEPH and Orthalis, reported to Romexis software

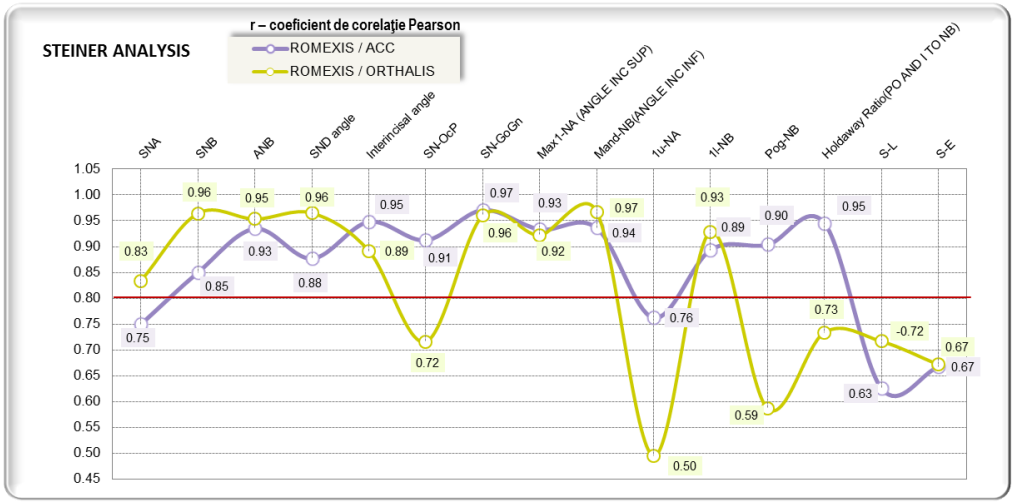

Fig. 4. Pearson correlation for values from Steiner Analysis, measured with Ax. CEPH and Orthalis, reported to Romexis software

DISCUSSION

The objective of this study was to assess the reliability of 2D cephalometric approaches by determining, comparing and evaluate three different softwares.

After a research in the literature, some of the papers with the similar purposes show a lack of criteria with regard to choice of cephalometric landmarks and the ideal linear/angular measurements to be used in studies (6, 7, 8, 9, 10).

Computer-assisted cephalometric analysis was found to be more precise and saving time; moreover, with digital cephalo-

grams obtained by digital radiography, the clinician identify the anatomic landmarks and let the program calculate automatically the cephalometric measurements (11).

Gravely and Benzies, in their research, sustain that, compare with hand tracing, computer-aided cephalometric analysis does not introduce less measurement errors in localization of the landmarks (12).

Recently, in 2004, Chen and co. revealed the fact that the difference between this two methods were statistically significant but clinically acceptable (6). A more recently study, concluded and presented a greater differences between this two methods for dental angular measurements (13).

In the present study, from the investigated measurements, 16 measurements were statistically significant: SNA, SNB, ANB, AO-BO, Z-angle, Upper lip thickness, Total chin thickness, SND, Posterior facial height, SN-OcP, SN-GoGn, Mand-NB, 1u-NA, 1l-NB, Pog-NB, PO and I – NB, S-E.

Statistical analysis founded significant difference between first method (Romexis – which is considerate gold standard in our study) and second method (Ax. CEPH) for 7 variables: Frankfort-mandibular plane angle, ANB, Occlusal plane, Z-angle, Upper lip thickness, Total chin thickness, S-E. Statistical analysis also revealed significant deifferences between second methods (Ax. CEPH) and third method (Orthalis) for 9 variables : SNA, ANB, Z- angle, SND, SN – GoGn, 1u – NA, 1l – NB, Pog – NB, S-E.

The computer method reduced, discretely, the possibility of differences, and it is clearly more secure than the manual method. However, in the present study, we found significant differences between three different computer methods.

For locating landmarks defined as being more inferior or deep in a given bone contour, points A, B and N, the computerized method proved to be more reliable than the traditional method (14).

Some studies sustain that cephalometric landmarks related to the location of N, B and A vertically, were difficult to reproduce them in the manual and computerized methods, but were similar (10, 15).

Linear measurements 1u-NA and 1l-NB, which require the location of points A and B show statistically significant differences in this study when comparing the measurements between Romexis / Ax. CEPH, with Pearson correlation r = 0.50; also we found no statistically significant differences when comparing first and second methods, r = 0.76. Other researcher found for this two linear measurements statistically significant differences when comparing manual with computer methods (10) and when comparing the manual tracing of Observer 2 with the computerized tracing of Observer 1 (16), and no significant differences when comparing the measurements of the manual and computerized tracings of Observer 1, when comparing interobserver computerized tracings, and when comparing the computerized tracing measurements of Observer 2 with the manual tracing measurements of Observer 1 (17).

Angular measurement SNGn, measurement that involves the landmark N, is difficult to locate in computer and traditional methods. With reference of this measurements, some of the previous study sustain that it is not statistically different in both methods of cephalometric tracing (17, 18).

The present study we did not measure the SNGn, but measure SNGoGn which show no statistical differences between this three methods, thus, for SN-OcP we found statistical differences between first method

(Romexis) and third method (Orthalis) with the Pearson correlation r = 0.72.

Soft tissue parameters used in the study, Upper lip thickness, Total chin thickness and Z-Angle, present significant differences. For Upper lip thickness the corelation between Romexis/Ax. CEPH was found more smaler (Mean Difference = -0.675, p = 0.013), with Pearson corelation r = 0.73, and the measurements between Ax. CEPH/Orthalis were more bigger, (Mean Difference=0.86, p<<0.01). For Total chin thickness the value from Ax. CEPH were smaller than Romexis, and the Pearson correlation between Ax. CEPH/ Romexis were r = 0.639 and between Romexis/ Orthalis were r = 0.371. For the last variable of the soft tissue, Z – angle, the measurements presented significant differences for all three methods. Between Romexis/Ax. CEPH (Mean Difference =2.76, p=0.015), and Romexis / Orthalis (Mean Difference=2.63, p=0.023), all this make visible a significant differences, resulting in that the values of Z – angle from Romexis are more bigger then other. Pearson corelation was poor for Ax. CEPH/ Romexis were r = 0.645 and Romexis/ Orthalis r = 0.68.

CONCLUSIONS

According to our results, Romexis and Ax. CEPH softwares are more reliable in measurements than Orthalis software.

The relevance of these differences will vary according to the objectives and purposes of the clinicians.

ACKNOWLEDGMENTS

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NEWS

IN VITRO COMPARISON OF THREE DIFFERENT IMAGE RECEPTORS FOR DETERMINING THE LENGTH OF ENDODONTIC FILES

The correct evaluation of working length is one of the most important conditions of successful endodontic treatment. Both digital and traditional radiographs have been used for these assessments. However, the accuracy of these methods is still controversial. The aim of this study was to evaluate the accuracy of endodontic file length measurements of E-speed radiographs, Digora storage phosphor plates, and Schick charge-coupled device sensors, with a secondary aim of assessing the influence of image enhancement on the measurement accuracy. Forty-seven extracted mandibular first premolar teeth were selected. ISO size 8, 10, and 15 files were inserted into each canal, and the files were fixed when the tip was seen at the apical foramen. The teeth were mounted in acrylic blocks and exposed using E-speed films, Digora storage phosphor plates, and Schick charge-coupled device sensors. Two radiologists and two endodontists measured the length of each file between the file stopper and tip on each image. Measurements were carried out on original and magnified images as well as on revealer images for Schick CDR. The actual lengths of the files were measured using a calipers to the nearest 0.01 mm and served as the "true length". Repeated measures analysis of difference and Tukey honestly significant difference tests were used to analyze the data ($P < 0.05$). E-speed films were superior to digital systems for the measurement accuracy of the size 08 file ($P < 0.05$). Revealer images gave equivalent results with E-speed films for the measurement of the size 10 file ($P > 0.05$). The most accurate results were obtained with the size 15 file regardless of the image receptor ($P > 0.05$). Size 15 or larger files should be used for endodontic working length determinations. Revealer images gave equivalent results with E-speed films and may be utilized for determining the file length of size 10 files (Betul Ilhan, Ilgın Akcay, Nesrın Dundar. In vitro comparison of three different image receptors for determining the length of endodontic files. *Journal of Dental Sciences* 2014; 9(4): 345-350).

Gianina Iovan

CBCT FINE PREOPERRATIVE EVALUATION OF INFLAMMATORY RADICULAR CYSTS AND POSTOPERATIVE LOCAL INTEGRATION APPRECIATION OF ALLOPLASTIC GRAFTS MATERIALS

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CBCT FINE PREOPERRATIVE EVALUATION OF INFLAMMATORY RADICULAR CYSTS AND POSTOPERATIVE LOCAL INTEGRATION APPRECIATION OF ALLOPLASTIC GRAFTS MATERIALS (Abstract): The purpose of this paper is to point out the value of CBCT exam in pre and postoperative diagnosis assessment of inflammatory radicular cysts together with full appreciation of local integration of alloplastic graft materials used to repair the osseous defects. There were statistically retrospective evaluated the pre and postoperative results of CBCT and x-ray examinations of 34 patients with inflammatory radicular cysts clinically, biologically and histopathologically assessed at Oral and Maxilo-Facial Surgery Clinic from Timisoara. In all cases we proceeded to surgical radicular cysts removement, extraction of the associated non-vital tooth together with alloplastic graft materials repairement of the osseous defects. The CBCT preoperative scans clearly showed the extent, the morphological characteristics and the topoanatomic reports, in all 34 cases of inflammatory radicular cysts together with regional endodontic status. The CBCT postoperative scans revealed the very local integration of alloplastic graft materials used to repair the osseous defects and, in some cases, the dental rehabilitation by metallic implants. CBCT scan is the imaging method of choice in pre and postoperative diagnosis assessment of inflammatory radicular cysts together with alloplastic graft materials repairement of the osseous defects and dental rehabilitation by metallic implants, due to high specific abilities in bone tissue 3D evaluation. **Keywords**: INFLAMMATORY RADICULAR CYSTS, ALLOPLASTIC GRAFT MATERIALS, CBCT SCAN

The dento-maxillary pathology is a major problem of medicine throughout the world due to the importance of this most anterior portion of the aero digestive tract with essential functions in: speech, mastication, taste, deglutition, aesthetics and general well being. The pathological conditions within the teeth and jaw bones will affect the integrity of the oral cavity with serious implications of all this functions. Any local disease could be the cause of regional and general complications which concern the whole organism with a great impact on health population (1).

The inflammatory radicular cysts are by far the most common pathological conditions within the teeth and jaw bones that will affect the integrity of the oral cavity

with serious implications of all his functions. The so common, inflammatory radicular cysts could be the cause of regional and general complications which concern the whole organism with a great impact on health population. Thus surgical radicular cysts removement and the associated non-vital tooth extraction are the treatment procedures of choice, with alloplastic graft materials repairment of the osseous defect and dental rehabilitation by metallic implants as the immediate required next steps (1, 2).

Examination and diagnosis of inflammatory radicular cysts by radiological and imaging modalities is the immediate of choice algorithm after clinical evaluation. Not only with the teeth and their surrounding bone the oral radiology concern, but also with the entire oral and maxillofacial complex (3, 4).

Among the imaging techniques of dental radiology the most common are panoramic and periapical radiographies together with the classical projections used in the diagnosis of maxilla and mandible pathology (4, 5).

Due to the ability of assessment of both the bone maxillary structures and the regional endodontic status with additional possibility of high-quality multiplanar reconstructions CBCT exam has proved to be an excellent procedure for characterizing the skeletal anatomy and related abnormalities of all oral regions being now the imaging modality of choice in pre and post-therapeutic daily practice (2, 3).

The aim of this paper is to point out the value of CBCT scan in pre and postoperative diagnosis assessment of inflammatory radicular cysts together with regional oral cavity extent, bone repair and dental rehabilitation by metallic implants evaluation.

MATERIAL AND METHODS

We retrospectively reviewed the pre- and postoperative results of CBCT and X-ray examinations of 34 patients with inflammatory radicular cysts clinically, biologically and histopathologically assessed at Oral and Maxilo - Facial Surgery Clinic from Timisoara. In all cases we proceeded to surgical removement, extraction of the associated non-vital tooth together with alloplastic graft materials repairement of the osseous defects and, in some cases, the dental rehabilitation by metallic implants.

All patients were pre and postoperative X-ray examined by dental radiographies obtained after the classical projection techniques protocol and by panoramic radiographies achieved with the usual orthopan tomographic procedure.

Preoperative and post terapeuthic surgical and grafting skeletal and dental procedures CBCT scans were done with the same unit with multiplanar and 3D reconstruction in order to precise regional oral cavity extent and dento-maxillary rehabilitation evaluation.

RESULTS AND DISCUSSION

From the 34 patients with inflammatory radicular cysts, 19 cases (56%) were detected in the mandible and 15 cases have maxillary localization (44%). In mandible 12 cases (63%) were found in aesthetic anterior regions and 7 cases (37%) have non aesthetic posterior localization. In maxillary regions 9 cases (60%) were revealed in aesthetic anterior regions and in 6 cases (40%) the sites were in non aesthetic posterior region.

In all cases radicular cysts removement and the associated non-vital tooth extraction ware the treatment procedures of choice, with alloplastic graft materials repairement

of the osseous defect and in 9 mandibular cases (47%) and only in 6 cases (40%) with maxillary localization we proceed to dental rehabilitation by metallic implants.

Fig. 1. The multiplanar and 3D CBCT- scans and X-ray exam in a pre and postoperative case of left posterior mandible inflammatory radicular cysts with surgical removal and alloplastic graft materials repairement of the osseous defect.

In all patients periapical and panoramic radiographies revealed complete bone lysis done by inflammatory radicular cysts and fine alloplastic graft materials repairement

of the postoperative osseous defect but the CBCT scans were a great deal more accurate in describing the orientation, vicinity and extent in all 3 plans of the lesions.

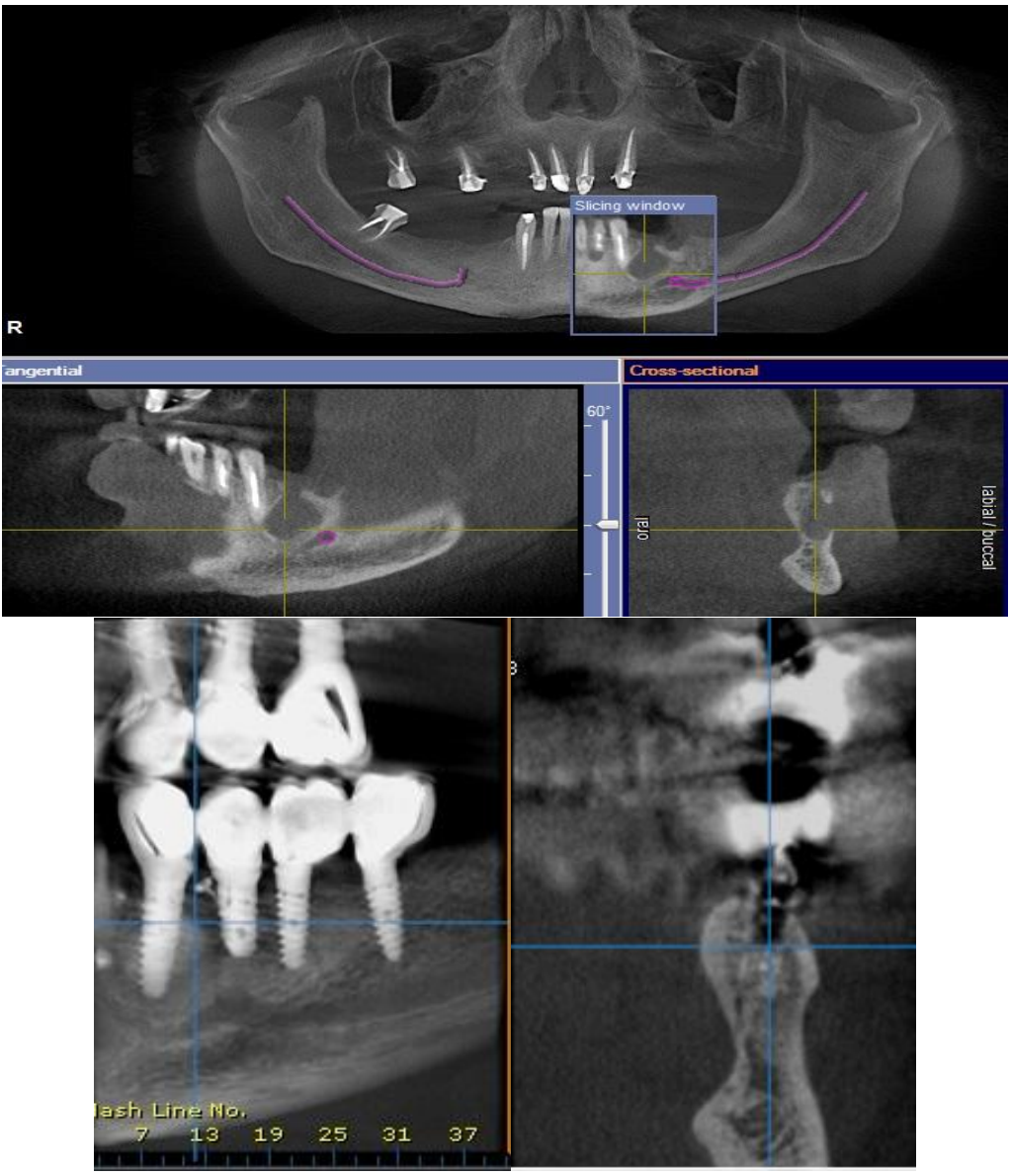

Fig.2. The multiplanar and 3D CBCT- scans in a pre and postoperative case of left anterior mandible inflammatory radicular cysts with surgical removal, alloplastic graft materials repairement of the osseous defect and dental rehabilitation by metallic implants.

In patients with dental rehabilitation by metallic implants we clearly evaluated by CBCT scans the proper alignment, parallelism, direction and mezodistal and

interproximal spacing in aesthetic zone in. The good locations and buccolingual angulations of the implants in non aesthetic zone were investigated with added extra information by postoperative CBCT scans tissue 3 D analyses.

CONCLUSIONS

The CBCT - exam offer a detailed evaluation of the dento-maxillary region together with the accompanying vital structures: the floor of the sinus, the floor of the nose, the roof of the mandibular canal and the position of the mental neurovascular bundle.

CBCT - exam is the imaging modality of choice in the diagnosis algorithms of inflammatory radicular cysts offering high abilities in the evaluation of bone tissue by multiplanar and 3D reconstruction.

CBCT - scan are of great importance value in patients with inflammatory radicular cysts being able to offer subtle and no doubt data for precise skeletal extent diagnosis and local dental status in order to proceed a clearly quantificated treatment with accurate capacities in postoperative evaluation.

The CBCT - exam offer a detailed precise evaluation of alloplastic graft materials integration used in the postoperative osseous defect repairement and proper alignment, parallelism, direction, spacing and angulations in patients with dental rehabilitation by metallic implants.

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ECHINOCANDINS - NEW ANTIFUNGAL AGENTS

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ECHINOCANDINS - NEW ANTIFUNGAL AGENTS (Abstract): Over the past 10-15 years, the number of clinically available antifungal agents has increased substantially, due to rise in the number of invasive fungal infections, which are a real problem for specialists. Echinocandins are the new class of antifungal agents available for clinical use. This class comprises over 20 natural echinocandins and several semisynthetic ones. Natural echinocandins are not of clinical utility due to their toxicity and low water-solubility (which does not allow obtaining parenteral pharmaceutical forms), although they have good antifungal activity against *Candida* species. Consequently, semisynthetic echinocandins with minimal toxicity, good antifungal activity and high water-solubility were obtained. All echinocandins inhibit β-1,3-glucan-synthase, an essential component of the fungal cell wall. Echinocandins exhibit potent antifungal activity against key pathogenic fungi, including *Candida* species, *Aspergillus* species and *Pneumocystis carinii*. The available echinocandins lack in vitro activity against *Cryptococcus neoformans*. The semisynthetic echinocandins have great advantages, among which low toxicity, fast antifungal activity, favorable pharmacokinetics that allow once-daily administration. The echinocandins recently available for clinical use are: caspofungin, micafungin and anidulafungin. **Keywords:** MICAFUNGIN, ECHINOCANDINS, CASPOFUNGIN, PNEUMOCANDIN, ANIDULAFUNGIN

Antifungal agents were discovered quite late, although some species of pathogenic fungi were discovered in 1835 (Beauvaria bassiana).

Despite intense research carried out in order to discover new antifungal agents, they appeared later than the antibacterial antibiotics on the pharmaceutical market. This happend because it was difficult to find good antifungal agents with low toxicity.

Antifungal agents can be categorized depending on how they are obtained as synthetic antifungal agents and natural antifungal agents (tab. I) (1).

Nowadays, the natural antifungal agents most often used in therapy are: polyenic macrolides (nystatin, natamycin, amphotericin B), spiranic antibiotics (griseofulvin) and cyclic lipopeptide antibiotics (candins- the most popular being echinocandins).

Other natural compounds are in different stages of clinical testing:

- papulacandins – glycolipid antibiotics produced by Papularia species;

- pradimicins and benanomycins – antracyclic antifungal agents isolated from

cultures of actinomycetes;

- nikkomycins and sardarins – antifungal agents isolated from cultures of actinomycetes.

TABLE I

Natural antifungal agents

No	Antifungal agent	Producing miroorganism	Pharmaceutical products
1.	Amphotericin B	Streptomyces nodosus	Fungizone, Fungilin
2.	Azaserin	Streptomyces spp.	-
3.	Candicidin	Streptomyces griseus	Levorin, Vanobid
4.	Dermostatin	Streptomyces viridogriseus	Viridofulvin, Dermastatin
5.	Fungicromin	Streptomyces cellulosae Streptomyces penticus Streptomyces roseoluteus Streptomyces griseus	Cantricin
6.	Filipin	Streptomyces filipenensis	Filimarisin
7.	Griseofulvin	Penicillium griseofulvum Penicillium janczewskii	Fulvicin, Grisactin, Fulcin, Grifulvin
8.	Hachimycin	Streptomyces hachijoensis	Trichomicin, Trichonat
9.	Hamycin	Streptomyces pimprina	Primamycin
10.	Lucensomycin	Streptomyces lucensis	Etruscomicyn,
11.	Natamycin	Streptomyces natalensis Streptomyces chattanoogensis	Pimaricin, Natacyn Pimafucin, Mycophyt
12.	Nystatin	Streptomyces noursei, Streptomyces aureus	Stamicin, Nistatin, Fungicidin, Mycostatin
13.	Partricin	Streptomyces aureofaciens	Ayfactin
14.	Pecilocin	Paecilomyces varioti Bainier var. antibioticus	Supral, Variotin
15.	Perimicin	Streptomyces coelicolor var. aminophilus	Aminomycin
16.	Pirrolnitrin	Pseudomonas pyrrocinia	Pyroace
17.	Siccanin	Helminthosporium siccans Drechsler	Tackle
18.	Trichomycin	Streptomyces hachijoensis	Trichomycin

Echinocandins were discovered in the 70's and 80's, being natural lipopeptidic antibiotics active against the resistant strains of Candida and Aspergillus. These are the most recently aproved antifungal agents, after years of intense research (2, 3).

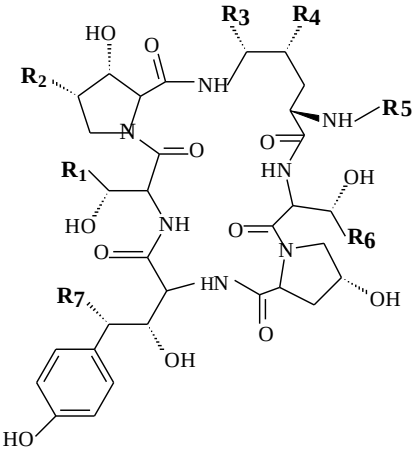
Candida species are the fourth leading cause of nosocomial infections in USA, mortality from candidiasis is reported to be 38% in imunocompromised persons, and complications could appear in over 15% of patients (4, 5). Moreover, during the past decade, the incidence of invasive aspergillosis has increased, especially in imunocompromised, cancer, and transplant patients (6).

Amphotericin B or fluconazole are the first-line therapeutic options in the treatment of such fungal infections, but the utility of amphotericin B is limited due to its nephrotoxicity. Fluconazole is relatively safe, but there are already Candida species resistant to it.

Therefore, because invasive candidiasis and aspergillosis are severe nosocomial infections, there was an urgent need for new antifungal agents. The recent discovery and development of echinocandins is an alternative to amphotericin B and fluconazole as first-line treatment for candidiasis.

Echinocandins are large amphiphilic cyclic hexapeptides molecules with the N-linked acyl fatty acid ("side chain") with a length of 14 to 18 carbon atoms (1).

Echinocandins are a class represented by over 20 isolated natural products, which are divided into several subclasses, and many semisynthetic analogues, derived from natural compounds (fig. 1).



No	Echinocandin	Fatty acid side chain (R5)
1.	Echinocandin B	Linoleic acid
2.	Echinocandin C	Linoleic acid
3.	Echinocandin D	Linoleic acid
4.	Aculeacin Aγ	Palmitic acid
5.	Mulundocandin	12-Methylmyristoic acid
6.	Sporiofungin A	10,12-Dimethylmyristoic acid
7.	Pneumocandin A0	10,12-Dimethylmyristoic acid
8.	Pneumocandin B0	10,12-Dimethylmyristoic acid

Echinocandin	R1	R2	R3	R4	R6	R7
Echinocandin B	CH3	CH3	OH	OH	CH3	OH
Echinocandin C	CH3	CH3	OH	OH	CH3	H
Echinocandin D	CH3	CH3	H	H	CH3	H
Aculeacin Aγ	CH3	CH3	OH	OH	CH3	OH
Mulundocandin	H	CH3	OH	OH	H	OH
Sporiofungin A	CH2CONH2	CH3	OH	OH	H	OH
Pneumocandin A0	CH2CONH2	CH3	OH	OH	CH3	OH
Pneumocandin B0	CH2CONH2	H	OH	OH	CH3	OH

Fig. 1. Natural echinocandins

All natural echinocandins are active by inhibiting enzyme 1,3-β-D-glucan synthetase, being selective and noncompetitive inhibitors of the essential components of the fungal cell wall biosynthesis, making it easy to lysate (1, 7). The enzyme is not present in the cell wall of higher animals, which explains the very few side effects

caused by echinocandins.

They have the advantage of low toxicity, fast antifungal activity and favorable pharmacokinetic that allows once-daily administration (having large molecules should only be given intravenously).

The spectrum of natural echinocandins is limited to Candida species (spp.), but some semisynthetic analogues have a wider spectrum that includes other endogenous fungi, such as: Aspergillus spp., Pneumocystis carinii etc. None of these compounds show activity againts Cryptococcus neoformans strains.

Echinocandin B was first isolated and identified as the major component of the class (which includes echinocandin C and D). It was isolated from the fermentation broths of various fungi of the genus Aspergillus nidulans and Aspergillus rugulosus in 1974, at the same time by the researchers from Ciba-Geigy, Sandoz, and Eli-Lilly. Researchers from Toyo Jozo have isolated echinocandins from aculeacin class, and fermentations broths of Aspergillus aculeatus, those from Hoechst have isolated mulundocandin from Aspergillus sydowi and sporiofungin class was isolated from Criptosporiopsis spp. by Sandoz researchers. The pneumocandin class, with the main component pneumocandin B0, was isolated from the fermentations broths of some fungi of the genus Zalerion arboricola and Glarea lozoyensis by Merck researchers in 1985.

All these compounds are substances with low water solubility, being discovered quite recently. Of these, only the echinocandins and pneumocandins were studied more closely for the development of semisynthetic derivatives.

Natural echinocandins and natural pneumocandins have no therapeutic utility due to their toxicity (they are hemolytic substances), although they have very good antifungal activity against Candida strains (8). They also have low water solubility, which makes them difficult for parenteral formulations. Thus, semisynthetic compounds, non-toxic, with high antifungal activity and enhanced water solubility were obtained. Today three semisynthetic echinocandins, caspofungin, micafungin and anidulafungin are licensed for clinical use (9).

Starting with the natural candins isolated from the culture medium of Glarea lozoyensis species a semisynthetic analogue of pneumocandin B0, called Caspofungin (fig. 2) was obtained and entered the U.S. market in 2001 by Merck (CANCIDAS - Merck, USA, vials 50 or 70 mg lyophilized powder). In Europe it was introduced in therapy in 2002 (CANCIDAS, Merck & Co. Inc., USA, MK-0991). The pharmaceutical product contains caspofungin acetate a water-soluble salt of caspofungin. ($C_{52}H_{88}N_{10}O_{15}\cdot 2C_2H_4O_2$).

It has a good pharmacokinetics: 100% bioavailability, high plasma protein binding (97%) and a half-life of 9-11 h. It has a slow hepatic metabolism and a slow elimination from plasma with a clearence of 10-12 mL/minute. Excretion of drug is through hepatic (34%) and renal (41%) routes. A small amount of caspofungin is excreted unchanged in urine (1.4%).

Caspofungin acetate inhibits the synthesis of 1,3-β-D-glucan synthetase, an essential component of the cell wall of many filamentous fungi.

It is active against Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei, Candida lusitaniae, Candida dubliniensis and Aspergillus species, but is also active against Pneumocystis jirovecii

(7, 10, 11, 12). It is less active against Candida parapsilosis and Candida guilliermondii, with higher minimum inhibitory concentrations for these species. Caspofungin has limited activity against other fungi (e.g. Trichosporon beigelii, Rhizopus arrhizus, Fusarium spp.) and its activity against other pathogens (e.g. protozoa) is undergoing clinical evaluation (13). The minimum inhibitory concentrations (MIC50) of caspofungin against Candida albicans ranges from 0.01 μg/mL to 8 μg/mL (14).

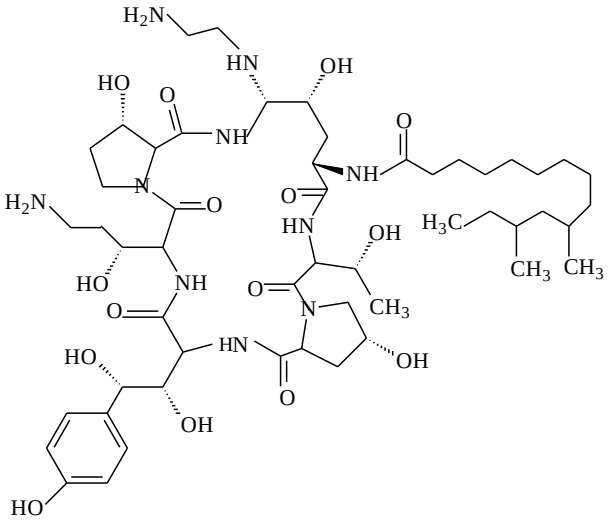


Fig. 2. Chemical structure of Caspofungin

It is administered only intravenously (i.v.), the solution is reconstituted by dissolving the powder exclusively in 0.9% NaCl solution. It is not compatible with dextrose, so diluents containing dextrose have to be avoided. Caspofungin is provided as a lyophilized powder and excipients include sucrose, mannitol, acetic acid, and sodium hydroxide. It can be stored (refrigerated) for up to 24 h after reconstitution (14).

Caspofungin (CANCIDAS) is administered intravenously as first-line treatment for Candida infections: intra-abdominal abscesses, peritonitis, pleural infections and in the treatment of esophageal candidiasis. It is indicated to treat adults, adolescents and children with invasive candidosis and invasive aspergillosis when the patient does not respond to or does not tolerate amphotericin B or itraconazole. (15, 16, 17, 18). It is used successfully in the treatment of invasive candidiasis in immunocompromised patients (19).

Another analog of pneumocandin B0 is Micafungin (fig. 3), a semisynthetic echinocandin obtained by conversion of the natural compounds isolated from fermentation broths of Coleophoma empetri F-11899.

Micafungin is manufactured in Japan and commercialized by Astellas Pharma under the trade name MYCAMINE (Micafungin sodium, 50 mg or 100 mg lyophilized powder per vial). The product has received U.S. market approval from Food and Drug Administration (FDA) on 16 March 2005. European Medicines Agency (EMA) has given approval for European market on 25 April 2008 (MYCAMINE, Astellas Pharma Inc., USA, FK-463).

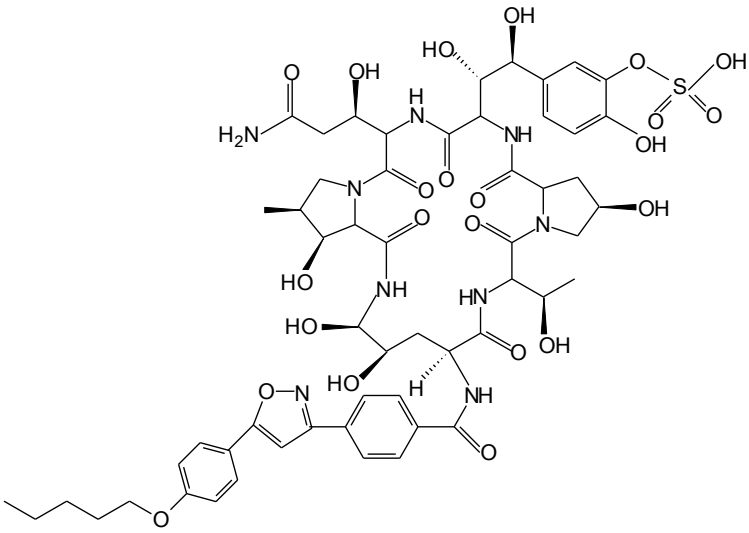


Fig. 3. Chemical structure of Micafungin

Micafungin is a semisynthetic echinocandin, presented as a white hygroscopic powder. The pharmaceutical product contains sodium micafungin a salt readily soluble in water ($C_{56}H_{70}N_9NaO_{23}S$).

It is active against strains of Candida albicans, Candida glabrata, Candida krusei and Candida tropicalis. It is less active against Candida parapsilosis and Candida guilliermondii, with higher minimum inhibitory concentrations for these species. The minimum inhibitory concentrations (MIC50) of micafungin against Candida albicans ranges from 0.01 μg/mL to 0.5 μg/mL (14). Micafungin is characterized by linear pharmacokinetics. It is metabolized into three metabolites which are excreted slowly over many days, mainly in the bile. It is highly bound to plasma protein (99%), and the half-life is 10-17 hours. Micafugin is eliminated via digestive (40%) and renal (15%) routes.

Micafungin is administered intravenously, once daily, to treat Candida infections such as intra-abdominal abscesses and peritonitis (20, 21). Micafungin is approved for use in neonates, children, adults and adolescents in the treatment of invasive candidosis. It is also used, from January 2008, in the prophylaxis of candida infection in patients undergoing allogeneic hematopoietic stem cell transplantation or patients who are expected to have granulocytopenia (22). In addition, micafungin is used in the treatment of esophageal candidosis in adults.

It has few side effects, like other semisynthetic echinocandins being very well tolerated. However, patients who develop abnormal liver function tests during treatment with micafungin should be evaluated for the risk/benefit ratio.

The vials contain sodium micafungin and excipients which include lactose, citric acid and sodium hydroxide. The lyophilized powder is ready for reconstitution by dissolving with NaCl 0.9% or dextrose 5%.

Reconstituted solution is stable at room temperatures for 48 h, if protected from light.

An analogue of echinocandin B is Anidulafungin (fig. 4), marketed by Pfizer (U.K.).

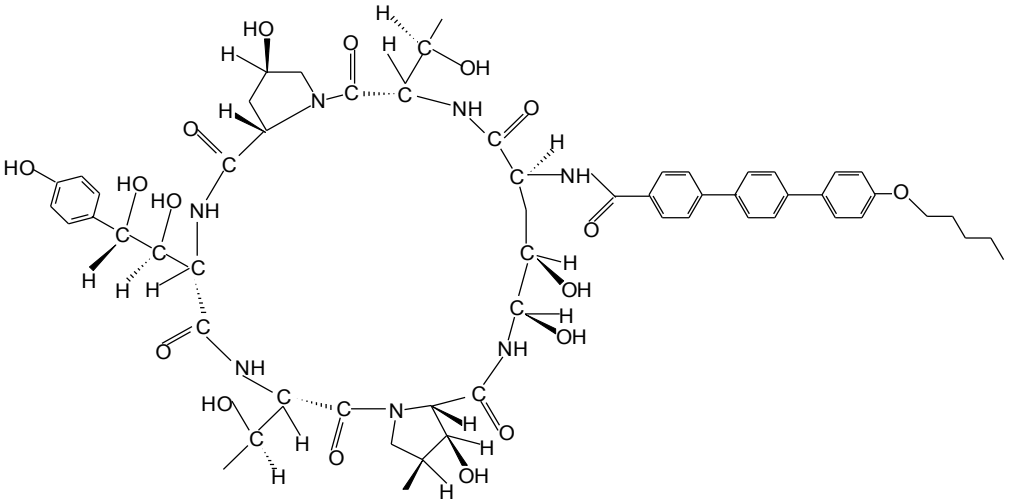


Fig. 4. Chemical structure of Anidulafungin

Pfizer received U.S. market approval from FDA for anidulafungin under the trade name ERAXIS (50 mg or 100 mg vials containing lyophilized powder) in February 2006. EMA has given approval for European market on November 2007 for anidulafungin under the trade name ECALTA (50 mg or 100 mg vial containing lyophilized powder, Pfizer Inc. UK).

It is a semisynthetic echinocandin obtained by converting natural echinocandin B isolated from Aspergillus nidulans species. It was initially researched by Eli Lilly, clinically developed by Vicuron company, and later marketed by Pfizer.

It is a white powder ($C_{58}H_{73}N_7O_{17}$), freely soluble in water, with an alkoxytriphenyl side chain which intercalates with the phosphor-lipid bilayer of the cell membrane.

It is active against strains of Candida albicans, Candida glabrata, Candida krusei and Candida tropicalis. It is less active against Candida parapsilosis and Candida guilliermondii, with higher minimum inhibitory concentrations for these species (23). The minimum inhibitory concentrations (MIC50) of anidulafungin against Candida albicans ranges from 0.01 μg/mL to 8 μg/mL (14). On other fungi anidulagfungin activity is limited (e.g. Acremonium spp., Phialophora spp., Rhizopus spp., Fusarium spp.).

Anidulafungin is slowly metabolized into an inactive open-ring peptide which is excreted in the bile. No liver metabolism has been observed. It is highly bound to plasma protein (99%), and the half-life is 0.5-1 hour. It is eliminated via digestive (30%) and renal (1%) routes.

Anidulafungin is administered intravenously to treat invasive candidosis, invasive aspergillosis in adults who are not

granulocytopenic, but not in children or adolescents below the age of 18 years (23). It is administered once-daily, the duration of treatment depending on patient response, usually at least 14 days. It has few side effects, like other semisynthetic echinocandins being very well tolerated. It can be used in patients with renal and hepatic impairment (14, 23, 24).

The vials contain anidulafungin and excipients as a lyophilized powder. The excipients include fructose, tartric acid, manitol, sodium hydroxide and hydrochloric acid. The lyophilized powder is ready for reconstitution with water for injections and subsequently diluted with NaCl 0.9% or glucose 5% for infusion. Reconstituted solution is stable at room temperatures for 24 h.

Echinocandins are the newest antifungal agents available for clinical use. The semisynthetic echinocandins have great advantages such as, low toxicity, great antifungal activity, high water solubility, and favorable pharmacokinetics, which allow once-daily intravenous administration. They exhibit potent antifungal activity against key pathogenic fungi, including Candida species, Aspergillus species and Pneumocystis species. All clinical trials demonstrate their efficacy in the treatment of invasive candidosis and invasive aspergillosis in patients not responding to other antifungal agents. They can be used successfully in the treatment of invasive candidiasis in immunocompromised patients, or in patients with renal and hepatic impairment.

The echinocandins recently available for clinical use are: caspofungin, micafungin and anidulafungin.

Their safe use, route of administration, fewest interactions with other drugs and their spectrum of action make echinocandins a class of antifungal agents with a secure future, although today they are less accessible due to the cost.

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POSSIBILITIES TO ACYLATE 7-AMINOCEPHALOSPORANIC ACID TO OBTAIN SOME CEPHALOSPORINS

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POSSIBILITIES TO ACYLATE 7-AMINOCEPHALOSPORANIC ACID TO OBTAIN SOME CEPHALOSPORINS (Abstract): **Aim**: Acylation of 7-aminocephalosporanic acid with an adequate acyl chloride in order to obtain cefotaxime sodium salt. **Material and methods**: Cefotaxime sodium salt was synthesized by acylating 7-amino cephalosporanic acid with 2-[2`-chloracetamidothiazole-4-yl]-2-(*syn*)-methoxy-imino acetic chloride in four steps. The melting point was determined, and IR spectral analysis and elemental analysis were performed to confirm cefotaxime structure. The quantitative determination was performed. **Results:** The reaction conditions were established. The yield of the synthesis phases (73-80%) and actual yield (45-47%) were very good. The structure of the obtained cefotaxime sodium salt was confirmed by the IR spectral analysis and by elemental analysis (C, H, N). The melting point was 163^0C. The purity of the synthesized cefotaxime sodium salt was 98.9%. **Conclusions:** Cefotaxime sodium salt was synthesized by acylation of 7-aminocephalosporanic acid with 2-[2`-chloracetamidothiazole-4-yl]-2-(*syn*)-methoxy-imino acetic chloride, in aqueous solution, then transformed into sodium salt with sodium 2-ethylhexanoate. The method proved to be very good, yields were good, it is reproducible and simple, and does not involve high risks, so it is also safe. **Keywords:** 7-AMINOCEPHALOSPORANIC ACID, CEFOTAXIME, CEPHALOSPORINS, ACYLATION.

Cephalosporins are often used to treat severe infections with Gram-negative and Gram-positive aerobic and anaerobic microorganisms (1). Cefotaxime is a third generation cephalosporin, being a broad spectrum beta-lactam antibiotic with high resistance to beta-lactamase inactivation. In therapeutic doses it has no toxicity, and it is used as antibacterial agent in severe infections caused by susceptible strains of Gram-positive and Gram-negative bacilli, Gram-positive and Gram-negative cocci. It is a parenteral cephalosporin used as "ex temporae" solution of cefotaxime sodium salt, 1-2 g/day every 8-12 hours. It can be used to treat severe infections in adults, children and infants (2).

Cephalosporins could be obtained by acylation of the 7-amino group of 7-amino cephalosporanic acid (7ACA) with acyl chlorides, mixed anhydrides, activated esters, activated acids, enzymatic acylation and transacylation (3, 4, 5, 6).

This paper presents the possibilities of acylating 7-aminocephalosporanic acid (7ACA) with an adequate acyl chloride in

order to obtain cefotaxime, a third generation cephalosporin.

MATERIAL AND METHODS

Apparata:

- Infrared Spectrophotometer UNICAM SP200;
- Melting point apparatus Mel-Temp Barnstead/Thermolyne 1002 (USA);
- Exeter-Analytical, CE-440 elemental analyzer with thermal conductivity detection.

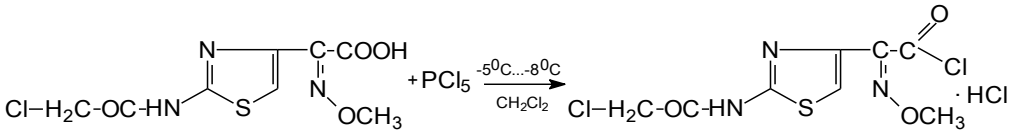
Materials and reagents:

7-aminocephalosporanic acid; 2-[2`-chloracetamidothiazole-4-yl]-2-(*syn*)-methoxy-imino acetic acid; thiourea; sodium 2-ethylhexanoate; phosphorus pentachloride; standard reagents and organic solvents (*p.a.* quality) purchased from Sigma Aldrich Company.

Chemistry

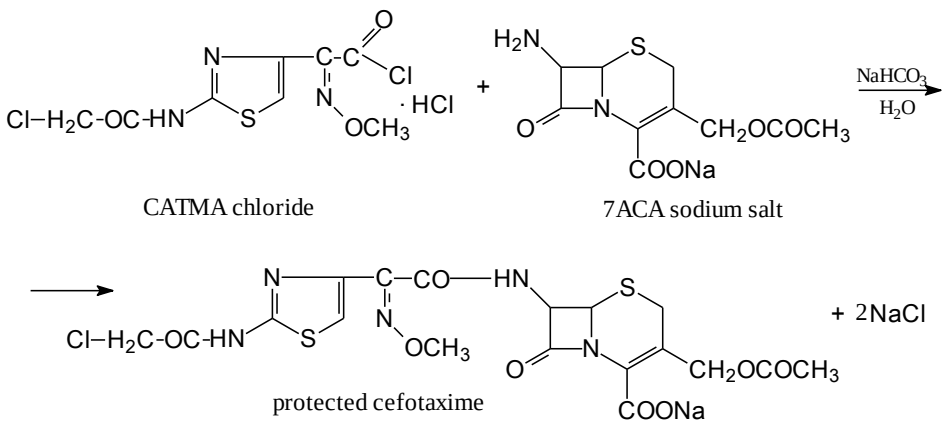
Cefotaxime sodium salt synthesis was performed in four steps:

1. 2-[2`-chloracetamidothiazol-4-yl]-2-(*syn*)-methoxy-imino acetic (CATMA) chloride obtained by treating the methylene chloride solution of the CATMA acid with phosphorus pentachloride at -5^0C (fig. 1);
2. 7ACA sodium salt acylation with CATMA choloride, obtained in the first step, at 10–15^0C, when a protected cefotaxime was formed (fig. 2);
3. deprotection of the second step obtained compound, by treating it with thiourea and then with formic acid untill pH=1.9–2.2 at -5^0C, when cefotaxime free acid was formed (fig. 3);
4. cefotaxime sodium salt obtained by treating the cefotaxime free acid with an isopropanol solution of sodium 2-ethylhexanoate (fig. 4).



CATMA acid CATMA chloride

Fig. 1. Synthesis of CATMA chloride



CATMA chloride 7ACA sodium salt

protected cefotaxime

Fig. 2. Synthesis of protected cefotaxime

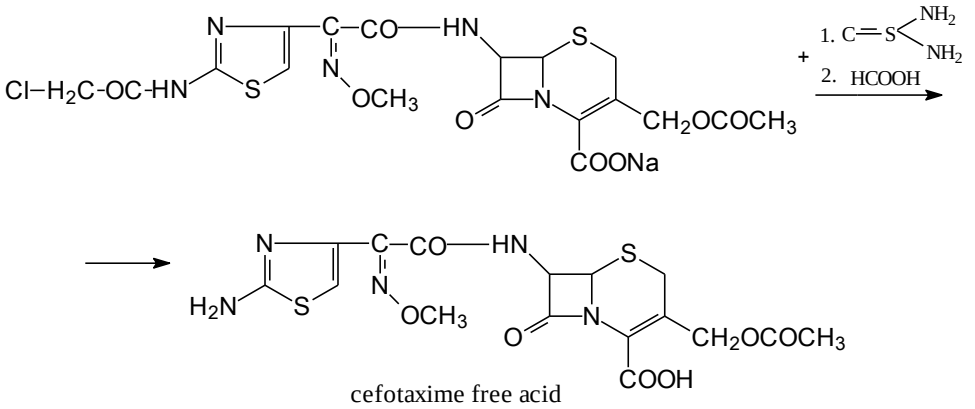


Fig. 3. Synthesis of cefotaxime free acid

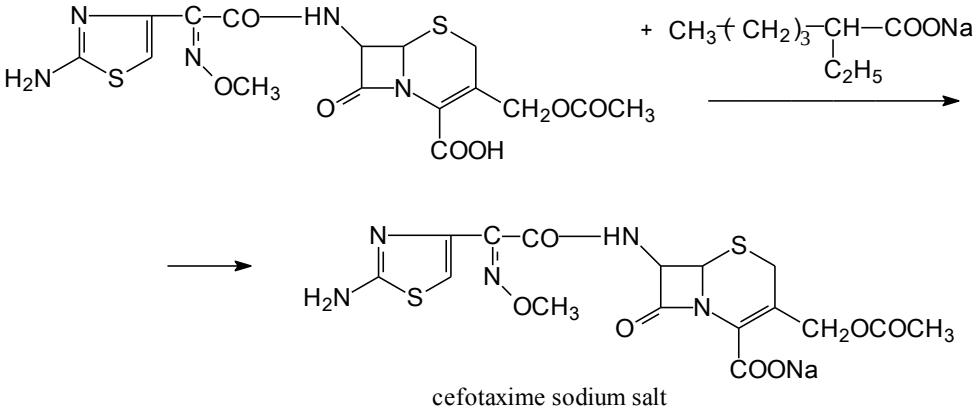


Fig. 4. Synthesis of cefotaxime sodium salt

In the first phase the CATMA chloride was obtained by treating the methylene chloride solution of the CATMA acid with phosphorus pentachloride at -5^{0}C…-8^{0}C. We suspended 5 g of 2-[2`-chloracetamido-thiazol-4-yl]-2-(*syn*)-methoxy-imino acetic acid in 125 ml of methylene chloride and cooled the suspension to -5^{0}C…-8^{0}C. Then, 5.8 g of phosphorus pentachloride were added, and after 30 minutes of stirring 63 ml of *n*-hexane were added. The suspension was filtered and the precipitate washed 2-3 times with *n*-hexane and then dried.

In the second phase, the 7ACA sodium salt was acylated with the previously obtained CATMA chloride. Four g of 7ACA acid were suspended in 72 ml of distilled water. The suspension was cooled to 10–15^{0}C and then 4.7 g of $NaHCO_3$ were slowly added. In 15–20 minutes a solution of 7ACA sodium salt was obtained. The CATMA chloride was slowly added and at

room temperature the acylation reaction was performed for 3 hours. The protected cefotaxime sodium salt was obtained.

In the third phase, the compound obtained in phase 2 was deprotected by treating it with thiourea and then with formic acid until pH=1.9–2.2 at -5^0C; cefotaxime free acid was formed. Over the solution obtained in phase 2, protected cefotaxime sodium salt, 2.8 g thiourea were added and stirred for 6 hours at room temperature. The solution was discolored with activated carbon charcoal and the suspension was filtered. The obtained filtrate was diluted with distilled water, cooled to -5^0C and acidulated with 80% formic acid solution until pH=1.9–2.2. The obtained suspension was filtered, and the precipitate was washed with cold water. The crystals were suspended in 95% ethanol, stirred for 2 hours, then filtered and dried for 3 hours at 25–40^0C.

In the fourth phase the cefotaxime sodium salt was obtained. The methanolic solution of cefotaxime (5 g of cefotaxime dissolved in 17 ml of methanol) was treated with stoechiometric quantities of isopropanolic solution of sodium 2-ethylhexanoate. The mixture was stirred for 10 minutes and then discolored with activated carbon, for other 10 minutes. The activated carbon charcoal was filtered and washed with methanol. After that, over 200 ml of isopropanol the cefotaxime sodium salt methanolic solution was added and the crystallization process of the cefotaxime sodium salt started. The suspension was stirred for 60 minutes and then filtered. The crystals were washed with 10 ml of isopropanol and dried.

The product was purified by recrystallization from 95% ethanol. The pure cefotaxime sodium salt was dried for 5 hours at 40–45^0C.

The IR spectra of the synthesized cefotaxime sodium salt and of the standard one were recorded with an Infrared Spectrophotometer UNICAM SP200. The spectra were acquired over the range of 650 and 5000 cm^{-1}. The elemental analysis was performed with an Exeter-Analytical CE-440. The quantitative determination of the synthesized cefotaxime sodium salt was carried out by titration in non-aqueous medium with perchloric acid in dioxane, according to pharmacopoeia (7, 8).

RESULTS AND DISCUSSION

Using the presented method we obtained cefotaxime sodium salt as a white, white-yellowish crystalline powder, with characteristic odor, bitter taste, and very slightly soluble in water.

The compound was characterized by melting point and elemental analysis (C, H, N) (tab. I).

TABLE I

Physicochemical characteristics of cefotaxime sodium salt

No	Substance	Molecular formula	Molecular weight	Melting point (^{0}C)	C, H, N calculated/ found (%)
1.	Cefotaxime sodium salt	$C_{16}H_{16}N_5NaO_7S_2$	Mr=477.45	163	C:40.24/40.18 H:3.37/3.34 N:14.66/14.61

The yields of the process phases were very good, over 70% for each phase, and also the overall yield of the general process was very good, over 45% (tab. II).

TABLE II
The yields of the process

Phase	**η (%)**			**η (%) average**
Phase 1	77.96	79.23	77.78	78.32
Phase 3	79.61	80.64	80.89	80.38
Phase 4	73.07	74.00	73.46	73.51
General process (overall yield)	45.35	47.27	46.21	46.27

These suggest that the method used to obtain cefotaxime sodium salt is a good one, is reproducible, and the obtained amounts are large. The method is easy to perform, is simple and does not involve high risks, so it is also safe.

The structure of the cefotaxime sodium salt obtained by the above described method was confirmed by IR spectral analyses and compared with the IR spectrum of cefotaxime sodium salt standard. The characteristic absorption bands of all functional groups were recorded (tab. III).

In the IR spectrum of the obtained cefotaxime sodium salt the characteristic absorption bands of all functional groups were observed. The presence of absorption bands at 1760 cm^{-1}, characteristic of CO lactonic group, proved the integrity of the β-lactam ring. The presence of absorption bands at 3320 cm^{-1} proved the presence of the free amine group in the 3-position of the thiazole ring.

The quantitative determination was done by titration in non-aqueous medium with perchloric acid in dioxane. In order to correspond to pharmacopoeia provisions, the obtained cefotaxime sodium salt must contain at least 98.0% and no more than 102.0% cefotaxime sodium salt related to the anhydrous substance (7, 8). The obtained cefotaxime sodium salt had a purity of 98.9%, so it meets the provisions of pharmacopoeia (tab. IV).

TABLE III
Characteristic spectral bands of cefotaxime sodium salt

Substance	**IR characteristic band (cm^{-1})**				
	-NH-	**-CO- lactonic**	**-CO- amidic I**	**-CO- amidic II**	**-C=N- thiazolic**
Synthesized cefotaxime sodium salt	3320	1760	1650	1540	1730
Cefotaxime sodium salt standard	3350	1770	1660	1540	1730

TABLE IV

Cefotaxime sodium salt concentration

No.	Substance	Concentration (%)
1.	Cefotaxime sodium salt	98.9

CONCLUSIONS

In this study we synthesized cefotaxime sodium salt by acylation of 7ACA sodium salt with CATMA chloride, in aqueous solution, when cefotaxime free acid was formed, followed by the transformation in sodium salt with sodium 2-ethylhexanoate.

The method proved to be a very good one, having good yields, is reproducible, and the obtained amounts are large. It is not difficult to perform, is simple, and does not involve high risks, so it is safe.

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IMPORTANCE OF SULFONAMIDE MOIETY IN CURRENT AND FUTURE THERAPY

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IMPORTANCE OF SULFONAMIDE MOIETY IN CURRENT AND FUTURE THERAPY (Abstract): Sulfonamides and their different derivatives are extensively used in therapy due to their pharmacological properties. Sulfa drugs were amongst the oldest synthesized antimicrobial agents and are still widely used today to treat different microbial infections. Clinical treatment with sulfonamides has regained confidence with the use of a combination of sulfamethoxazole and trimethoprim to treat urinary tract bacterial infections. Today, they are widely used as antimicrobial agents, chiefly because of their low cost, low toxicity and excellent activity against bacterial diseases. Over the course of time, the application of sulfonamides has been extended from their use as antimicrobial agents to anticancer agents, antiglaucoma agents, inhibitors of γ-secretase, cyclooxgenase-2 and lipoxygenase, anticonvulsivant agents, hypoglycemic agents. **Keywords:** SULFONAMIDES, ANTIMICROBIAL, LOW TOXICITY.

The introduction of sulfonamides in 1935 marked a turning point, a true „therapeutic revolution", and opened the era of ''miracle drugs''. After the discovery of penicillin followed by other antibiotics sulfonamides became less used, but later they started to attract attention for their synergic activity in the combination with trimethoprim.

Sulfonamides proved to be the starting point for a series of structural changes and this has led to the discovery of new drugs or classes of drugs used today not just in combating bacterial infections, but also in other diseases. They have widespread use in a variety of applications including antibacterials, antitumor agents, diuretics, carbonic anhydrase inhibitors, hypoglycaemic agents, thyroid inhibitors, and protease inhibitors (1). In recent years, molecules containing sulfamide group have also been investigated as inhibitors of ATP-sensitive potassium channels, carboxy-peptidase A, γ-secretase, glycosidase, HCV polymerase (NS5B), HIV-1 integrase, HIV-1 protease, histone deacetylase, human chymase, human leukocyte elastase, kinesin spindle protein, monoamine reuptake, plasma cell membrane protein-1, and thrombin; as agonists of androgen receptor, β_3-andrenergic receptor, and as antagonists of CXCR2 (2).

The chemical class of sulphonamides shares a common sulfanilamide moiety with

an aromatic amino group at the C_4-position, differing in the substitution of sulfamoil group at the C_1-position. A tetrahedral sulfur atom and multiple vectors for substitution of the nitrogen atoms lead to conformational and structural diversity (fig. 1).

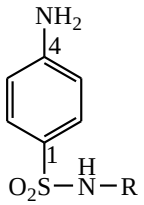

Fig. 1. The backbone of the sulphonamides

The characterization of sulfonamides as chemotherapics is more than half a century old. Since then, the sulfonamide group -*SO_2NH*- has been found as a key structural motif shared by a large number of bioactive compounds, spanning a wide variety of biological effects, such as antimicrobial activity, specific enzyme inhibition, hormone regulation, among others. They are usually classified as short-acting sulfonamides, with half lives (in serum) of 5–10 hours (i.e. sulfisoxazole, sulfadiazine and sulfamethizole); intermediate-acting sulfonamides, with half lives of 10–12 h (i.e. sulfamethoxazole, sulfadimethoxine and sulfamethazine); and finally long-acting sulfonamides, with half lives from 40 h onwards (i.e. sulfadoxine and sulfamethoxypyridazine) (3). Sulfadiazine and more recent compounds, including sulfafurazole (sulfisoxazole), sulfa-methoxazole, sulfametrole, sulfacitine and sulfamethizole, are quickly absorbed and eliminated. Compared with the older generation they are more soluble, less toxic, and probably less allergenic. Long-acting sulfonamides include sulfametoxydiazine, sulfadimethoxine, and other compounds, of which many are no longer available, due to the fact that they were associated with severe hypersensitivity reactions.

SULFONAMIDES AS ANTIVIRAL AGENTS

The effectiveness of sulfonamides has also been proven over time in the context of viral infections. A large number of structurally novel sulfonamide derivatives have ultimately been reported to show substantial antiviral activity *in vitro* and *in vivo*. Hepatitis C virus (HCV) is a common pathogen that can lead to cirrhosis, hepatocellular carcinoma (HCC), and liver failure. It is estimated that 170 million people were infected worldwide by the year 2000, and that the virus is responsible for at least 10.000 deaths annually in the United States alone. Moreover, existing therapies are hampered by drug-related toxicities. Therefore, there is a particular need for new therapies directed to genotype 1 HCV infection. An attractive strategy for the treatment of liver infection with hepatitis C virus was the synthesis of some compounds with sulfonamidic structure (4). On the other hand, Delavirdine (Figure 2), a second-generation bis(heteroaryl) piperazine, is licensed for the treatment of HIV infection and it is used in combination with other anti-HIV agents (5).

SULFONAMIDES AS ANTIBACTERIAL AGENTS

They are used in urinary tract infections, meningitis, streptococcal pharyngitis, bacillary dysentery, trachoma, chancroid, malaria, toxoplasmosis, nocardiasis, and conjunctivitis. The most popular sulfonamides are p-aminobenzensulfonamides, or sulfanilamides, which are bacteriostatic due to their resemblance to p-aminobenzoic acid (PABA), used by bacteria in the biosynthesis of folic acid required for their growth (6). They interfere with the use of *p*-aminobenzoic acid (PABA) in the bio-

synthesis of tetrahydrofolic acid, which is a growth factor that is vital for bacterial metabolism. Sulfonamides have a wide range of antimicrobial activity against both *Gram-positive* and *Gram-negative* bacterial strains. These drugs act on the bacteria themselves and either prevent their growth (bacteriostatic) or act as germicides (bactericides).

The topical use of sulfonamides has been limited, because of the high risk of hypersensitivity.

Nevertheless, sulfacetamide and sulfadicramide are still used topically for eye infections.

Because systemic antibiotics are ineffective in reducing bacterial counts in granulation wounds, the use of a suitable topical antibacterial agent may substantially decrease wound sepsis and benefit overall management. The actions of topical 1% silver sulfadiazine and of topical 5% mafenide acetate in *Acinetobacter strains* wound infection have been investigated (7). Metal-based drugs for the treatment of many ailments have gained much attention over the last decade. The ability of metal ions to bind *in vivo* with proteins and peptides is an important feature of metal-based drugs. Simple and *N*-substituted sulphonamides have attracted much attention in this context. Modified toxicological and pharmacological properties have been observed when some of these sulphonamides are administered in the form of their metal complexes. For example, Ag(I)-sulfadiazine, Ce(III)-sulfadiazine, Ni(II)-sulfadimethoxine and Cu(II)-sulfacetamide have shown higher antimicrobial activity than free ligands. Several authors have conductingstudies on the coordination forms of sulphonamides with Cu (II), reported the versatility of these ligands and the importance of their complexes in coordination chemistry (8). Some sulphonamide complexes have proved to be relevant catalysts in chemical reactions, such as Zn-sulfonamide complexes that catalyze enantio selective cyclopropanations, and also reagents for the cleavage of nucleic acids. On the other hand, numerous sulphonamide complexes have been studied as simple models of metal-protein interactions. In addition, some metal sulfonamides have drawn much attention due to the fact that they are mainly used in medicine as antibacterial drugs (9).

Sulfonamides and their different derivatives are among the most widely used antimicrobial agents, chiefly because of their low cost, low toxicity and excellent activity against bacterial diseases.

SULFONAMIDES AS ANTICANCER AGENTS

Among the wide range of compounds tested as antitumor agents, sulphonamides have attracted great attention, as many sulphonamide derivatives were reported to have interesting antitumor activity.

Recently, it has been showed that β-carbonic anhydrase from *Mycobacterium tuberculosis*is inhibited by several sulfonamides at low concentration. Sulfa methoxazole was shown to inhibit dihydropteroate synthase of *Mycobacterium avium* and affected the growth of three strains of *Mycobacterium avium* complex with MIC50/80 about 30 mg/mL. Also other sulfonamides were active against *Mycobacterium avium* complex (10).

There are a variety of mechanisms for their anticancer action, such as disruption of microtubule assembly, cell cycle arrest in the G1 phase, functional suppression of the transcriptional activator NF-Y, angiogenesis and carbonic anhydrase inhibition (11). The most prominent of these mechanisms was the inhibition of carbonic anhydrase isozymes (12). It has been shown that

two carbonic anhydrase isozymes (CA IX and CA XII) are prominently associated in many tumors. They are involved in crucial processes associated with cancer progression and response to therapy. Aromatic or heteroaromatic sulfonamides have been shown to reverse the effect of tumor acidification; consequently they inhibit the growth of cancer cells and supress carbonic anhydrase-mediated tumor invasion (13).

Methanesulfonamides ($CH_3SO_2NH_2$) are also used in drug industry because of their biological activity on a large scale. Methanesulfonamide derivatives have DNA binding ability, show cytostatic effects, and some of them, such as Amsacrine (Fig. 2), are used in cancer chemotherapy. In addition, some sulfonyl hydrazines are known to have antineoplastic effects which prevent malignant cells growth and spread (14).

SULFONAMIDES AS ANTIGLAUCOMA AGENTS

Several sulfonamides, such as acetazolamide, methazolamide, ethoxzolamide or dichlorophenamide, are clinically used as systemic antiglaucoma agents for more than 50 years. In the mid 90s, the first clinically used, topically effective antiglaucoma sulfonamide, dorzolamide, was discovered (15). More recently, another topically acting antiglaucoma sulfonamide is also available: brinzolamide (Figure 2). However the main drawback of these compounds, and of many sulfonamides reported so far in the literature, is their lack of selectivity, as they usually inhibit most of the catalytically active isoforms of carbonic anhydrase in the low nano/ micromolar range (16).

SULFONAMIDES IN ALZHEIMER'S DISEASE

Furthermore, recent studies suggest that carbonic anhydrase activation may provide a novel therapy for Alzheimer's disease (17), the most prevalent form of dementia that affects approximately 24 million people worldwide. A number of structurally diverse γ-secretase inhibitors have been reported and several advanced into clinical trials. Recently, the structure–activity relationships of a series of N-bridged bicyclic sulfonamides as inhibitors of γ-secretase have been reported (18). Begacestat (Fig. 2) is a novel thiophene sulfonamide gamma-secretase inhibitor that selectively inhibits cleavage of amyloid precursor protein (APP) (19).

SULFONAMIDES AS ANTICONVULSANT AGENTS

A new class of anticonvulsant structures that belong to the family of sulfamides has been designed. In recent years, several compounds, such as topiramate and zonisamide (Fig. 2) have been approved as anticonvulsant drugs. Their anticonvulsant action is probably due to CO_2 retention secondary to inhibition of red cell and brain carbonic anhydrase enzyme, but other mechanisms of action, such as sodium channels blockade were proved for some of them (20).

SULFONAMIDES AS COX-2 AND LIPOXYGENASE INHIBITORS

Sulfasalazine (salicylazosulfapyridine), a compound in which sulfapyridine is linked to 5-aminosalicylate by a diazo bond has been and still is widely used to treat ulcerative colitis and regional ileitis (Crohn's disease). Sulfasalazine is not used for the antibacterial properties of the sulfapyridine, but for the local anti-inflammatory effect of 5-aminosalicylate. Selective cyclooxygenase-2 inhibitors (COX-2) (Celecoxib, Parecoxib, Valdecoxib) are compounds with sulfonamide struc-

ture that are indicated for the relief of signs and symptoms of osteoarthritis and rheumatoid arthritis but also for the treatment of primary dysmenorrhea.

Recently, sulfonamide group has been found to be the key constituent of a new class of compounds as potential inhibitors of lipoxygenase (21), enzyme that catalyzes the oxidation of polyunsaturated fatty acids.

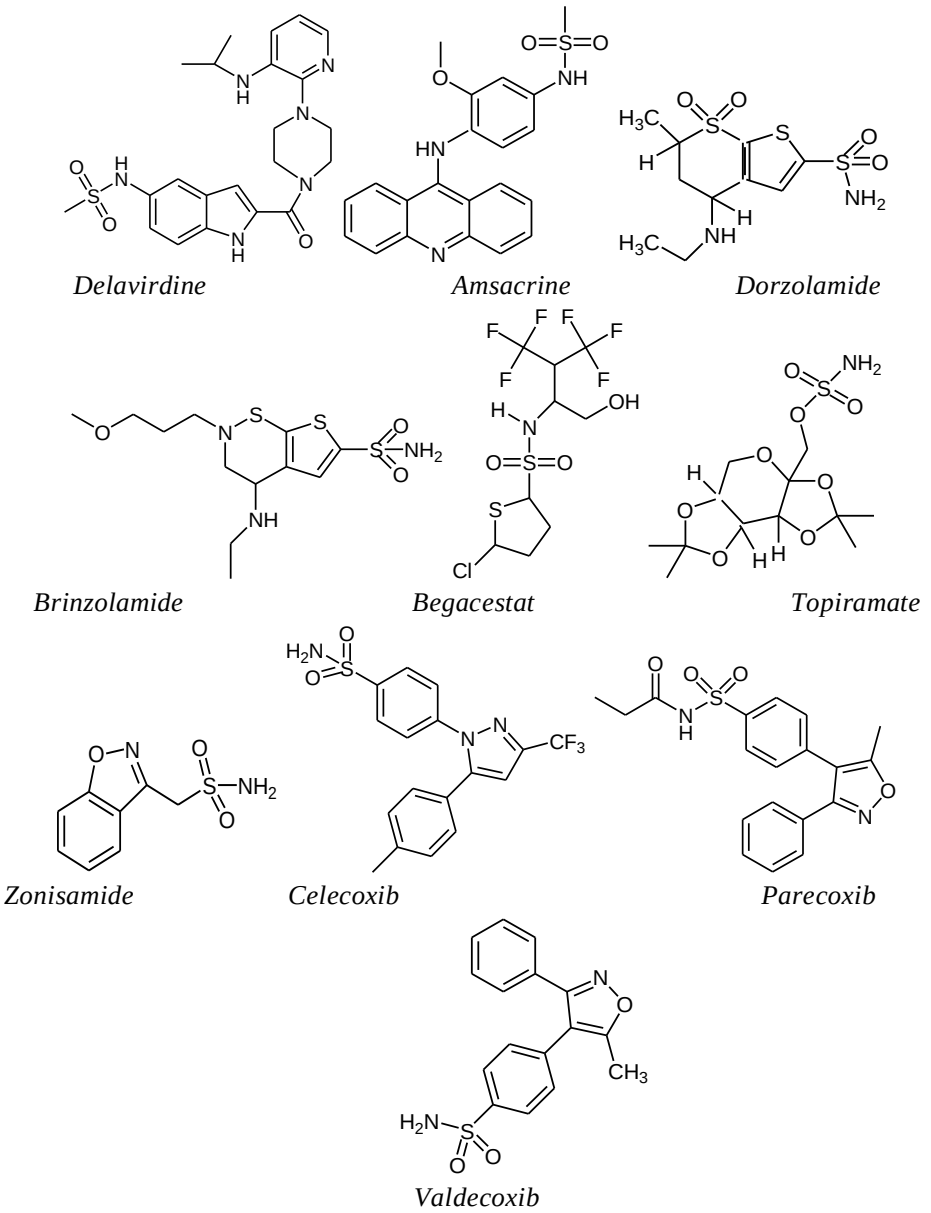

Fig. 2. Drugs with sulfonamide structure

SULFONAMIDES AS HYPOGLYCEMIC AGENTS

Sulfonylureas are known to act by closing the adenosine triphosphate-sensitive potassium (KATP) channels in the pancreas through binding to the sulphonylurea receptor (SUR) 1 (an integral component of pancreatic KATP channels), leading to beta cell insulin release. The beta cells of patients taking these drugs are chronically stimulated. Sulfonylureas have been regarded as being unsuitable for overweight diabetics, who primarily need to lose weight. However, KATP channels are also found in coronary vascular smooth muscle cells and cardiomyocytes, but here the SUR components are of the SUR 2A and SUR 2B subtypes, respectively (22).

OTHER APPLICATIONS OF SULFONAMIDES

Today, one of the most widely used sulfonamide drugs is *sildenafil*, marketed as Viagra®. It is a drug used to treat erectile dysfunction and pulmonary arterial hypertension by inhibiting cGMP specific phosphodiesterase type 5, an enzyme that regulates blood flow in the penis. Sildenafil, as a selective PDE5 inhibitor, inhibits cGMP degradation and improves the relaxation of the smooth muscles in the corpus cavernosum (23).

CONCLUSIONS

Compounds with sulfonamide structure enjoy a vast applicability. In this context, they have been widely studied for many years, for their chemotherapeutic activity. The application of sulfonamides has greatly been extended over the years from their function as antimicrobial agents to COX-2 inhibitors, diuretics, carbonic anhydrase inhibitors and even as an anti-impotence drug. In conclusion, sulfonamides represent a diverse and relevant class of therapeutic drugs. The sulfonamide group remains a important strating point for the discovery of new compounds to treat an increasing portfolio of diseases.

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RESEARCH STUDIES ON THE QUALITY OF BIO-MUCOADHESIVE TABLETS CONTAINING MICONAZOLE NITRATE

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RESEARCH STUDIES ON THE QUALITY OF BIO-MUCOADHESIVE TABLETS CONTAINING MICONAZOLE NITRATE (Abstract): Biomucoadhesive tablets are widely used to formulate topical antifungal drugs for treating acute oral candidiasis. The research focuses on the pharmaco-technological control of certain bio-mucoadhesive tablets containing miconazole nitrate, formulas developed and prepared by the authors based on the original formulas. The analyzed parameters were: macroscopic aspect, physical-chemical (pH) and pharmacotechnical properties (hardness, friability, mass uniformity, in vitro disintegration).The used methods are described in literature, most of them included in the Romanian and European Pharmacopoeia. The obtained results allow us to conclude that of the five original formulas, two (formulas IV and V) meet the criteria for oral mucosa drug by their tolerance (pH 5.77 to 5.84) and retention time (65-90 min.), the intimate contact of the tablet with the oral mucosa determining a high concentration of active substance. **Keywords**: MICONAZOLE NITRATE, BIOMUCOADHESION, ORAL BIOADHESIVE TABLETS.

During the last years, bioadhesive tablets have been developed to improve oral drug bioavailability. Drug administration through epithelial routes avoids first-pass effect (or presystemic metabolism), is safe and efficient (1-3). Oral cavity is a convenient area for placing them and is well-accepted by users. Five formulas of bio-mucoadhesive tablets containing miconazole nitrate in a concentration of 0.25 g have been developed and analyzed (4). Quality control of the obtained biomucoadhesive tablets was done by macroscopic, physical-chemical (pH) and pharmacotechnical (hardness, friability, mass uniformity, *in vitro* disintegration) tests.

MATERIAL AND METHODS

Five formulations of mucoadhesive tablets containing miconazole nitrate in a concentration of 0.25 g were prepared and evaluated using quality control tests:

Macroscopic Characteristics: the following visual and olfactory characteristics have been analyzed: aspect, uniformity, colour, smell, taste, shape and size.

Tablets Size: diameter (d2) and height (h) were determined using PHARMA-TEST equipment, Germany. Tablet area calculation was done by the formula:

$$S = \frac{\pi * d^2}{4}$$, where: S- tablet area (mm^2). r - tablet radius (mm).

Mass Uniformity was determined according to FR X and Eur. Ph. on a BD 202 Mettler Toledo analytical balance.

Tablets Hardness was determined by using PHARMA TEST equipment (Germany), the average value for 10 tablets from each formulation batch being obtained.

Tablets Friability for exposing tablets to rolling and vibration was analysed by using a ROCHE brittling machine. Friability was calculated by using the formula:

Friability = Lost Mass/Initial Mass x 100

The procedure consists in weighing 10 tablets that are tumbled in the brittling machine. The unit was set at 100 RPM for 4 minutes.

Tablet pH. The pH value of the biomucoadhesive tablets containing miconazole nitrate was obtained by potentiometric analysis (Cyberscan 2500 with pH electrode using data fitting software)

Disintegration Time of tablet was determined using method B from Romanian Pharmacopoeia 10th edition. Disintegration test was carried out on 6 tablets of each formula in a glass with 500 ml distilled water at a temperature of 37°C ± 2ºC.

For processing the experimental data, we used the descriptive statistics of the ANOVA analysis of variance.

RESULTS AND DISCUSSION

The resulting biomucoadhesive tablets have a lenticular disc shape, are flat, uniform, with full margins, flat, white, uniform surface, spotless and odourless.

The biomucoadhesive tablets are 8.99 mm in diameter and 2.75 mm in height, with small differences between the five formulations (tab. I, II).

The degree of asymmetry of distribution (skewness) around the mean, compared to the normal distribution, is represented by a frequency distribution:

- as an asymmetrical ”tail”, in the ”positive” values range for formulas I, III, IV, and V

- as an asymmetrical ”tail”, in the ”negative” values range for the IInd formula, explained by the presence in the tablet of CMC-Na 60 mg, and PEG 6000 40 mg.

TABLE I

The Physical Characteristics of Biomucoadhesive Tablets Containing Miconazole Nitrate

Formula	Bulk (h) (mm)	Diameter (mm)	Area $S = \frac{\pi * d^2}{4}$	Hardness (N)	Friability %	pH	Disintegration Time (min)
I	2.753 [1](0.0033)	8.988 [1](0,008)	63.447	60.06	0.9404	5.487	25
II	2.518 [1](0.008)	9.002 [1](0.002)	63.645	54.98	0.9268	5.500	45
III	2.56 [1](0.01)	9.039 [1](0.019)	64.169	72.44	3.0495	5.720	60
IV	2.666 [1](0.024)	9,04 [1](0.000)	64.184	76.14	0.237	5.770	65
V	2.568 [1](0.002)	9.047 [1](0.003)	64.283	70.15	0.626	5.840	90

TABLE II

Descriptive Statistics of Tablets Area

	Formula I	Formula II	Formula III	Formula IV	Formula V
Mean	63.448	63.646	64.171	64.184	64.283
Standard Error	0.001685744	0.001247111	0.030864644	0.0020164	0.002433266
Median	63.476	63.617	63.971	64.184	64.326
Mode	63.476	63.617	63.900	64.184	64.326
Standard Deviation	129.8362	111.6733	555.2999	115.9425	95.8427
Sample Variance	16857.43	12470.93	308358	13442.67	9185.822
Kurtosis	3.356387	-1.08507	2.747375	-1.39286	-0.28299
Skewness	1.55241	-0.40064	1.688115	0	0.433637
Range	0.424	0.283	1.851	0.284	0.284
Minimum	63.335	63.476	63.617	64.042	64.184
Maximum	63.759	63.759	65.468	64.326	64.468
Sum	634.479	636.456	641.707	641.840	642.834
Count	10	10	10	10	10

The "flattening" degree of distribution (Kurtosis), compared with the normal distribution, indicates a relatively flat curve for formulations II, IV and V and a curve with a higher peak than the normal distribution for formulations I and III.

TABLE III

Tablet Hardness (N) Descriptive Statistics

	Formula I	Formula II	Formula III	Formula IV	Formula V
Mean	60.060	54.980	72.380	76.140	70.150
Median	61.000	54.100	74.050	77.150	71.900
Mode	62.800	53.500	0	67.700	73.500
Standard Deviation	3304.6096	5746.651	10434.537	5858.555	3529.322
Standard Error	1.092453333	3.3024	10.88795556	3.432265556	1.245611111
Sample Variance	10920444	33024000	108879556	34322667	12456111
Kurtosis	-1.307846	0.027506	-0.422346	-0.67949	-1.14133
Skewness	-0.5757	0.594507	-0.525311	-0.10268	-0.7295
Range	8.800	19.000	32.600	17.600	9.400
Minimum	54.600	46.700	53.000	67.700	64.100
Maximum	63.400	65.700	85.600	85.300	73.500
Sum	600.600	549.800	723.800	761.400	701.500
Count	10	10	10	10	10

Mass Uniformity. Compared to the calculated average mass, the individual mass agrees with percentage deviations provided by Romanian Pharmacopoeia 10th

edition (219 mg / tablet ± 7.5%).

Tablet Hardness. Tablet hardness values are shown in table I and statistical calculation in table III. The differences are explained by the polymers used in the formulas.

The degree of asymmetry of distribution (skewness) around the mean value, compared to the normal distribution, is represented as a frequency distribution:

- as an asymmetric"tail", in the "positive" values range for formula II;

- as an asymmetric"tail", in the "positive" values range for formulas I, III, IV, and V.

The degree of "flattening" of the distribution (Kurtosis), compared with the normal distribution, indicates a relatively flat curve for formulas I, III, IV and V (higher hardness for these four formulae) and a curve with a higher peak than the normal distribution, for formula II, that is a lower hardness for this formula.

Higher mechanical strength (hardness) values have been obtained for formulas III, IV and V.

Tablet Friability. Mechanical strength of the tablets (tab. IV) is unsatisfactory when a maximum limit of 1% is exceeded. Statistical calculation is shown in table IV.

TABLE IV

Tablet Friability (%). Descriptive Statistics

	Formula I	**Formula II**	**Formula III**	**Formula IV**	**Formula V**
Mean	0.9242	0.9268	3.0506	0.2376	0.6264
Median	0.926	0.925	0.717	0.22	0.449
Mode	0.881	0	0	0.044	0
Standard Deviation	0.045523	0.008385	3.319278	0.198244	0.244786
Standard Error	0,00041446	0,00001406	2,20352186	0,00786016	0,026306012
Sample Variance	0.002072	7.03E-05	11.01761	0.039301	0.05992
Kurtosis	-2.98068	0.276745	-3.32187	-3.00978	-3.323805
Skewness	0.015806	0.678451	0.606655	0.110428	0.610101
Range	0.093	0.022	6.271	0.396	0.455
Minimum	0.881	0.917	0.489	0.044	0.446
Maximum	0.974	0.939	6.76	0.44	0.901
Sum	4.632	4.633	15.253	1.188	3.132
Count	5	5	5	5	5

The degree of asymmetry of distribution (skewness) around the mean compared to the normal distribution is represented by a frequency distribution:

- as an asymmetrical"tail", in the "positive" values range for all the five formulas.

The degree of "flattening" of distribution (Kurtosis) compared with the normal distribution indicates a relatively flat curve for formulas III, IV and V (lower friability for the fourth formulas) and a curve with a higher peak than the normal distribution for formula, fact that implies a higher hardness for this formula.

The biomucoadhesive tablets with high hardness have low friability. After analysing the formulas, it can be noticed that formulas IV and V have friability less than 1%. Formulas IV and V have an adequate friability of 0.237% and 0.626, respectively.

pH. As seen in table I, pH values ranged between 5.487 and 5.840.

Disintegration time. The analysis of the results obtained following the disintegration of the five tablet-formulas containing miconazole nitrate (tab. V) revealed different disintegration times for the five tablet-formulas.

TABLE V

Disintegration Time (min.) of Miconazole Nitrate

Descriptive Statistics

	F I	F II	F III	F IV	F V
Mean	25.015	45.035	60.06	65.54	90.03666667
Standard Error	0.000005	0.000005	0.00002	0.04232	0.000044444
Median	25.015	45.035	60.06	65.54	90.04
Mode	25.01	45.03	60.05	65.08	90.05
Standard Deviation	0.005477226	0.005477226	0.010954451	0.503904753	0.016329932
Sample Variance	3E-05	3E-05	0.00012	0.25392	0.000266667
Kurtosis	-3.33333333	-3.333333333	-3.33333333	-3.333333333	-0.3
Skewness	6.66134E-17	2.91887E-12	1.45943E-12	-6.34826E-14	-0.85732141
Range	0.01	0.01	0.02	0.02	0.04
Minimum	25.01	45.03	60.05	65.08	90.01
Maxi-mum	25.02	45.04	60.07	66	90.05
Sum	150.09	270.21	360.36	393.24	540.22
Count	6	6	6	6	6

Faster disintegration was recorded for formula I (25 min.), while formulas II, III, IV and V have very long disintegration times (45 min., 60 min., 65 min. and 90 min, respectively).

The long disintegration time of formulas II, III, IV and V is explained by the CMC-Na and miconazole nitrate polymer ratio, which is: 0.6: 0.25 for formula II and 1.00: 0.25 for formulas III, IV and V.

CONCLUSIONS

This study deals with the five formulas of miconazole nitrate biomucoadezive tablets developed by the authors, such as statistical analysis, proved that these formulas meet the quality requirements regulations regarding macroscopically characteristics, hardness, friability, mass uniformity, pH, and disintegration. The obtained data and especially the long disintegration time recommend formulas IV and V for further studies.

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NEWS

SILIBININ - PROTECTIVE ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* PNEUMONIA

Staphylococcus aureus, a virulent Gram-positive pathogen, is responsible for nosocomial and community-acquired diseases which threaten human health such as soft tissue infections, septicemia and pneumonia. In the last decade, the emergence of methicillin-resistant *S. aureus* challenged the potency of traditional antibiotics, imposing major restrictions on the treatment options of *S. aureus* pneumonia. Recent reports have established and demonstrated in animal models that α-toxin plays a key role in the pathogenicity of staphylococcal pneumonia. Silibinin is the main biologically active ingredient in silymarin, a flavonolignan complex extracted from *Silybum marianum* fruits. Silibinin is known for its anti-inflammatory, antioxidant, hepatoprotective and anticancerigenic activity. This study shows that silibinin inhibits the expression of α-toxin in a dose-dependent manner and alleviates α-toxin-induced lung cells injury. Moreover, silibinin provides effective protection on the lung injury of staphylococcal pneumonia. Therefore, silibinin may potentially be used in combination with β-lactam antibiotics to treat *S. aureus* infections (Wang X, Dong J, Dai X, Zhang Y, Wang J, Li H, Lu C, Tan W, Gao X, Deng X, Bu S, Niu X. Silibinin *in vitro* protects A549 cells from *Staphylococcus aureus*-mediated injury and *in vivo* alleviates the lung injury of staphylococcal pneumonia. *Planta Med* 2013; 79: 110–115).

Adriana Trifan

CONTRIBUTION OF CERTAIN INDIVIDUAL FACTORS AND OF THE ENTOURAGE TO THE HEAVY SMOKER STATUS IN TIMIS COUNTY STUDENTS

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CONTRIBUTION OF CERTAIN INDIVIDUAL FACTORS AND OF THE ENTOURAGE TO THE HEAVY SMOKER STATUS IN TIMIS COUNTY STUDENTS (Abstract): The study aimed for a hierarchy of factors associated to the heavy smoker status in young people. **Material and methods**: The representative sample of students included into the study totalized 2076 young subjects in Timis County universities, with urban residence, 62.5% girls and 37.5% boys, aged between 18-25 years. The working method was the transversal population study based on the CORT 2004 questionnaire regarding health risk behaviors in adolescents and young people. Data processing used the *EpiInfo* software. The strongest predictors of the heavy smoker status in young people are the existence of suicidal thoughts, the increased number of friends who smoke the smoker status without the wish to quit. **Results and conclusions**: Students with an increased intensity of smoking more frequently experienced suicidal thoughts as compared to students smoking with a medium intensity. Students with a medium smoking intensity have significantly more friends who smoke as compared to students who are light smokers. Students, who tried to quit smoking, smoke significantly less cigarettes a day than those who did not wish to quit smoking**.** **Keywords**: STUDENTS, HEAVY SMOKER STATUS, ASSOCIATED FACTORS

In a study conducted in New Zealand (1), the authors demonstrated that early tobacco consumption was a important predictor for suicidal ideation but lost significance when increased levels of stress and depression, as well as low attachment for parents, were included into the multivariate model.

The affiliation of adolescents and young adults with friends who practice risk behaviors, especially substance consumption, is a strong predictor for the adherence to such behaviors (2, 3). The smoking habit in friends and the perception of smoker friends are associated to the smoking behavior in adolescents (4, 5, 6).

Individuals who cease smoking benefit from a significant decrease of the risk for all fatal disturbances (7). There is a clear relation between the age when a smoker quits and the risk of premature death. While smoking cessation has benefits over the general mortality, the benefit degree varies depending on the disease (8).

MATERIAL AND METHODS

The representative sample of Timis County students totalized 2976 young subjects from Timis County universities, urban residents (the size of the stratified cluster sample was determined with the the *EpiInfo* software). The gender distribution

was: 62.5% girls and 37.5% boys. The students were aged between 18-25 years, the highest proportion being aged 21 years, 27.2%.

The working method was the transversal population study based upon the CORT 2004 Questionnaire regarding health risk behaviors in adolescents and young people, designed on the occasion of the type A CNCSIS grant, code 1167, 2003-2005, entitled: "Assessment of the dimensions of risk behaviors in high school pupils and young subjects in undergraduate, postgraduate, occupational and university teaching systems in Timis County". The CORT 2004 Questionnaire represents an original working instrument belonging to the research team of the project and it was built upon other questionnaires used for the investigation of risk behaviors, mainly the European ESPAD and the American YRBSS. The Questionnaire was validated by the Commission of Ethics of the "Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania.

High school pupils were only included into the study after freely expressed informed consent was obtained from each participant. The general response rate in students was 40%. Data processing and interpretation used advanced modern methods of medical statistics, with the *EpiInfo* software, version 3.5.1, 2008.

RESULTS AND DISCUSSION

1. The heavy smoker status. The logistic regression test was applied to determine the impact of multiple personal and entourage-linked factors on the heavy smoker status (high intensity smoking means over 10 cigarettes/day) in young people. The model includes 19 independent variables (gender, last school graduated by the father, last school graduated by the mother, satisfaction concerning the financial situation of the family, number of friends who smoke, number of friends who get drunk, number of friends who consume marijuana, smoking status of the father, mother and siblings, number of days he/she skipped classes, school grades, age when he/she smoked the first cigarette, the wish to quit smoking, number of days of binge drinking, marijuana consumption, feelings of sadness, suicidal thoughts, knowledge on the effects of smoking). The model including these predictors is statistically significant, $\chi^2(19)=110.36$, $p<0.001$, showing that the proposed model may discriminate between young people who smoke and those who do not. The model may explain between 21.7 and 30.0% of the heavy smoker status variance and may correctly classify 73.8% of the cases (tab. I).

The strongest predictor of the heavy smoker status in young people is the presence of suicidal thoughts, those reporting these thoughts having 2.18 times more chances to be high intensity smokers. The increased number of friends who smoke is an important predictor, young people with a high number of smoker friends having 2.1 times more chances to smoke with high intensity than those without friends who smoke, provided the other factors in the model are constant. The smoker status without the wish to quit is also an important predictor, young people who do not have the wish to cease smoking having 1.7 times more chances to smoke with increased intensity. The other statistically significant predictors are: the increased number of friends who smoke marijuana, the high number of days he/she skipped school, the non-smoker status of the mother and the female gender.

TABLE I

Logistic regression for predicting the heavy smoker status in young people

	B	S.E.	Wald	df	Sig.	OR	95% C.I. for EXP(B)	
							min	max
Gender (1)	-.528	.266	3.95	1	.047	.590	.350	.992
Last school graduated by the father	-.116	.128	.83	1	.362	.890	.693	1.143
Last school graduated by the mother	-.056	.134	.17	1	.674	.945	.727	1.229
Satisfaction regarding the family financial status	-.117	.123	.90	1	.342	.890	.699	1.133
Smoker status of the father (1)	-.123	.239	.26	1	.606	.884	.554	1.412
Smoker status of the mother (1)	-.499	.248	4.04	1	.044	.607	.373	.988
Smoker status of siblings (1)	.077	.236	.10	1	.743	1.080	.681	1.714
Number of friends who smoke	.744	.207	12.89	1	.000	2.105	1.402	3.159
Number of friends who get drunk	.313	.184	2,88	1	.089	1.367	.953	1.961
Number of friends who smoke marijuana	.550	.273	4.05	1	.044	1.733	1.015	2.960
Number of days he/she skipped school	.384	.091	17.81	1	.000	1.468	1.228	1.754
School grades at the end of the last semester	.177	.145	1.47	1	.224	1.193	.897	1.586
Age when he/she smoked the first cigarette	.135	.118	1.30	1	.254	1.144	.908	1.442
Attempt to cease smoking	.567	.273	4.32	1	.038	1.763	1.033	3.008
Number of binge drinking days	.137	.107	1.63	1	.201	1.147	.930	1.414
Marijuana consumption	-.037	.319	.014	1	.907	.964	.515	1.802
Sadness	.264	.274	.93	1	.334	1.303	.762	2.227
Suicidal thoughts	.781	.354	4.87	1	.027	2.184	1.092	4.370
Knowledge on the effects of smoking (1)	-.165	.282	.34	1	.557	.847	.488	1.472
Constance	-3.30	.835	15.63	1	.000	.037		

Legend: (1)= first category that was taken as reference to categorical variables introduced in the model; B= β coefficient; SE= standard error of the β coefficient; Wald= Wald coefficient; df= degrees of freedom; Sig.= p value; OR=odds ratio

The strongest predictor of the heavy smoker status in young people is the presence of suicidal thoughts, those reporting these thoughts having 2.18 times more chances to be high intensity smokers. The increased number of friends who smoke is an important predictor, young people with a high number of smoker friends having 2.1 times more chances to smoke with high intensity than those without friends who smoke, provided the other factors in the model are constant. The smoker status without the wish to quit is also an important predictor, young people who do not have the wish to cease smoking having 1.7 times more chances to smoke with increased intensity. The other statistically significant predictors are: the increased number of friends who smoke marijuana, the high number of days he/she skipped school, the non-smoker status of the mother and the female gender.

2. Suicidal thoughts during the previous year. In the non-smoker group 7.3%

(102) students experienced suicidal thoughts during the previous year. In the smoker group, the percent of students with suicidal thoughts is 10.2% (65). The smoker group had 1.42 times more frequent suicidal thoughts during the previous year as compared to the non-smoker group, χ^2=4.61, p<0.05 (fig. 1).

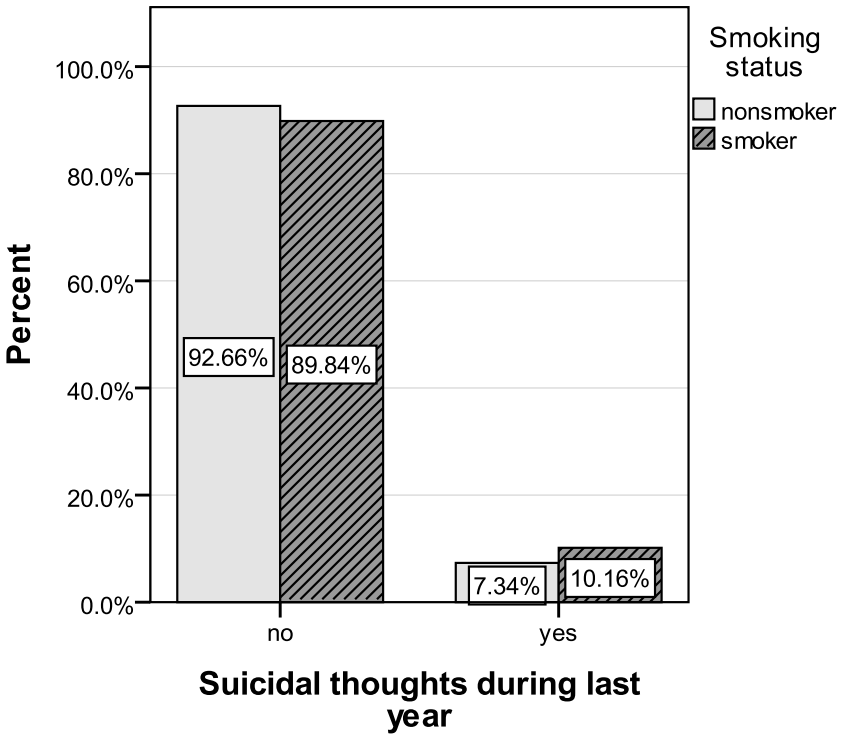

Fig. 1. Percent distribution of students according to the existence of suicidal thoughts and to the smoker status

Researchers conducted by Choi et al. (9) revealed that persons without depression and who did not smoke increased their chances to develop depression if they started smoking. Further studies (10) found that smoker adolescents and those who were ex-smokers at the beginning of the study had 1.5-2 times higher chances to develop depression symptoms in the future as compared to non-smokers.

3. Smoking among friends. Of the friends of non-smoker students, several smoke in a percent of 56.5% (787), most of them smoke in a percent of 31.1 (433) and all of them in a percent of 3.3 (46). 9.1% (127) of the non-smoker students have no friends who smoke. Of the friends of smoker students the majority smoke in a percent of 63.3 (407), several in a percent of 26.0 (167) and all in a proportion of 9.2% (59).

1.6% (10) of the smoker students has no friend who smokes. Smoker students have significantly more friends who smoke as compared to non-smoker students, U=267197, z=-16.11, p<0.001, the association being of medium size (fig. 2).

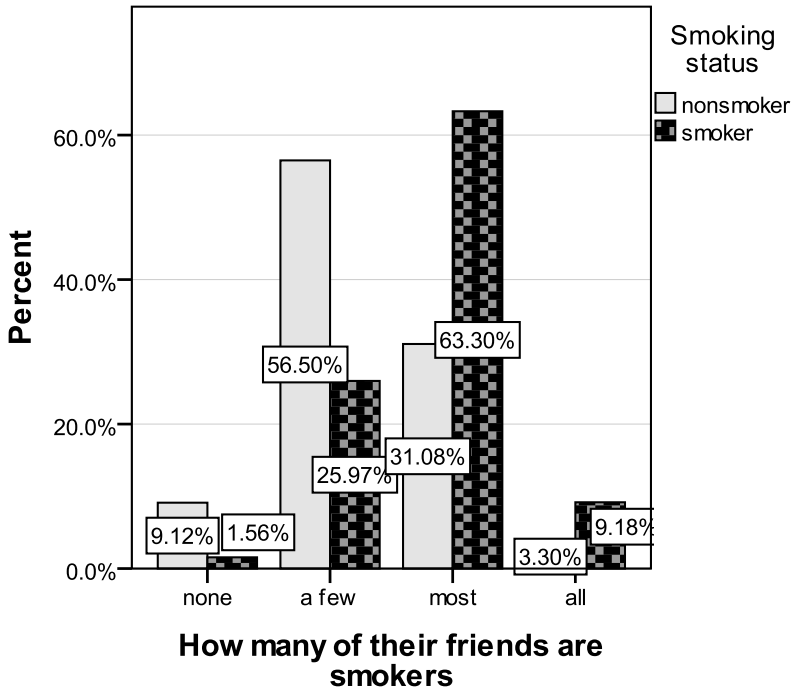

Fig. 2. Percent distribution of students according to the smoker status of friends and to their own smoker status

Regarding the non-smoker students, we found that smoking intensity is influenced by the number of friends who smoke, $H(2)=37.26$, $p<0.001$. We applied the Mann-Whitney test to investigate this result. We applied the Bonferroni correction and the effects were reported to a level of significance of 0.0167. We found that students with a medium smoking intensity have significantly more friends who smoke, as compared to light smoker students, $U=15342.5$, $z=-4.07$, $p<0.001$, and there are no differences between students who smoke with medium or high intensity regarding the number of friends who smoke (fig. 3).

The similarity or homogeneity of tobacco consumption patterns in adolescents and their peers, led researchers to the conclusion (6, 11) that the entourage of friends influences the smoking habit in adolescents. The most often incriminated mechanism is social learning (12), by which adolescents learn about the use of tobacco by observing friends who smoke and they are encouraged to use tobacco by perceiving apparent advantages such as gaining group acceptance or establishing an apparent social identity. Other transmission mechanisms from friends are direct pressure to smoke and offering cigarettes and other tobacco products.

4. Attempt to cease smoking. Regarding the attempt to cease smoking, 25% (513) of respondents admit they tried to quit smoking and 9.1% (186) did not try to quit smoking (fig. 4).

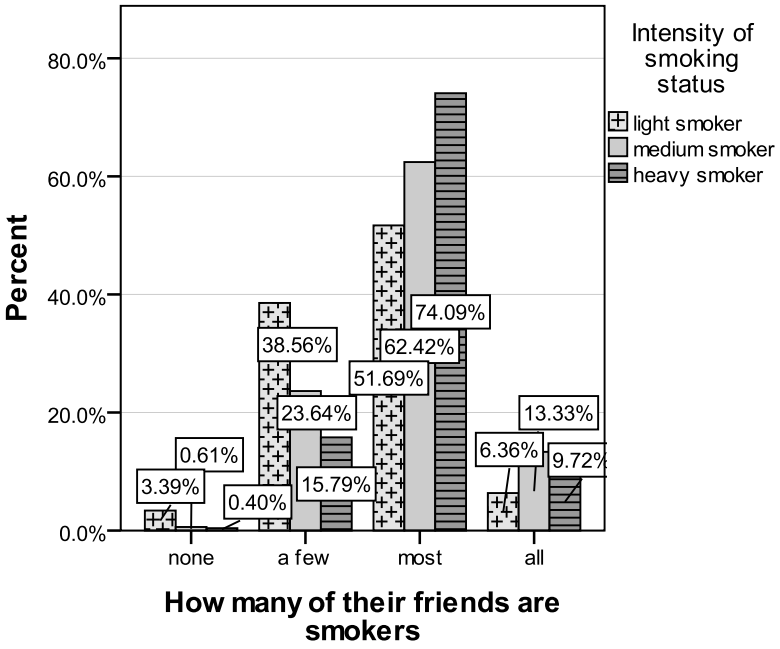

Fig. 3. Percent distribution of smoker students according to the number of friends who smoke and to the intensity of smoking

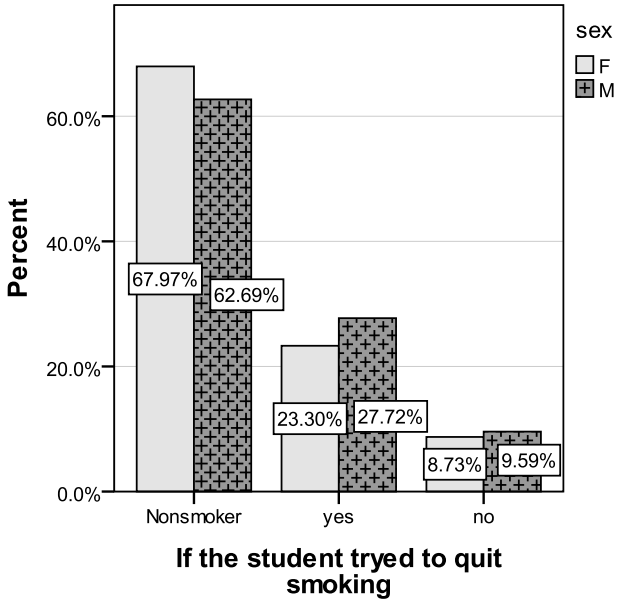

Fig. 4. Percent and gender distribution of students according to their attempt to quit smoking

After excluding the students who do not smoke, we wanted to find out if there were gender differences regarding the attempt to quit smoking. There are no significant differences in the distribution of gender related responses, $p>0.05$.

Using only the smoker students, we found out that those who tried to quit, smoked their first cigarette at a significantly older age than those who did not try to quit smoking, $U=38623$, $z=-4.08$, $p<0.001$. These results are concordant to other studies (13), concerning the fact that young age at the onset of smoking is associated to the permanent smoker status at adult age.

Naive smoking experiments in adolescents frequently transform them into regular users, frequently causing a strong addiction which counteracts smoking cessation efforts. Efforts to reduce smoking in future adults must focus on experimental as well as on regular smoking.

In smoker students, we found that those who tried to quit, smoke significantly less cigarettes a month than those who did not wish to cease smoking, $U=38931$, $z=-4.13$, $p<0.001$. Using only smoker students, the results showed that those who tried to cease smoking, smoke significantly less cigarettes a day than those who did not wish to cease smoking, $U=36473$, $z=-4.77$, $p<0.001$.

Previous research (14, 15) demonstrated that the earlier individuals start smoking the higher are their chances to develop pulmonary carcinoma or to have an altered health during adult life. Globally, one third of the young people who become regular smokers to adult age will die due to consequences of smoking (16). There is evidence that sometimes smoking is the first step to triggering the abuse of other substances, and preventing or delaying this first step will decrease the risk for the use of other substances (17-19).

CONCLUSIONS

The strongest predictors of the heavy smoker status in young people are the existence of suicidal thoughts, the increased number of friends who smoke the smoker status without the wish to cease smoking.

In non-smokers / smokers we obtained the following results: 7.3% / 10.2% of the students had suicidal thoughts during the previous year. Students who smoke at high intensity more frequently experienced suicidal thoughts as compared to medium intensity smokers.

The friends of non-smoker / smoker students also smoke: several in 56.5% / 26% of cases; most in 31.1% / 63.3%; all in 3.3% / 9.3%. Students who smoke with medium intensity have significantly more friends who smoke as compared to light smoker students.

Depending on the attempt to cease smoking, 25.0% of respondents tried to cease smoking and 9.1% did not. Students, who tried to cease smoking, smoke significantly less cigarettes a day than those who did not wish to quit.

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DIAGNOSIS OF MENINGOCOCCAL INFECTION BY QPCR: DETECTION AND QUANTIFICATION OF DNA / *NEISSERIA MENINGITIDIS*

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DIAGNOSIS OF MENINGOCOCCAL INFECTION BY QPCR: DETECTION AND QUANTIFICATION OF DNA/*NEISSERIA MENINGITIDIS* (Abstract): We present the case of a patient with clinical signs of meningococcemia and negative bacterial culture. Microbiological diagnosis was possible only by testing with Real Time PCR technique the cerebrospinal fluid and blood samples. Both pathologic samples were positive for *Neisseria meningitidis* by this rapid and sensitive diagnostic method. **Key words:** MENINGOCOCCEMIA, REAL TIME PCR DIAGNOSIS..

Neisseria meningitidis is an important cause of morbidity and mortality worldwide, causing a spectrum of diseases ranging from asymptomatic porting to meningitis, and even septicaemia (meningococcemia), sometimes with fulminant development (1). Because of the difficulty of early diagnosis and rapid progression, the invasive meningococcal disease (sepsis and /or meningitis) is a potentially fatal disease, which can also cause long-term sequelae (2, 3). Despite the advances in resuscitation and intensive care, case fatality rate remained at 10% in developed countries and can reach 50% in developing countries (4, 5). Of those who survive, 11% have severe complications (6).

CASE REPORT

We present the case of a 22 months old child, male, from a family of average condition from rural area, transferred from The "*St Marie*" Universitary Clinical Children's Hospital, where he was hospitalized for 24 hours with a diagnosis of "febrile syndrome, acute nasopharyngitis, dispeptic syndrome". The onset of the disease was sudden, 12 hours prior to his admission to Children's Hospital with fever (39.9°C), chills, coldness, phenomena which occurred on a background of vomitting, diarrhea and watery rhinorrhea (which evolved about 14 days and were treated symptomatically in ambulatory). In pediatrics there was rapid worsening of the general condition and the petechiaes, rash, exanthema appearing on the chest, with rapid generalization throughout the body, having shock and meningeal contracture syndrome. Biohumoral test revealed 6280 leukocytes /mmc with PMN 72.4%, the decreasing

number of thrombocytes (from 257000/ mmc to 83000/mmc), increased TQ and PTTK clotting time, decreased prothrombin activity and the lumbar puncture reveals 8 elements / mmc. Ceftriaxone was administered (1 g), and then the patient was referred to the Clinic of Infectious Diseases. Family history record: parents apparently healthy, smokers. Personal history of pathology: insignificant for the present disease; the only one to mention is the nasopharyngitis on which the present disease started. The clinical examination performed at admission in our unit showed a bad general condition, weakness, generalised necrotic petechial, rash exhantema (fig. 1), hemodynamic instability (BP 87/64 mmHg, 173/min PP AV, cold limbs) and meningeal contracture syndrome. Prognostic score (determined according to Stiehm-Damroch and Niklasson criteria) was 5 (very severe).

Fig. 1. Petechial rash

The petechial necrotic rash with rapid expansion, accompanied by fever, shock phenomena and the meningeal syndrome suggested a meningococcal infection (purpura fulminans, acute meningococcemia or meningococcal meningitis). Biohumorally leukocytosis with neutrophil polynucleosis (NL 14820/mmc, 81%), thrombocytopenia (Tr 67000/mmc). The prognostic score 24 hours after admission was thus 2, a good prognostic indicator. Blood culture, direct bacteriological culture and latex agglutination (LA) of cerebrospinal fluid (CSF) were negative. A sample of CSF and one of blood collected on admission were sent to the Virology Laboratory of the Microbiology Discipline for RT-PCR analysis.

Real Time PCR Diagnosis: DNA / *Neisseria meningitidis* was purified using the kit *PrimerDesign Precision*^{TM} *Gram Negative Bacterial DNA extraction.* The kit has the advantage of rapidity (a sample of DNA can be purified in 16 minutes) and required the use of *Beckman Coulter* microcentrifuge and of the *Biosan* thermoblock. The purity and concentration of DNA were analysed with *NanoDrop Pearl* and RT PCR amplification used *Mx3005P Stratagene* thermocycler.

The first stage of the RT - PCR analysis, according to the protocol established in the project application was the detection of DNA / *N. meningitidis* through the method *qualitative end point PCR.* This meant the inclusion in the RT-PCR plate of a positive control and of negative controls (between 1 and 3) to find out the contamination that which can lead to false positive results. If the positive control shows significant fluorescence, it certifies the fact that all the negative results of the tested samples are truly negative, and are not just the result of a PCR experiment failed because of primers, samples quality or polymerase. In the case of qualitative *end point PCR method*, the final calls are based on the value of the final fluorescence; the analysis was made within the experiment of *Quantitative Analysis Plate Read,* which allows the software to mark the positive and negative samples with "+" and "-". This software performs the calculation of a *t test* based on the final values of the fluorescence, the final normal-

ized fluorescence and the fluorescence change pre - and post-experiment. The DNA purified from CSF and blood has been detected as positive for *Neisseria meningitidis* and in fig. 2. a), can be observed the amplification plots of the experiment (for positive control, DNA/meningococci from CSF and from blood respectively).

The second stage involved testing the purified DNA from the same patient through the *Quantitative PCR method*, which uses the standard curve.

The *PrimerDesign*™ genesig kit for *N. meningitidis* use the *TaqMan* principle. Preparation of the standard curve was done by performing 10-fold dilution of the positive control (from 2 x 10^6/µl up to 2 copies/µl). The pathogen detection mix contained 10 µl of 2 x *Precision*^{TM} *Mastermix*, 1 µl *N.meningitidis* Primer/Probe mix, 1 µl internal extraction control primer/probe mix and 3 µl RNAse/DNAse free water for a final volume of 15 µl. Five µl of diluted DNA template was added into each well. To confirm extraction of a valid biological template, endogenous actin beta (ACTB) was quantified in parallel through the FAM channel. The amplification protocol included one cycle for enzyme activation for 10 min at 95°C and 50 cycles of 10 sec denaturation at 95°C, 60 sec annealing and extension at 60°C (7). The *N. meningitidis* specific primer and probe mix was detected through the FAM channel and the internal control were detected through the VIC channel (11). The parameters of the standard curve (efficiency, slope, Rsq) were in the accepted limits by the *Stratagene*'s software and the values obtained were: for CSF sample – 6.801 x 10^3 DNA copies / ml, Ct = 28.47 and for blood sample: 3.406 x 10 DNA copies / ml, Ct = 37.26 (fig. 2, b).

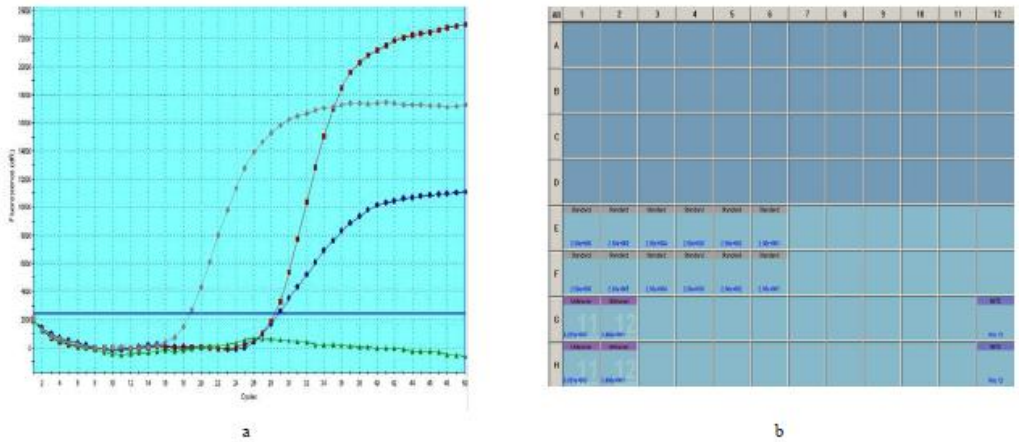

Fig. 2. a) Amplification plots for positive control, CSF and blood sample – end point PCR

b) Initial template quantity of DNA/meningococci in CSF and blood sample – absolute quantification

Under the treatment with Ceftriaxone, 1.5g/day, 10 days, the pathogenetic treatment for shock and cerebral edema, with symptomatic and hygienic-dietary regime, the evolution was favorable, with remission of fever in the 3rd day of hospitalization; he became hemodynamically stable after two days from admission and the meningeal contracture syndrome disappeared after the

7th day. During hospitalization there were no new cutaneous elements. It is still worth mentioning a fever attack (39.2°C) on day 10 of hospital stay without other signs of severity, considered to be of immunological origin, which resolved rapidly under corticotherapy. The final diagnosis, based on the previous clinical data correlated with the laboratory data (RT-PCR of blood and CSF positive for *Neisseria meningitidis*) was that of „Meningococcemia (acute form) with meningitis". The patient was discharged, with improved status after 10 days of treatment, with the persistence of a single petechia (faded) on the trunk.

DISCUSSION

Invasive meningococcal disease (IMD) is a potentially fatal disease which requires prompt treatment and prevention measures to close contacts. In the absence of a rapid diagnostic test for determining causation it is necessary to recognize the typical clinical signs (8). Clinical suspicion of meningococcal infection is based on the presence of hemorrhagic rash associated with symptoms of meningitis / sepsis. Unfortunately, the rash is not present in each case. Therefore, in more than half of the cases presented in the initial stages of the disease the opportunity of an early diagnosis is missed. Also, the initial stages of this infection, signs and symptoms are nonspecific; they are common with the ones specific to simple viral illness, thus making their positive predictive value for IMD to be low. Certain signs, such as cold limbs and pallor may be specific for early diagnosis of sepsis, but their diagnostic value remains to be proven. The number of leukocytes and the C-reactive protein (CRP) are often used by clinicians to differentiate invasive bacterial infection from self-limiting viral diseases, but their diagnostic value in the initial stages of IMD is reduced, none of these investigations being sufficiently sensitive or specific. Procalcitonin (PCT) is an excellent biomarker for invasive bacterial infections. It typically grows in the first 2 h of symptom onset, much earlier than CRP, and can be detected quickly and accurately to bedside (12). PCT was found to be more sensitive and specific than the number of leukocytes and the CRP in the prediction of IMD in children. CSF examination (biochemical data and PMN counting) may be useful for differentiating acute viral meningitis from the bacterial ones. On the other hand, the gold-standard of the microbiological diagnostic remains cultivation from a sterile *situs* such as blood or CSF. Currently, it becomes increasingly difficult to confirm the diagnosis of meningococcal infection by conventional microscopy and culture techniques, because of the early antibiotic treatment, which is recommended by current guides, to improve management and case prognosis (9).

The rate of positive blood cultures ranges from 25-50% and decreases to about 5% when given antibiotics before admission (10, 11). Direct microscopic examination and culture of CSF are positive in 80-90% of untreated cases of meningococcal meningitis, but this rate also decreases in the case of previous administration of antibiotics. In addition, for many patients lumbar puncture it is not possible in the early stages of the disease due to fears of inducing clinical deterioration. Other limitations of conventional diagnostic methods include the time interval until culture positivity and poor sensitivity and specificity of rapid tests such as LA (12).

PCR has the potential to overcome these limitations, through the detection of small

amounts of bacterial DNA in the absence of viable bacteria and under antibiotics treatment. Detection or confirmation of meningococcal infection by DNA amplification was very efficient so that about 50% of meningococcal infections in the UK for which no isolation is available are now confirmed by PCR. The proportion of cases confirmed by non-culture techniques is also increasing in many other countries. Furthermore, PCR can be used to define sero subtypes of meningococci that were previously nontypeable. The screening for the DNA specific to meningococci is carried out currently using the *ctrA s*equence (segment of meningococci capsular biosynthesis locus) specific for clinically significant serogroups (A, B, C, Y, W135, X and 29E) (13).

CONCLUSIONS

RT-PCR is potentially more sensitive than conventional PCR. RT-PCR tests allow rapid and accurate quantification of bacterial load (made of viable and nonviable bacteria). The case shown above proves the fact that the RT-PCR technique is a rapid and sensitive method for the diagnosis of cases of meningococcal infection with classical negative diagnosis following the administration of antibiotics before admission to hospital.

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CARACTERISTICILE DESPICĂTURILOR ORO-FACIALE ÎN COLECTIVITĂȚI ÎNCHISE

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THE CHARACTERISTICS OF OROFACIAL CLEFTS IN SMALL COMMUNITIES (Abstract): **Aim**: Establishing the prevalence of orofacial clefts in a community of institutionalized children and the correlations with sex, weight at birth, life environment, maternal age and children's associated diseases. **Material and methods**: The study was conducted on a batch of 37 living newly-born (15 boys and 22 girls) from 1987 to 1997, with orofacial clefts, institutionalized in placement centers from Iasi, who survived and were treated medical and surgical. The data were collected from the records found in the archives of these centers and were statistically processed with the EpiInfo 3.4.3. software a Center for Disease Control and Prevention C.D.C. (U.S.A.). **Results**: In 1987, children with orofacial clefts represented 7.42% and in 1997, 2.18% from the total of all children institutionalized. The average rate of prevalence of live births with orofacial clefts was 3.7/year. The frequency of clefts lip and palate in children who survived was 54.06%, clefts lip was 37.84% and clefts palate of 8.10%. Statistically significant correlations were found between the types of orofacial clefts and sex, weight at birth, life environment, maternal age and children's associated diseases ($F_{calculat} > F_{Fisher}$). **Conclusions:** The results confirm the decreased incidence of children with orofacial clefts, the high prevalence of clefts lip and palate the existence of orofacial cleft complications and the need for orthodontic treatment. **Key words:** OROFACIAL CLEFTS, PREVALENCE OF CLEFTS LIP AND PALATE, RISK FACTORS, SURGERY, ORTHODONTIC TREATMENT

Despicăturile oro-faciale sunt tulburări de coalescență a mugurilor faciali, care apar în timpul embriogenezei, fiind prezente la naştere, la nivelul buzei, palatului sau a ambelor, în forme izolate sau în cadrul unor sindroame cromozomiale.

Centrul pentru Prevalența şi Controlul bolilor din S.U.A. (CDC-NBDPN) a consemnat o incidență a despicăturilor de buză cu/fără palat de 10,63 şi de palat de 6,35 per 10.000 nou-născuți vii, 70% reprezentând formele cu despicături izolate (1).

Cauzele acestor malformații nu sunt elucidate, fiind combinația factorilor genetici cu alți factori (mediul, dieta, fumatul şi medicamentele maternale).

Tratamentul este complex, de lungă durată, depinzând de severitate şi de asocierea sindroamelor genetice sau a altor defecte (2). Acesta se impune datorită dificultății de alimentație a sugarului, urmând restabilirea funcționalității şi esteticii maxilofaciale. În primele luni de viață, repararea defectului se face chirurgical, apoi copiii

au nevoie de chirurgie adiţională, dar în special de terapie ortodontică.

Aspectul facial monstruos încă de la naştere produce traume psihice asupra familiilor acestora, iar copiii şi adolescenţii cu despicături au problemele psihice critice (3). Specialiştii din unele ţări consideră despicăturile oro-faciale ca serioase handicapuri, iar după apariţia metodelor precoce de diagnostic fetal (4), au permis avortul.

În acest sens, scopul studiului este de a stabili prevalenţa despicăturilor oro-faciale dintr-o colectivitate de copii instituţionalizaţi, de a determina corelaţiile cu sexul, greutatea la naştere a nou-născuţilor, mediul de proveniență, vârsta maternă şi cu afecţiunile asociate ale copiilor şi de a compara aceste date cu rezultatele studiilor anterioare.

MATERIAL ŞI METODE

Studiul a fost efectuat pe datele din foile de observaţie ale unui lot de 37 de nou-născuţi vii (15 băieţi şi 22 de fete) în perioada 1987 - 1997, cu despicături oro-faciale, proveniţi din Centrele de Plasament din Iaşi ("Sf. Parascheva" nr. 1 şi 2, "Sf. Andrei" şi Bucium). După domiciliul mamelor, nou-născuţii cu despicături oro-faciale proveneau din judeţele Iaşi (n = 16), Botoşani (n = 9), Neamţ (n = 5), Vaslui (n = 3), Bacău (n = 2) şi Slobozia (n = 2), 59% din mediul urban şi 41 % din mediul rural. Copiii au fost diagnosticaţi de la naştere şi au supravieţuit. Lotul de studiu a beneficiat de tratamente chirurgicale la Spitalul "Sf. Maria" din Iaşi, au fost monitorizaţi medical, dar nu şi trataţi ortodontic.

Pentru prelucrarea şi interpretarea statistică s-a utilizat programul *EpiInfo 3.4.3.*, cu analiza variabilelor prin statistică descriptivă, testul Fisher, regresie liniară şi multiplă.

REZULTATE

În 1987, cei 160 copii cu despicături oro-faciale reprezentau 7,42% din totalul copiilor instituţionalizaţi (n = 2150), respectiv 1:13,44 copii, comparativ cu 1997, când cele 25 cazuri reprezentau 2,18% (n = 1146), respectiv 1:45,80 copii (fig. 1). În 1987, cel mai mare procent l-au înregistrat despicăturile de buză 65,30%, cu o distribuţie aproape egală pe sex (50,84% masculin şi 49,16% feminin), iar în 1997, despicăturile de buză au avut o prevalenţă de 42,50% (41,07% masculin şi 58,93% feminin), iar despicăturile de buză şi palat de 57,50% (33,65% masculin şi 66,35% feminin). Distribuţia despicăturilor de palat pe sex a fost de 61,21% la masculin şi de 38,79% la feminin.

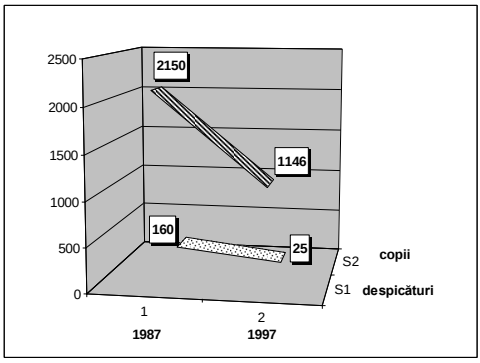

Fig. 1. Dinamica frecvenţei copiilor cu despicături oro-faciale (1987-1997)

Media anuală pentru perioada 1987 - 1997 a nou-născuţilor vii cu malformaţii oro-faciale, a avut o rată de frecvenţă de 3,7/an, respectiv de 1,4/an a despicăturilor de buză, de 2,0/an a despicăturilor de buză şi palat şi de 0,3/an a despicăturilor de palat.

Prevalenţa celor de buză şi palat a fost de 54,06%, a despicăturilor de buză de 37,84% şi a despicăturilor de palat de 8,10%. Despicăturile de buză şi palat sau numai de palat au fost mai frecvente la

sexul feminin, iar despicăturile de buză au fost mai frecvente la sexul masculin. Despicăturile oro-faciale unilaterale au fost mai frecvente de partea stângă (tab. I).

TABELUL I

Distribuția formelor anatomo-clinice ale despicăturilor oro-faciale

Tipul despicăturii	Cazuri		Băieți		Fete	
	n	%	n	%	n	% *
Buză incompletă unilaterală	3	8,11	3	8,11	-	-
Buză incompletă bilaterală	7	18,92	4	10,81	3	8,11
Buză completă unilaterală	4	10,81	1	2,70	3	8,11
Buză şi palat unilaterale	14	37,84	5	13,52	9	24,32
Buză şi palat bilaterale	6	16,22	3	8,11	3	8,11
Palat incompletă	1	2,70	-	-	1	2,70
Palat completă	2	5,40	-	-	2	5,40
Total	37	100,00	16	43,25	21	56,75

* Pentru valorile mai mici de 10, proporțiile sunt orientative

Greutatea la naştere a nou-născuților vii cu despicături oro-faciale a fost normală la 54,06% de cazuri, 18,91% au fost distrofici şi 27,03% au fost supraponderali (tab. II).

Vârsta mamelor care au născut copii vii cu despicături oro-faciale a fost sub 20 de ani în proporție de 5,40%, între 21 şi 34 de ani de 91,89% şi peste 34 de ani de 2,71% (tab. III).

TABELUL II

Formele anatomo-clinice ale despicăturilor oro-faciale în funcție de greutatea la naştere a nou-născuților

Greutate (g)	Despicături de buză		Despicături de buză şi palat		Despicături de palat		Total	
	n	%	n	%	n	%	N	% *
< 2900	2	5,41	4	10,80	1	2,70	7	18,91
3000 – 3900	10	27,03	8	21,62	2	5,41	20	54,06
> 4000	2	5,41	8	21,62	-	-	10	27,03

* Pentru valorile mai mici de 10, proporțiile sunt orientative

TABELUL III

Formele anatomo-clinice ale despicăturilor oro-faciale în funcție de vârsta maternă

Vârsta maternă	Despicături de buză		Despicături de buză şi palat		Despicături de palat		Total	
ani	n	%	n	%	n	%	n	% *
< 20	1	2,70	1	2,70	-	-	2	5,40
21 - 34	12	32,43	18	48,65	4	10,81	34	91,89
> 35	-	-	1	2,71	-	-	1	2,71

* Pentru valorile mai mici de 10, proporțiile sunt orientative

Utilizând testul Fisher ($F_{calculat} > F_{Fisher}$), s-a constatat o pantă de regresie liniară pozitivă pentru corelația semnificativ statistic a formelor anatomo-clinice ale despicăturilor oro-faciale cu vârsta maternă şi pante de regresie liniară negative pentru corelația formelor anatomo-clinice ale despicăturilor oro-faciale cu sexul, greutatea nou-născuților vii şi cu mediul urban/rural al mamelor (tab. IV).

TABELUL IV

Corelațiile formelor anatomo-clinice ale despicăturilor oro-faciale cu variabilele independente

Variabile	r (coeficientul de corelație)	β (panta dreptei de regresie)	F (testul Fisher)
Sex	- 0.31	- 0.8090	3.5902
Greutatea la naştere	- 0.04	- 0.0009	0.0487
Mediul de proveniență	- 0.20	- 0.5363	1.4920
Vârsta maternă	0.22	0.0764	1.8615

Prin regresie multiplă s-au calculat corelațiile formelor anatomo-clinice ale despicăturilor oro-faciale cu afecțiunile asociate ale copiilor care au supraviețuit şi cu unele analize de laborator şi s-au găsit valori semnificativ statistic direct proporționale cu hipodezvoltarea musculară facială, asimetriile faciale, tahicardia, extrasistolelor, timpul de sângerare şi invers proporționale cu tulburările nervilor faciali şi trigemeni şi hipoplazia osoasă facială (tab. V).

TABELUL V

Corelațiile formelor anatomo-clinice ale despicăturilor oro-faciale cu afecțiunile asociate ale copiilor

Variabile	β (panta dreptei de regresie)	F (testul Fisher)
Hipodezvoltare musculară	0.6449	1.04 < F < 1.3999
Asimetrii faciale	0.8601	5.62 < F < 18.3784
Sistem osos	- 0.7593	3.96 < F < 6.1258
Sistem cardio-vascular	0.4717	1.04 < F < 1.6832
Sistem nervos	- 0.4020	1.04 < F < 12.6176
Timp de sângerare	0.4447	0.22 < F < 0.6401

DISCUȚII

Un studiu epidemiologic realizat în 34 de state şi 30 de țări între 2002 - 2006 a arătat o creştere în S.U.A. şi o scădere la nivel internațional a prevalenței nou-născuților vii cu despicături de buză cu/fără palat sau a altor malformațiilor congenitale (5).

În lotul studiat, scăderea incidenței copiilor cu despicături oro-faciale, în decursul anilor, este conform datelor de la nivel internațional, dar şi a scăderii numărului de copii instituționalizați în Centrele de Plasament din Iaşi. Dacă în 1987, distribuția

pe sex era aproximativ egală, în 1997, sexul feminin a avut o frecvenţă mare a despicăturilor de buză cu/fără palat, iar cel masculin a despicăturilor de palat.

Studiile epidemiologice estimează că în fiecare an se nasc în S.U.A. 2.651 copii cu despicături de palat şi 4.437 copii cu despicături de buză cu/fără palat (1). Ratele prevalenţei raportate la nou-născuţii vii cu despicături oro-faciale au fost diferite după grupurile rasiale: nativii americani, 3,74/1000; japonezi, 0,82/1000 şi 3,36/1000; chinezi, 1,45/1000 şi 4,04/1000; caucazieni, 1,43/1000 şi 1,86/1000; latinii americani, 1,04/1000; africanii americani, 0,18/1000 şi 1,67/1000. Prevalenţa despicăturilor de luetă a variat între 0,02% şi 18,8%, cea mai mare fiind la indieni şi cea mai mică la africanii americani (6).

În lotul studiat, media anuală a ratei frecvenţei nou-născuţilor vii cu despicături oro-faciale a fost similară cu nativii americani, fiind rata cea mai ridicată din grupurile rasiale a studiilor prezentate.

Despicăturile oro-faciale prezintă variate forme anatomo-clinice, astfel că în 1976, Tessier a descris 16 linii de despicături orofaciale, însă frecvenţa lor de apariţie este mult mai rară (7). În general, sunt definite trei entităţi separate: despicături de buză, despicături de palat şi despicături de buză şi palat. Despicăturile de buză apar mai frecvent de partea stângă sau bilateral. Despicăturile de palat sunt mai frecvente la sexul feminin şi afectează toate rasele umane. Despicăturile de buză şi palat, în care există o comunicare dintre gură şi nas, se numesc şi insuficienţe velo-faringiane şi sunt mai frecvente la sexul masculin, de partea stângă, bilaterale, la asiatici şi la africanii americani.

În studiul nostru, la sexul feminin au fost mai frecvente despicăturile de buză şi palat sau numai de palat, iar la masculin despicăturile de buză. De asemenea, despicăturile unilaterale au fost mai frecvente de partea stângă, aşa cum arată şi datele din literatură.

Cauzele despicăturilor oro-faciale sunt multifactoriale, incluzând factorii genetici, de mediu, chimici/medicamente. Unii autori au menţionat ca factori de risc hipoxia maternală determinată de fumat, alcoolismul şi hipertensiunea maternală.

Prezentul studiu analizează greutatea la naştere a nou-născuţilor vii şi vârsta mamelor la naştere, pentru a găsi o cauzalitate cu despicăturile oro-faciale. Procentul ridicat al cazurilor cu despicături oro-faciale cu greutate normală la naştere a demonstrat că dezvoltarea intrauterină a fătului nu a fost afectată de existenţa unei embriopatii. Numărul mic de copii cu despicături orofaciale aparţinând mamelor cu vârste extreme, nu permite concluzii definitive privind factorii de risc ai despicăturilor. Nu am avut date despre vârsta taţilor şi posibilelor con-sanguinităţii dintre părinţi.

Testul Fisher a arătat o corelaţie semnificativ statistic între despicăturile de palat şi sexul feminin, greutatea mică la naştere a nou-născuţilor şi mediul rural maternal. De asemenea, Testul Fisher a arătat o corelaţie semnificativ statistic între despicăturile de buză şi palat şi greutatea mare la naştere a nou-născuţilor, mediul urban maternal şi vârsta maternă sub 20 ani la naştere.

Complicaţiile care apar la copiii cu despicături oro-faciale sunt multiple. Dezvoltarea maxilo-facială a copiilor este aproape normală în despicăturile oro-faciale necorectate chirurgical, în cele operate apare un deficit de dezvoltare tridimensională, datorită cicatricelor postoperatorii şi a hipoplaziei osoase, cu consecinţe grave asupra funcţiilor complexului dento-maxilar (8).

CONCLUZII

Datele obținute concordă cu cele din literatură, ele demonstrează că despicăturile oro-faciale respectă frecvența populației generale. Valorile mai mari sunt probabil rezultatul structurii populației cercetate din Centrele de Plasament din Iaşi. Realizarea unor astfel de studii oferă date pe termen lung a monitorizării medico-chirurgicale a populației în studiu. Este posibilă extinderea unor studii în alte domenii de interes, pentru unele facilități corecționale.

Mulțumiri

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OVERWEIGHT AND GASTROINTESTINAL SYMPTOMS AMONG ADULTS OF WORKING AGE IN IASI CITY, ROMANIA

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OVERWEIGHT AND GASTROINTESTINAL SYMPTOMS AMONG ADULTS OF WORKING AGE IN IASI, ROMANIA (Abstract): The objectives were to determine the presence of gastrointestinal symptoms and overweight in a sample of working age adults, from Iasi city (North-East Romania) and relationship with eating habits. **Material and methods:** For this survey a sample was randomly chosen from family doctors records and 158 adults participated (65 males and 93 females, aged 19-64 years). Eating habits and food-frequency questionnaires were conducted, and *Body Mass Index* (BMI), waist and *Body Fat Percent* (FAT) were measured. For recent symptoms we used *Gastrointestinal Symptom Rating Scale* (GSRS). **Results**: 43.1 % of male and 47.3% of female were overweight and 21.5 % of male and 21.6 % of female were obese. The total body fat exceeded its normal limits in 70.8 % of men and in 32.3 % of women. Waist was high (abdominal obesity) in 49.5 % of women and in 26.2 % of men. Present gastrointestinal symptoms were: reflux (23.4%), abdominal pain (8.9%), indigestion (32.9%), diarrhea (6.3%) and constipation (8.9%). Overweight or obese subjects accused reflux, indigestion ($p<0.01$) and constipation ($p<0.05$) more frequently than those with normal weight. *Odd ratio* value and 95 % confidence interval for obese versus normal weighted subjects was 8.23 (2.15-37.58) for reflux and 11.65 (3.34-45.5) for indigestion. **Conclusions**: Our findings up-date epidemiological data and suggest the need to promote healthy eating habits in order to reduce the tendency of obesity and to improve digestive function in population. **Key words**: OVERWEIGHT, GASTROINTESTINAL SYMPTOMS, ADULT AGE.

Many studies in the literature refers to the association between obesity and liver disease, but less has been studied the relationship between nutrition, obesity and gastrointestinal disorders. The objectives of the current work were to determine the presence of gastrointestinal symptoms in a sample of overweight adults of working age, from Iasi City (North-East Region of Romania) and the relationship with eating and life habits.

MATERIAL AND METHODS

For this survey a sample was randomly chosen from family doctors records. The sample size was calculated using population survey or descriptive study random sampling formulas in *EpiInfo 3.5.1*. Final-

ly, 158 adults participated (65 males and 93 females, aged between 20-65 years). A questionnaire about life and eating habits and a food-frequency questionnaire was conducted (1). *Body Mass Index* (BMI), waist and *Body Fat Percent (*FAT) were measured. Obesity was defined by BMI over 30 kg/m^2 and overweight by BMI between 25 and 30 kg/m^2. Waist over 88 cm in women and over 102 cm in men defined abdominal obesity. FAT was measured by bioelectric impedance (OMRON BF306) and classified into low, normal, high and very high categories. Age- and sex- specific body fat cut-offs were used to define excess body fat (2, 3).

For recent symptoms we used Gastrointestinal Symptom Rating Scale (GSRS). The GSRS is a 15-item instrument combined into 5 symptom clusters depicting reflux, abdominal pain, indigestion, diarrhea, and constipation. The GSRS uses a 7-point, Likert-type scale with 1 representing absence of bothersome symptoms and 7 representing very bothersome symptoms. Respondents rated the severity of symptoms over the past week. The reliability and validity of the GSRS are well-documented for use in measuring a range of gastrointestinal symptoms and disorders including gastro-esophageal reflux disease and dyspepsia (4, 5).

For statistical analyses we have used *SPSS 17.0* and *EpiInfo 3.5.1*. Spearman`s rank correlation, cross tabulation chi square test and *odd ratio* revealed relationship between variables (6-8).

RESULTS

Prevalence

Using *BMI criteria*, 28 male (43.1%) and 44 female (47.3%) were overweight and 14 male (21.5%) and 20 female (21.6%) were obese (fig. 1).

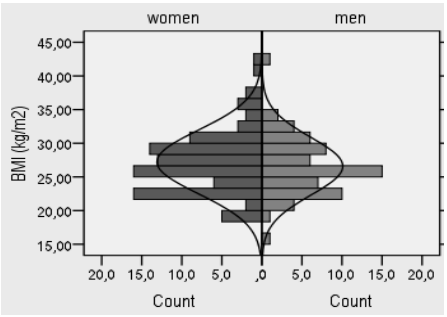

Fig. 1. BMI distribution, by gender

The total body fat was higher in female than male and exceeded its normal limits in 46 men (70.8%) and in 30 women (32.3%), with very high category in 21 men (32.3%) and 8 women (8.6 %) (fig. 2).

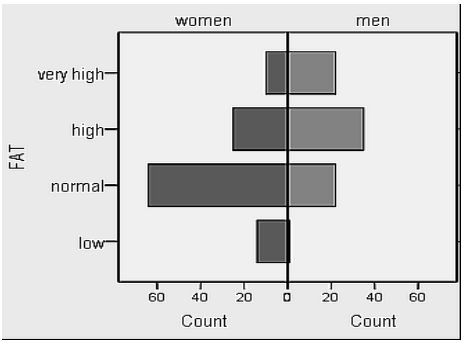

Fig. 2. Body fat categories

Abdominal obesity was present in 46 women (49.5%) and in 17 men (26.2%) (fig. 3).

Fig. 3. Waist categories

Gastrointestinal symptoms were present in the last 7 days preceding the survey: reflux (23.4%), abdominal pain (8.9%), indigestion (32.9%), diarrhea (6.3%) and constipation (8.9%), predominantly in women than men (fig. 4).

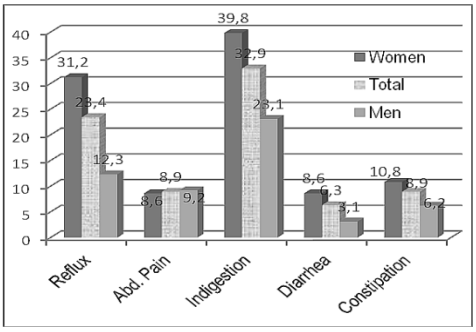

Fig. 4. Gastrointestinal symptoms (%)

Using of Spearman`s rank correlation coefficient and chi square test revealed multiple relationship between variables. All gastrointestinal symptoms were more frequent in the obese than in normal weighted subjects (2 to 5.7 times). Overweight or obese subjects accused indigestion ($p<0.001$), reflux ($p=0.001$), and constipation ($p=0.026$) more frequently than those with normal weight (table I).

Odd ratio value and 95 % confidence interval for obese versus normal weighted subjects was 11.65 (3.34-45.5) for indigestion and 8.23 (2.15-37.58) for reflux.

In our cross-sectional study, using Spearman's rank correlation test, we found statistically significant correlation between frequency of consumption of different food and gastrointestinal symptoms or nutritional status (tables II and III).

TABLE I

Gastrointestinal symptoms and nutritional status cross-tabulation (percent within BMI categories)

			BMI categories			*Total*	*p value ($\chi 2$ test)*
			normal	*over weight*	*obese*		
GASTROINTESTINAL SYMPTOMS	Reflux	-	47 92.2%	53 73.6%	20 58.8%	120 76.4%	0.001
		+	4 7.8%	19 26.4%	14 41.2%	37 23.57%	
	Abdominal pain	-	49 96.1%	66 91.7%	28 82.4%	143 91.08%	0.091
		+	2 3.9%	6 8.3%	6 17.6%	14 8.92%	
	Indigestion	-	46 90.2%	44 61.1%	15 44.1%	105 66.88%	0.000
		+	5 9.8%	28 38.9%	19 55.9%	52 33.12%	
	Diarrhea	-	48 94.1%	69 95.8%	30 88.2%	147 93.63%	0.322
		+	3 5.9%	3 4.2%	4 11.8%	10 6.37%	
	Constipation	-	48 94.1%	68 94.4%	27 79.4%	143 91.08%	0.026
		+	3 5.9%	4 5.6%	7 20.6%	14 8.92%	

TABLE II

Food frequency and gastrointestinal symptoms

Spearman correlation		*Reflux*	*Abdominal*	*Indigestion*	*Diarrhea*	*Constipa-*
Milk, yogurt	*r*	.048	-.155	-.076	-.170	-.276
	p	.549	.052	.341	.033	.000
Cheese	*r*	-.056	-.159	-.119	-.159	-.133
	p	.485	.046	.138	.046	.096
Butter, Lard	*r*	-.039	-.088	-.117	-.180	-.216
	p	.629	.272	.144	.023	.006
Fish	*r*	.017	-.203	-.006	-.037	-.250
	p	.832	.010	.938	.645	.002
Canned vegetables	*r*	.062	-.136	-.052	-.161	-.213
	p	.440	.088	.513	.043	.007
Vegetables 5% CH	*r*	-.017	-.098	-.107	-.119	-.226
	p	.835	.219	.180	.138	.004
Pulses	*r*	.189	.116	.004	-.044	-.123
	p	.018	.145	.959	.580	.125
Cereals	*r*	.020	-.146	-.095	-.140	-.160
	p	.802	.066	.236	.079	.045
Fast-food	*r*	-.031	-.200	-.172	-.186	-.219
	p	.703	.012	.031	.020	.006
Herb teas	*r*	.223	.145	.187	.081	.026
	p	.005	.070	.018	.313	.749

TABLE III

Food frequency and nutritional status

Spearman correlation		**BMI**	**WAIST**	**FAT**
Red meat (pork, beef)	*r*	-.221	-.214	.032
	p	.005	.007	.692
Smoked meat	*r*	-.183	-.253	-.025
	p	.021	.001	.754
Milk, Yogurt	*r*	-.160	-.070	-.168
	p	.045	.385	.035
Cheese	*r*	-.258	-.064	-.213
	p	.001	.422	.007
Butter, lard	*r*	-.179	-.234	-.034
	p	.024	.003	.672
Fish	*r*	-.202	-.068	-.196
	p	.011	.397	.014
Canned vegetables	*r*	-.220	-.230	-.037
	p	.006	.004	.647
Vegetables 5% CH	*r*	-.170	-.215	-.137
	p	.033	.007	.086
Pulses (bean, pea, lentils)	*r*	-.008	-.055	.031
	p	.918	.490	.696
Cereals	*r*	-.344	-.281	-.249
	p	.000	.000	.002
Fast-food	*r*	-.334	-.397	-.097
	p	.000	.000	.225
Confectionary	*r*	-.189	-.267	-.238
	p	.017	.001	.003
Herb teas	*r*	.117	.161	-.127
	p	.143	.043	.111
Alcoholic beverages	*r*	-.061	-.246	.204
	p	.444	.002	.010

We have found a significant inversely correlation between dairy products, animal fats, vegetables, cereals, fast food and constipation or diarrhea. Reflux was positively related with consumption of herb tea or pulses (table II).

All nutritional status indicators were inversely correlated with consumption of cereals or confectionary. BMI and waist were inversely correlated with meat, animal fats, vegetables and fast-food. FAT percentage was inversely related with dairy products and fish (table III).

DISCUSSION

Our results were compared with national and international studies and we found higher prevalence of overweight / obesity. WHO reported for Romania (2000): overweight 33.1% (28.6% females and 38.1% males) and obesity 8.6% (9.5% females and 7.7 % males) (9). In 2003, a study conducted in our area (Iasi county) revealed 28.7% obesity and 57.8% abdominal obesity, emphasizing with age (between 42.8 and 73%) (10).

An increased prevalence of upper and lower gastrointestinal symptoms was associated with obesity /overweight, as it emerged from previous studies published (11, 12).

The cross-sectional study cannot establish causality, only the relationship between the studied elements. A correlation can have several possible explanations:

1. Frequent consumption of a particular food may positively or negatively influence the presence of disease (for example, pulses are positively correlated with gastro-esophageal reflux, vegetable are inversely related with overweight or constipation);

2. A disease can lead to a certain life-style, sometime in compliance with dietary recommendations (frequently use of herb teas, rarely consumption of animal fat, confectionary and fast-food in obesity);

3. Both factors (disease and diet) may be dependent on a third factor - for example socio-economical status.

CONCLUSIONS

Our findings update epidemiological data and suggest the need for strategies and coordinated efforts at all levels to promote healthy eating habits to reduce obesity and high body fat and to improve digestive function in population.

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MODIFICĂRILE MORFOPATOLOGICE ALE REŢELEI TRABECULARE ÎN GLAUCOM

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MORPHOPATHOLOGICAL CHANGES IN GLAUCOMA-INDUCED TRABECULAR MESHWORK (Abstract): Glaucoma causes trabecular meshwork and Schlemm canal changes, resulting in the obstruction of the aqueous flow at this level. **Aim**: To show the histological changes in the surgically removed trabecular meshwork tissues. **Material and method**: Trabecular meshwork fragments were histologically processed for histological diagnosis. **Results**: 29.87% of all patients had ordered structure with high eosinophilia, 20.12% a rare structure with high eosinophilia, 21.34% a voluminous trabecular meshwork with fusiform cells, 21.03% tissue fibrosis and 7.62% had tissue necrosis. **Conclusions**: Aqueous flow obstruction in trabecular meshwork is produced by the histological changes at this level, found in all tissue specimens removed during surgery. **Key words**: GLAUCOMA, TRABECULAR MESHWORK, PATHOLOGY, IRIS STROMA

Glaucomul cu unghi deschis (GUD) este o formă tipică caracterizată prin următorii parametri: presiune intraoculară (PIO) mai mare de 21 mm Hg la cel puţin un ochi; un unghi deschis al camerei anterioare, de aspect normal, cu nici o modificare aparentă oculară sau sistemică, care ar putea determina o presiune intraoculară crescută; câmp vizual tipic, de tip glaucomatos şi/sau afectarea capului nervului optic.

Conjunctiva, corneea şi sclera în general au aspect normal. Uneori, în stadiu mai avansat, se constată dilatarea vaselor episclerale. Pe faţa endotelială a corneei pot fi găsite granule pigmentare iar în stadiu mai avansat, irisul prezintă zone întinse de atrofie care se manifestă printr-o rarefiere difuză a stromei anterioare şi a stratului pigmentar cu atrofia coleretului pigmentar, irisul prezintă în unele locuri orificii, iar la nivelul corpului ciliar, musculatura este înlocuită cu ţesut fibros (2,5).

Reţeaua trabeculară în glaucomul cu unghi deschis (GUD) nu are o afectare singulară. Creşte numărul de teci care înconjoară fibrele elastice, uneori umplând regiunea cribriformă. Studii cantitative au arătat întecuirea fibrelor elastice din regiunea cribriformă la 12% din ochii normali şi aproximativ 22% din ochii cu glaucom (1). O altă afectare a reţelei trabeculare în GUD este îngroşarea traveelor corneosclerale şi uveale. Unirea traveelor trabeculare se poate întâmpla în spaţiile unde membrana bazală este expusă umorii apoase, obliterând spaţiile apoase la acest nivel. O altă ipoteză poate fi acumularea de material amorf

care întăreşte reţeaua trabeculară şi nu ii permite expansiunea şi mişcarea indusă de contracţia muşchiului ciliar (2). Aceste întecuiri pot fi groase în ochi cu PIO maximală de 26 mm Hg sau pot fi normale la ochi cu presiune maximală de 36 mm Hg. Îngroşarea reţelei trabeculare acompaniază glaucomul dar nu este cauza singulară a creşterii PIO (2). Materialul care îngroaşă traveele trabeculare se găseşte şi la nivelul peretelui extern al canalului Schlemm şi în spaţiile interstiţiale dintre prelungirile anterioare ale fibrelor muşchiului ciliar (1).

Studiu histologic urmăreşte să evidenţieze modificările primare de cele secundare care pot fi cauzate de terapia medicamentoasă, modificări reactive din timpul intervenţiilor chirurgicale sau fibroză reactivă. Este posibil ca îngroşarea traveelor trabeculare să nu fie urmare a tratamentului medicamentos sau a fibrozei reactive (5).

Lucrarea urmăreşte să evidenţieze modificările morfopatologice, pe preparatele pentru biopsie trabeculare obţinute prin trabeculectomie.

MATERIAL ŞI METODĂ

Am efectuat un studiu retrospectiv pe 328 de pacienţi cu glaucom internaţi în Clinica II Oftalmologie Iaşi, în perioada 1 ianuarie 2001-31 decembrie 2010, care au fost supuşi trabeculectomiei ca urmare a unor glaucoame decompensate tensional (PIO peste 21,5 mm Hg sub triplă asociere). Biopsiile rezultate au fost examinate în serviciul de anatomie patologică din spitalul Prof. „N. Oblu" Iaşi iar rezultatele au fost interpretate în relaţie cu gradul de afectare glaucomatoasă şi cu durata în care a acţionat o PIO crescută. Prelucrarea datelor a fost realizată prin sisteme MS Excel 2007 şi *SPSS 16.0*.

REZULTATE

Lotul a fost format din 328 de pacienţi dintre care 166 (50,60%) bărbaţi, iar pe grupurile de vârstă 60-79 (28,4% feminin şi 24,6, masculin) (tab. I).

TABELUL I.

Repartizarea pacienţilor după grupe de vârstă şi sex

Grupuri de vârstă (ani)	Feminin		Masculin		Total cazuri	
	Nr.	%	Nr.	%	Nr.	%
0-9	18	6,89	27	10,37	45	17,26
10-19	1	0,38	5	1,91	6	2,29
20-29	1	0,38	1	0,38	2	0,76
30-39	9	3,44	6	2,29	15	5,73
40-49	5	1,91	12	4,59	17	6,50
50-59	21	8,04	28	10,72	49	18,76
60-69	42	16,09	45	17,24	87	33,33
70-79	51	19,54	36	13,79	87	33,33
80-89	12	4,59	6	2,29	18	6,88
90-100	2	0,76	0	0	2	0,76
Total	162	-	166	-	328	100,00

Pacienţii au fost examinaţi clinic, oftalmologic: acuitatea vizuală; gonioscopia; ex. FO şi a CV.

Pacienţii care prezentau PIO peste 21,5 mm Hg sub triplă asociere sau la care aceasta era normală (sub 21,5 mm Hg) dar cu modificări ale nervului optic (creşterea excavaţiei papilare) sau cu o evoluţie nefavorabilă a câmpului vizual, au fost supuşi trabeculectomiei.

Fragmentele de trabecul excizate au fost fixate în formol 15%, incluse în parafină, realizându-se secţiuni longitudinale de 50 şi grosimea de 4 microni. Secţiunile au fost colorate cu hematoxilin-eozină şi examinare de microscopul optic. (fig. 1, 2, 3, 4, 5).

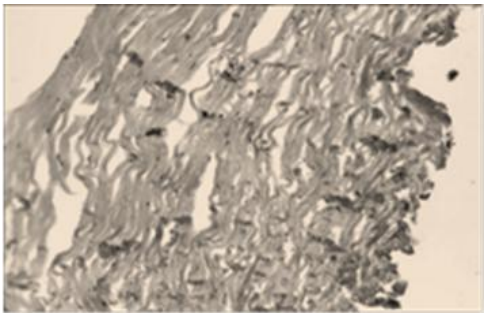

Fig. 1. Fragment de sclerotică.
Se observă structura ordonată a straturilor de ţesut conjunctiv dens.

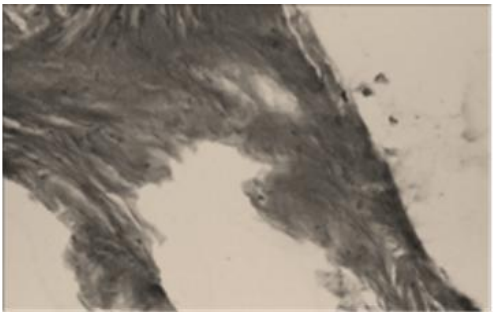

Fig. 2. Fragment mic trabecular slab celularizat.
Se observă o densitate rară a celulelor.

Fig. 3. Se observă numeroase celulele fuziforme, cu citoplasmă eozinofilă şi nuclei alungiţi situaţi în centrul celulei

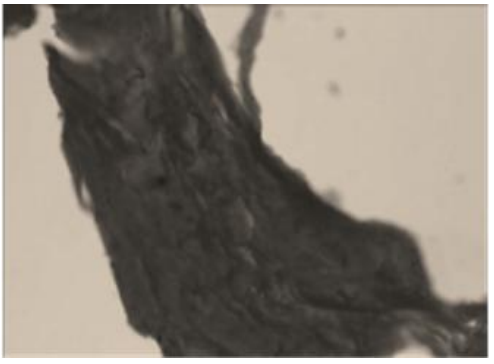

Fig. 4. Aspect de sclerozare a ţesutului conjunctiv prin bogăţia de colagen.

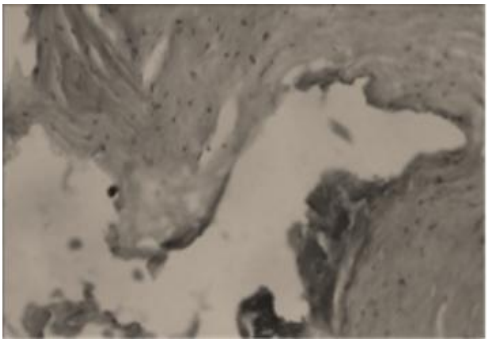

Fig. 5. Lamele trabeculare si spatii intertrabeculare cu modificări minime.
Se evidenţiază o zona necrozată întinsă pe una din margini.

TABELUL II.

Modificările histopatologice evidenţiate pe fragmentele de trabecul excizate chirurgical

Modificări histopatologice	Nr.	%
Structură ordonată a trabeculului cu eozinofilie	98	29,87
Structură celulară rară cu eozinofilie	66	20,12
Trabecul voluminos cu structură celulară densă, celule fuziforme	70	21,34
Fibroză tisulară	69	21,03
Necroză tisulară	25	7,62

Modificările histologice determinate în apariţia glaucomului au fost evidenţiate la nivelul unghiului camerular (tab. II). Constatările histomorfologice ale unghiului formează baza teoriilor referitoare la etiopatogenia bolii. Aplazia sau hipoplazia canalului Schlemm, se observă destul de rar şi apare ca fenomen secundar. În unghiul camerular se descrie persistenţa unei membrane mezodermale care acoperă sistemul trabecular.

Cercetările noastre au evidenţiat abundenţa colagenului cu sclerozarea trabeculului, creşterea importantă a celularităţii urmată de îngroşarea lamelor şi pensarea spaţiilor, aspecte de necroză pe întinderi variate, pe una din feţele trabeculului.

DISCUŢII

Preparatele rezultate prin trabeculectomie de la pacienţi care nu beneficiază de tratament înaintea intervenţiei chirurgicale au fost similare: îngroşarea traveelor şi a lamelelor cu prezenţa abundentă a colagenului(3). Alt studiu (2) a arătat că ochii cu GUD enucleaţi la autopsie sunt cu un grad de afectare glaucomatoasă mai redus decât cei care sunt supuşi intervenţiei chirurgicale. Comparaţia ochilor trataţi cu diverse medicaţii topice (beta blocanţi, pilocarpină sau analogi prostaglandinici) nu a arătat diferenţe histologice(5). Dacă există o supresie cronică a fluxului apos care determină modificări secundare ale reţelei trabeculare (compactarea debriurilor şi a materialului extracelular la acest nivel), acestea devin aparente în regiunea cribriformă şi compactarea lamelelor în regiunea corneoscleralã.

Rohen et al. (3) a observat că celulele trabeculare sunt în număr scăzut la ochii cu GUD dar fără a fi posibilă menţionarea stării anterioare în ce priveşte densitatea celulelor trabeculare la naştere. Gottanka et al. (2) consideră că un proces patologic determină creşterea pierderii celulelor trabeculare în glaucom, descoperirea şi stoparea sa ar favoriza eficienţa tratamentului. În contrast, dacă ochii cu GUD au mai puţine celule trabeculare, pierderea legată de vârstă poate cauza glaucomul printr-o simplă lipsă a celulelor trabeculare pentru a menţine un flux apos fiziologic.

Lutgen-Drecoll et al. (1) menţionează rezultatele unui studiu realizat pe ochii enucleaţi la necropsie şi pe preparatele obţinute prin trabeculectomie, când a evidenţiat modificări celulare şi artefacte ca urmare a manevrelor impuse de examinarea histologică.

În studiile noastre anatomopatologice am observat prezenţa de colagen abundent cu sclerozarea trabeculului (70 preparate), creşterea importantă a celularităţii şi îngroşarea lamelor cu pensarea spaţiilor intertrabeculare (98 preparate), aspecte de ne-

croză pe arii mai mici sau extinse pe una din feţele trabeculului (25 preparate).

CONCLUZII

Rezultatele studiului nostru sunt în concordanţă cu cele ale altor autori, evidenţiază blocarea dinamicii umorii apoase la nivel trabecular, datorită modificărilor histologice care determină creşterea PIO cu modificări consecutive la nivelul papilei nervului optic, urmate de scăderea parametrilor funcţionali. Stoparea sau anularea acestor modificări histologice ar putea fi realizată printr-o terapie farmacologică care să determine ameliorarea evoluţiei fără intervenţie chirurgicală.

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NOUTĂȚI

SIRT3: ŢINTIND DIRECT PROCESUL DE ÎMBĂTRÂNIRE

O enzimă, vsirtuina sau Sirt3, reprezentând o diacetilază mitocondrială, ar putea revoluţiona, prin combaterea extrem de activă a oxidărilor celulare, întârzierea îmbătrânirii. Studii efectuate pe animale de laborator au arătat faptul că restricţionarea aportului caloric are ca efect direct o creştere substanţială a concentraţiei Sirt3 în organism. Sirt3 elaborată în exces va interveni prin reducerea nenumăratelor specii reactive de oxigen (produse în mitocondrii în cadrul reacţiilor declanşate de stresul oxidativ), care stau la baza îmbătrânirii. La rozătoare,scăderea aportului caloric determină scăderea potenţialului de membrană la nivel mitocondrial, cu prăbuşirea ratei de producere a radicalior liberi şi neutralizarea acţiunii lor de către Sirt3. Stoparea de către Sirt3 a proceselor complexe de degradare celulară ar putea duce la o încetinire considerabilă a îmbătrânirii. (Liu Y.,Zhang D.,Chen D.SIRT3: Stricking at the heart of aging. *Aging* 2011;3(1):1-3).

Carmen Zamfir

STUDIU PRIVIND STAREA DE SUPRAFAȚĂ A RĂŞINILOR ACRILICE ÎN RAPORT CU METODELE DE PRELUCRARE MECANICĂ

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SURFACE CHARACTERISTICS OF THE ACRYLIC RESINS ACCORDING TO THE POLISHING METODS (Abstract): **Aim:** The objective of this study was to evaluate the effect of the polishing technique and glazing on the porosity of the dental resins. **Material and methods:** The studied resins were: Castapress/Vertex, Prothyl Hot/Zermack, Rapid Simplified/Vertex, Duracryl Plus/Spofa Dental, Vertex-Soft/Vertex, Superacryl Plus/Spofa Dental. Thirty specimens, five for every resin, of 50/25/2 cm in size were done. One surface of each sample was polished with extrahard tungsten carbide burs, the other surface being polished with extrahard extrafine and diamond burs. The final polishing was done using a conventional method: pumice, water and lathe bristle brush for 90 seconds, 1500 rpm and soft leather polishing wheel for 90 seconds, 3000 rpm. Twenty surfaces were glazed after polishing with Glaze™/Bosworth. Vertex Soft specimens were not polished because this is a resilient material. Surface porosity of the acrylic resin specimens was measured by optical microscopy. **Results:** The lowest porosity was obtained by conventional polishing combined with glazing techniques. No differences between glazed and non/glazed self-curing resin specimens were noticed, but there were differences between self-curing and heat-curing resins. **Conclusions:** Conventional lathe polishing method is an effective and reliable technique for polishing dental resins. Specimens of self-curing resin had a higher porosity than heat curing resin following the same surface treatment. Higher surface smoothness was obtained by conventional lathe polishing completed by glazing. **Key words**: ACRYLIC RESINS, POROSITY, POLISHING, GLAZING

Protezele dentare trebuie să prezinte suprafețe externe netede, perfect lustruite pentru a preveni colonizarea microorganismelor, acumularea plăcii bacteriene, modificările cromatice şi structurale, menținând astfel confortul şi sănătatea țesuturilor orale (1).

Porozitatea răşinilor este un fenomen complex cu origine multifactorială (2). Deşi numeroase studii au avut ca obiectiv evaluarea calitativă şi/sau cantitativă a porozității răşinilor acrilice, încă nu a fost impusă o standardizare unanim acceptată a dimensiunilor porilor. Conform Asociației Dentare Americane (ADA) "nu trebuie să existe bule sau goluri când sunt vizualizate fără magnificație", subliniind faptul că evidențierea clinică este dificil de realizat. Literatura de specialitate incriminează porozitățile

rășinilor acrilice ca factor favorizant al stomatopatiilor paraprotetice şi al fracturilor bazelor protezelor, obiectivată de prezența acestora pe linia de fractură şi la nivelul interfeței dinte artificial/baza protezei. Referitor la porozitatea rășinilor acrilice, rezultatele studiilor experimentale au fost extrem de variabile, dimensiunile încadrându-se între 10 şi 300 μm (3) fiind raportate însă şi valori mult mai mari - 500μm (4). Clasificarea acceptată şi utilizată în prezent îi diferențiază în pori mici (circa 10 μm diametru), pori medii (10-30 μm diametru) şi pori mari (peste 30 μm diametru).

Scopul studiului a constat în analiza prin microscopie optică a morfologiei suprafețelor (prezența impresiunilor mecanice rezultate în urma şlefuirii), a porozităților şi a incluziunilor, urmărind dimensiunea şi repartiția acestora, precum şi starea de suprafață după procesul de glazurare, a şase rășini acrilice cu regim de polimerizare diferit (autobaropolimerizare şi termopolimerizare) care au fost supuse la prelucrări mecanice diferite.

MATERIAL ŞI METODĂ

Rășinile studiate au fost alese dintre cele mai frecvent utilizate în protetica dentară: Castapress/Vertex, Prothyl Hot/Zermack, Rapid Simplified/Vertex, Duracryl Plus/ Spofa Dental, Vertex - Soft/Vertex, Superacryl Plus/Spofa Dental. S-au realizat treizeci de probe, împărțite în şase grupe (A-F) formate din cinci plăcuțe pentru fiecare tip de rășină.

Probele au fost realizate sub formă de plăcuțe paralelipipedice cu lungimea de 50 mm, lățimea 25 mm şi grosimea 2 mm. Machetele plăcuțelor au fost transformate în probe finale respectând algoritmul tehnologic specific fiecărei clase de rășină acrilică: termopolimerizare (B, C, D, F) şi autobaropolimerizare (A, E).

Prelucrarea şi lustruirea probelor s-a făcut în mod diferit pe fiecare suprafață a plăcuțelor, viteza şi timpul de prelucrare fiind identice (30 secunde, 1500 rpm). Suprafețele au fost notate cu literele A-F, respectiv A'-F'.

Prelucrarea mecanică s-a făcut cu o freză extradură din carbură de tungsten cu granulație fină (galbenă) pentru suprafețele: A, B, C, D, E, F iar pentru suprafețele A', B', C', D', E', prelucrarea s-a făcut atât cu o freză extradură cu granulație extrafină de 53-63 μ de formă conică cât şi cu o freză diamantată cu granulație fină (roşie) de formă cilindrică, cu vârful rotunjit.

Lustruirea finală a avut ca obiectiv obținerea unui "luciu de oglindă" şi s-a realizat cu praf abraziv pentru lustruit (Polishing powder/Astar Fedent) şi pastă abrazivă (High lustre polishing paste) acționate de perii şi filțuri.

Proba F', fiind dintr-un material rezilient, care se utilizează doar pe suprafața mucozală a protezei, a beneficiat de o prelucrare uşoară cu praf Vertex™ Soft. Lustruirea a fost făcută cu praf abraziv (Astar-Fedent) şi pastă abrazivă, încărcate pe un con din pâslă (filț) şi puf de bumbac (tab. I).

Glazurarea a fost realizată în cazul probelor: C, C', D' şi E, cu agentul de glazurare Bell Coat/K B Mutsumi (glazură fotopolimerizabilă pentru rășini acrilice şi materiale compozite). Acesta a fost aplicat prin pensulare în strat subțire, după ce suprafața a fost în prealabil uscată foarte bine, şi apoi fotopolimerizat timp de patru minute

Examinarea probelor s-a realizat prin microscopie optică în scopul analizării morfologiei suprafețelor, porozităților şi incluziunilor, urmărind dimensiunea şi repartiția acestora, precum şi starea de suprafață după procesul de glazurare. Am utilizat microscopul optic cu lumină fluorescentă şi polarizată Leica SM 2500 M, cu magnificație

x20 şi x50. Stocarea şi prelucrarea datelor a fost realizată cu ajutorul programului Apllication Leica. Inițial s-a realizat o inspecție de ansamblu a întregii suprafețe a probelor pentru aprecierea omogenității stării de suprafață. Deoarece starea de suprafață la magnificație x20 a fost omogenă pe toată aria, analiza detaliilor a fost realizată la o magnificație x50, pe trei câmpuri alese aleator.

TABELUL I
Regimul de polimerizare pentru fiecare probă

Proba	Denumirea comercială	Regimul de polimerizare
A, A’	Castapress/Vertex	30 minute la 55º C şi 2.5 bar
B, B’ C (glazurat) C’(glazurat)	Prothyl hot/Zermack	5 minute de la 80° la 100°C + 20 minute la 100°C
D D’(glazurat)	Rapid Simplified/Vertex	20 minute la 100°C
E (glazurat) E’	Duracryl Plus/Spofa Dental	30 - 40°C / 0,2 - 0,3 MPa.
F’	Vertex- Soft/ Vertex	3 ore la 70º C, urmat de 30 de minute la 100º C
F	Superacryl Plus/Spofa Dental	Temperatura creşte în 30 minute până la 70ºC, 30 minute se menține la 70ºC, urcă în 30 minute până la 100ºC, 30 minute ramâne la 100ºC.

REZULTATE ŞI DISCUȚII

În cazul răşinii Castapress/Vertex, suprafețele A au prezentat numeroşi pori de dimensiuni de până la 2 µm, repartizați uniform, şi incluziuni de dimensiuni mari (până la 24,5 µm) cu repartiție neuniformă în câmpurile analizate (fig.1). S-a constatat faptul că incluziunile de dimensiuni mari au o formă neregulată spre deosebire de porozitățile caracterizate de forma sferică. Suprafețele A’ s-au caracterizat, în general, prin absența incluziunilor de dimensiuni mari şi o prezență redusă a porilor, cu păstrarea liniilor de prelucrare mecanică.

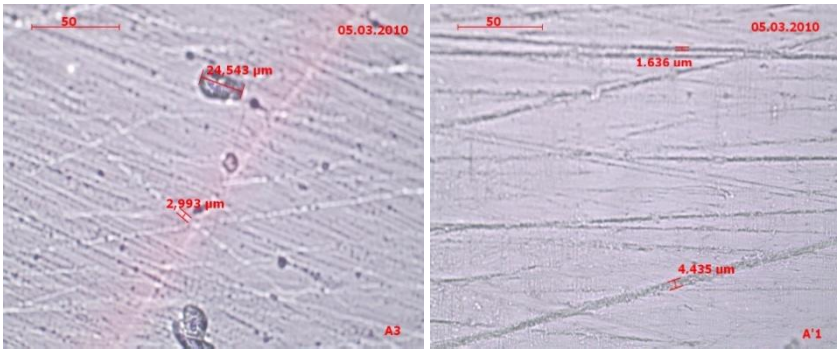

Fig. 1. Aspecte microscopice ale suprafețelor răşinii Castapress/Vertex X50

În cazul răşinii Prothyl Hot/Zermack, suprafețele B şi B’ rezultate consecutiv celor două tipuri de prelucrare, s-au caracterizat printr-o prezență redusă a porilor pe suprafață. În cazul suprafețelor B s-au evidențiat mai multe impresiuni mecanice (fig. 2).

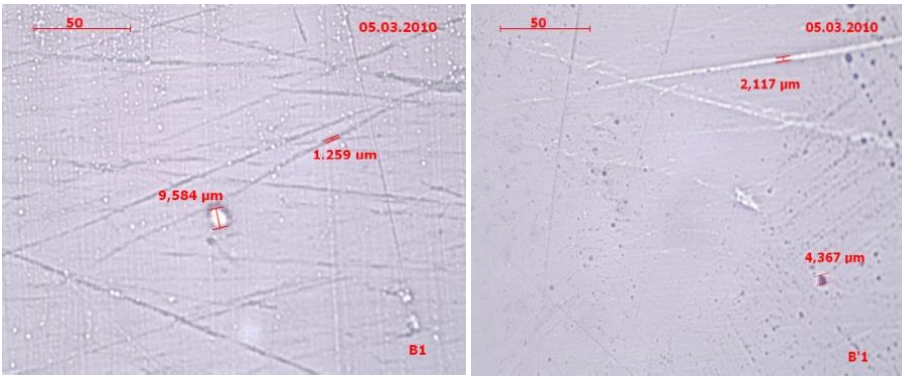

Fig. 2. Aspecte microscopice ale suprafețelor rășinii Prothyl Hot/Zermack X50

În cazul probelor din rășina Prothyl Hot/Zermack, la care ambele suprafețe au fost glazurate cu Bell Coat, s-a observat că după prelucrarea cu freza de carbură de tungsten pelicula de glazură acoperă impresiunile mecanice produse de instrumentarul abraziv, prezentând în acelaşi timp ea însăşi pori de dimensiuni foarte mici, de aproximativ 2 µm (fig. 3). Suprafața C', prelucrată cu freze extrafine şi freze diamantate a prezentat doar porii de mici dimensiuni (circa 3 µm) ai peliculei de glazură.

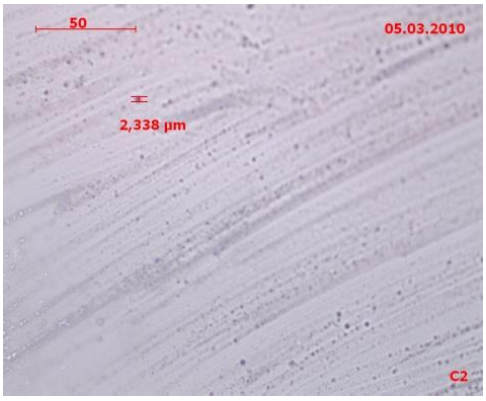

Fig. 3. Aspecte microscopice ale suprafețelor C ale rășinii Prothyl Hot/ Zermack (glazurată) X50

Aceleaşi aspecte s-au regăsit şi în cazul probelor realizate din rășina Rapid Simplified/ Vertex (fig.4).

În cazul rășinii Duracryl/Spofa Dental analiza microscopică a suprafețelor glazurate cu Bell Coat a evidențiat faptul că incluziunile de mari dimensiuni nu au putut fi acoperite de agentul de glazurare. In cazul imaginilor suprafețelor E' (neglazurate) s-a constatat prezența porilor cu dimensiuni de aproximativ 6,5 µm şi prezența impresiunilor de şlefuire (fig.5).

Imaginile captate în cazul rășinii Superacryl Plus/Spofa Dental au evidențiat suprafețe regulate, cu puțini pori, de mici dimensiuni şi absența impresiunilor consecutive prelucrării mecanice. Suprafețele F' căptuşite cu materialul rezilient Vertex Soft, au prezentat o textură spongioasă din cauza încorporării de incluziuni de aer în material (fig.6).

În studiul nostru, repartiția incluziunilor nu a fost uniformă la toate tipurile de rășini luate în studiu, rășinile autobaropolimerizabile prezentând astfel de incluziuni şi o porozitate mai mare, rezultatele fiind concordante cu cele ale altor studii (5). Incluziunile pot fi de diferite forme şi dimensiuni, repartizate în toată grosimea acrilatului, având drept cauze: repartiția defectuoasă a monomerului, variația prea mare a masei moleculare a polimerului sau lipsa omogenizării pastei de acrilat (6).

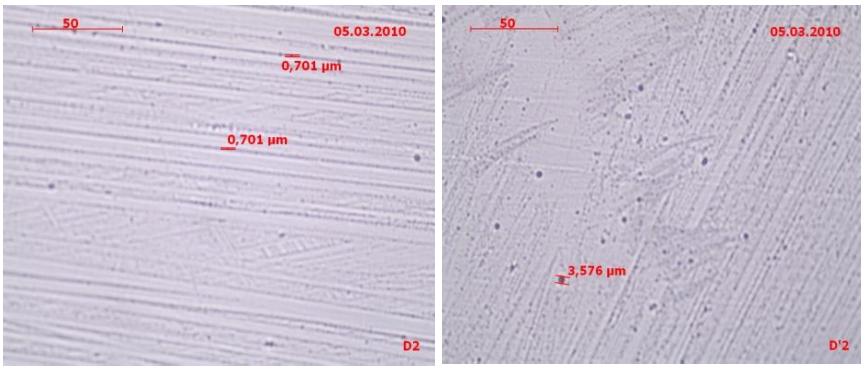

Fig. 4. Aspecte microscopice ale suprafețelor rășinii
Rapid Simplified/ Vertex X 50 (D- neglazurată, D - glazurată)

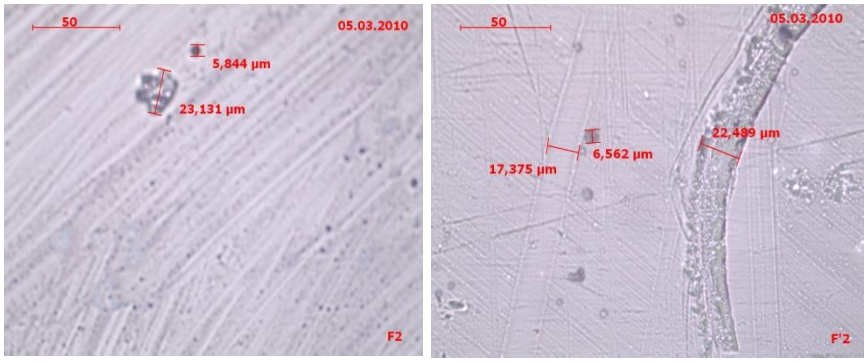

Fig. 5. Aspecte microscopice ale suprafețelor rășinii
Duracryl Plus/Spofa Dental- E glazurată, E' neglazurată X50

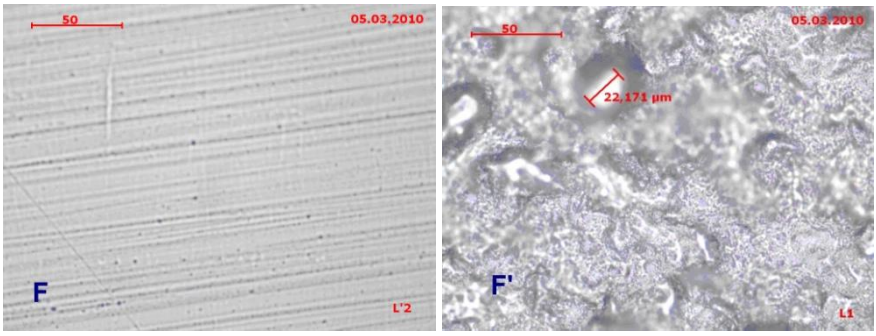

Fig. 6. Aspecte microscopice ale suprafețelor rășinii Superacryl Plus (F) și a rășinii Vertex Soft (F') x50

Indiferent de porozitatea materialului, s-a observat că prelucrarea și lustruirea până la "luciu de oglindă" determină o reducere a dimensiunii și numărului porilor de suprafață, valorile obținute fiind de până la 10 µm diametru. La examinarea prin microscopie optică s-a observat că prin prelucrarea cu ambele tipuri de freză - extradură și diaman-

tată -, porozitatea a fost mai mică decât în cazul prelucrării doar cu freza extradură. In acelaşi timp, prelucrarea numai cu freza extradură induce existența unor impresiuni mecanice mult mai evidente. Analizând aceste două aspecte calitative ale stării de suprafață, apreciem că prelucrarea cu freză extradură şi freză diamantată induce o suprafață cu calități mult mai bune comparativ cu prelucrarea doar cu freză extradură.

Referitor la glazurare, pe baza imaginilor obținute prin microscopie optică putem afirma că starea de suprafață după procesul de lăcuire prezintă calități superioare suprafețelor nelăcuite sub aspectul porozității şi impresiunilor mecanice postprelucrare.

CONCLUZII

S-a observat că indiferent de porozitatea materialului, prelucrarea şi lustruirea determină o reducere a dimensiunii şi numărului porilor de suprafață.

Prelucrarea realizată atât cu freza extradură cât şi cu freza diamantată, conduce la reducerea numărului de impresiuni mecanice.

În studiul nostru, repartiția incluziunilor a fost diferită pentru rășinile luate în studiu, cele autobaropolimerizabile prezentând incluziuni şi porozități mai numeroase. Prezența incluziunilor poate influența negativ rezultatul prelucrării mecanice a suprafeței, ele apărând evidente în timpul contactului dintre instrumentarul rotativ şi rășină, determinând creşterea rugozității materialului.

Utilizarea procedurii de glazurare permite optimizarea parametrilor de suprafață.

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CONTRIBUŢII LA ALEGEREA FAZEI MOBILE ÎN VEDEREA SEPARĂRII PRIN HPLC ŞI IDENTIFICAREA – DETERMINAREA CANTITATIVĂ A UNOR ANTIINFLAMATOARE NESTEROIDIENE FOLOSIND DETECTORUL UV-VIS

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CONTRIBUTION TO THE MOBILE PHASE SELECTION FOR HPLC SEPARATION IN ORDE TO MAKE THE IDENTIFICATION AND QUANTITATIVE DETERMNATION USING THE UV-VIS DETECTOR (Abstract): The aim of this study is to elaborate a HPLC with UV detection method for the analysis of some AINS, method which can be transferred in a system with mass spectrometry detection. **Material and methods**: More AINS drugs (diclofenac sodium, ibuprophen, ketoprofen, meloxicame, piroxicame, tenoxicame, nimesulide, phenylbutazone and indomethacin) were studied by UV spectrophotometry and by HPLC. **Results**: The UV spectra of the studied AINS were recorded in pure solvents or in a mixture of solvents in order to establish the absorbance that will be used for HPLC detection in UV. To establish the optimum chromatographic parameters, the influence of the flow and of the composition of the mobile phase (both of the aqueous and organic phases) were studied too. **Conclusions**: The proposed procedure is easy and relatively with low cost regarding the MS detection. **Key words**: AINS, HPLC, SPECTROPHOTOMETRY.

Pentru medicamentele antiinflamatoare nesteroidiene (AINS), care sunt de importanţă majoră în terapeutică, în literatura de specialitate studiată nu există o singură metodă analitică pentru separarea şi determinarea cantitativă a fiecărui component din amestec, ci numai metode pentru fiecare compus în parte sau pentru un număr restrâns dintre AINS (1-7). O asemenea metodă poate fi aplicată pentru analiza AINS din probe biologice, determinând componentul administrat dintr-o gamă mai largă de AINS. Ne-am propus realizarea unei metode de separare prin HPLC a unui amestec de AINS cu detecţie în UV în scopul transferării acestei metode pentru detecţie cu spectrometrie de masă (MS).

MATERIAL ŞI METODĂ

Reactivi utilizaţi: diclofenac sodic (etalon secundar Mark Int'l S.R.L.), ibuprofen, ketoprofen (etaloane secundare Terapia S.A.), piroxicam, meloxicam şi tenoxicam (etalone secundare Labormed Pharma S.R.L.), apă Chromasolv V®Plus pentru HPLC (Sigma – Aldrich), metanol şi acetonitril gradient grade pentru HPLC (Merck), acid acetic p.a., acetat de sodiu p.a.

Aparatură: spectrofotometru UV-VIS Agilent Technologies, cromatograf de lichide 1100 Agilent Technologies, coloană cromatografică StableBond SB C 18 (4,6 x 150 mm, 5 mm), balanţă analitică Kern, pH-metru Hanna Instruments.

Metode: s-au înregistrat spectrele de absorbţie pe domeniul 200 – 400 nm pentru

TABELUL I
Date privind solubilitatea unor antiinflamatoare nesteroidiene

	Diclofenac Na	Ibuprofen	Ketoprofen	Piroxicam	Meloxicam	Tenoxicam
Apa	> 9	0,1	10,0	1,0	0,012	0.045
Glicerol	-	-	-	-	0,138	-
Metanol	> 24	60-70	50,0	10,0	0,382	< 1
Etanol	-	60-70	50,0	5,0	0,354	< 1
Etilen-glicol	-	2-2,5	-	-	0,094	-
PEG	-	< 0,1	-	-	3,763	-
Acetona	6	60-65	50,0	20	-	2
DMF	-	55-60	50,0	-	na	-
Cloroform	-	65-70	50,0	250	-	8
Ciclohexan	< 1	-	-	-	-	-
Acetat de etil	-	-	50,0	12,5	-	-
DMSO	547,0	4121,0	542,0	58,0	na	63

AINS luate în studiu folosind diverşi solvenţi sau amestecuri ale acestora pe un spectrofotometru tip Hewlett Packard 8453 în cuvă de 1 cm faţă de mediul de dizolvare. S-au înregistrat cromatogramele amestecului unor AINS folosind un HPLC tip 1110 Agilent Technologies, pe o coloană Stable-Bond SB C 18 (4,6 x 150 mm, 5 mm) cu diverse faze mobile, la debite diferite şi cu detecţie în UV.

REZULTATE ŞI DISCUŢII

Analizând datele prezentate în tabelul I se constată faptul că antiinflamatoarele nesteroidiene luate în studiu (diclofenac sodic, ibuprofen, ketoprofen, piroxicam, meloxicam şi tenoxicam) prezintă o solubilitate mai mare în solvenţi polari de tipul apă, metanol, etanol. Acest lucru prezintă importanţă deoarece aceşti solvenţi sunt folosiţi de obicei în compoziţia fazelor mobile utilizate cel mai des în HPLC.

Având în vedere aceste date am înregistrat spectrul de absorbţie în UV-VIS pentru compuşii studiaţi folosind ca solvent pentru dizolvarea acestora apă, metanol, acetonitril şi soluţie tampon acetat pH = 5). Analizând spectrele obţinute (fig. 1) se constată mai multe aspecte. În primul rând, pentru toţi compuşii studiaţi se obţin mai multe maxime de absorbţie, unul în jurul valorii de 200 nm, iar altele la valori mai mari ale lungimii de undă. Având în vedere domeniul de lungimi de undă 200 – 210 nm unde prezintă absorbţie maximă compuşii analizaţi dar şi alte substanţe, am luat în studiu maximele de absorbţie ce se obţin la valori mai mari ale lungimii de undă.

În al doilea rând, maximele de absorbţie alese (tab. II) se găsesc la aceleaşi valori ale lungimii de undă (sau foarte apropiate) în cazul folosirii ca solvent a apei sau a soluţiei tampon pH = 5, în timp ce în cazul folosirii metanolului sau acetonitrilului ca solvent maximele rămân la aceleaşi valori ale lungimii de undă (pentru ibuprofen) sau se deplasează spre valori mai mari (pentru diclofenac sodic) sau mai mici (pentru ketoprofen, piroxicam, meloxicam şi tenoxicam). Pe de altă parte, valorile absorbanţelor la aceste lungimi de undă prezintă şi o serie de modificări (crescătoare sau descrescătoare, în funcţie de compusul studiat şi de solventul în care a fost dizolvat acesta). De exemplu, diclofenacul şi ibuprofenul prezintă absorbanţe mai mari în metanol,

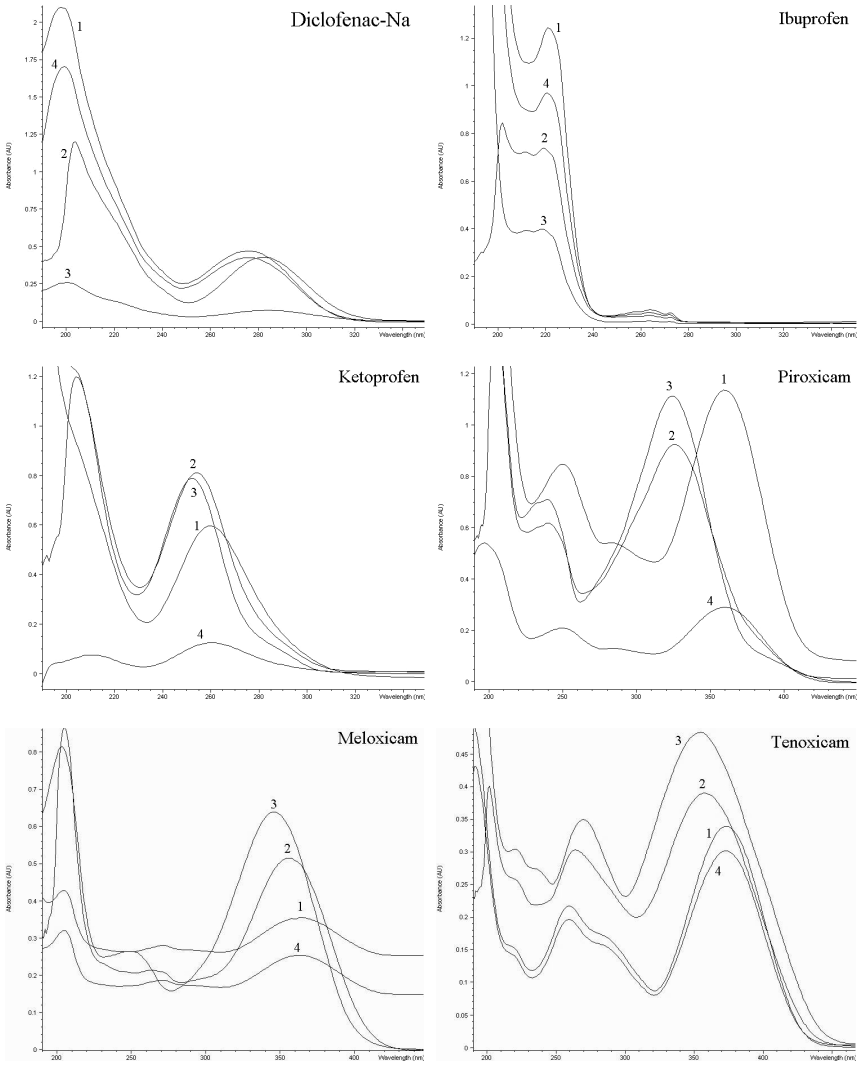

Fig. 1. Spectrele de absorbţie pentru diclofenac sodic, ibuprofen, ketoprofen, piroxicam, meloxicam şi tenoxicam în 1 - apă, 2 - metanol, 3 - acetonitril şi 4 - tampon acetat pH = 5

cei trei oxicami în acetonitril, iar ketoprofenul are absorbanţe asemănătoare în metanol şi respectiv acetonitril. În orice caz, folosind datele obţinute se pot trage o serie de concluzii referitoare la lungimile de undă de detecţie ce vor fi utilizate în cazul separării prin HPLC. Lungimile de undă optime vor fi stabilite abia după ce se va optimiza compoziţia fazei mobile, ştiut fiind faptul că spectrul de absorbţie poate suferi modificări în ceea ce priveşte lungimea de undă ce corespunde maximului de absorbţie funcţie de solvent (în acest caz de compoziţia fazei mobile).

Având în vedere faptul că scopul final urmărit constă în elaborarea unei metode de separare a compuşilor luaţi în studiu prin HPLC este foarte posibil ca faza mobilă să fie constituită din amestecul mai multor solvenţi.

TABELUL II

Maxime de absorbţie pentru diclofenac sodic, ibuprofen, ketoprofen, piroxicam, meloxicam şi tenoxicam în apă, metanol, acetonitril şi tampon acetat pH = 5

Compus studiat	Solvent			
	Apă	Metanol	Acetonitril	Tampon pH 5
Diclofenac Na	276	282	284	276
Ibuprofen	221	219	219	221
Ketoprofen	260	254	252	260
Piroxicam	359	326	324	331
Meloxicam	364	357	346	364
Tenoxicam	374	358	355	373

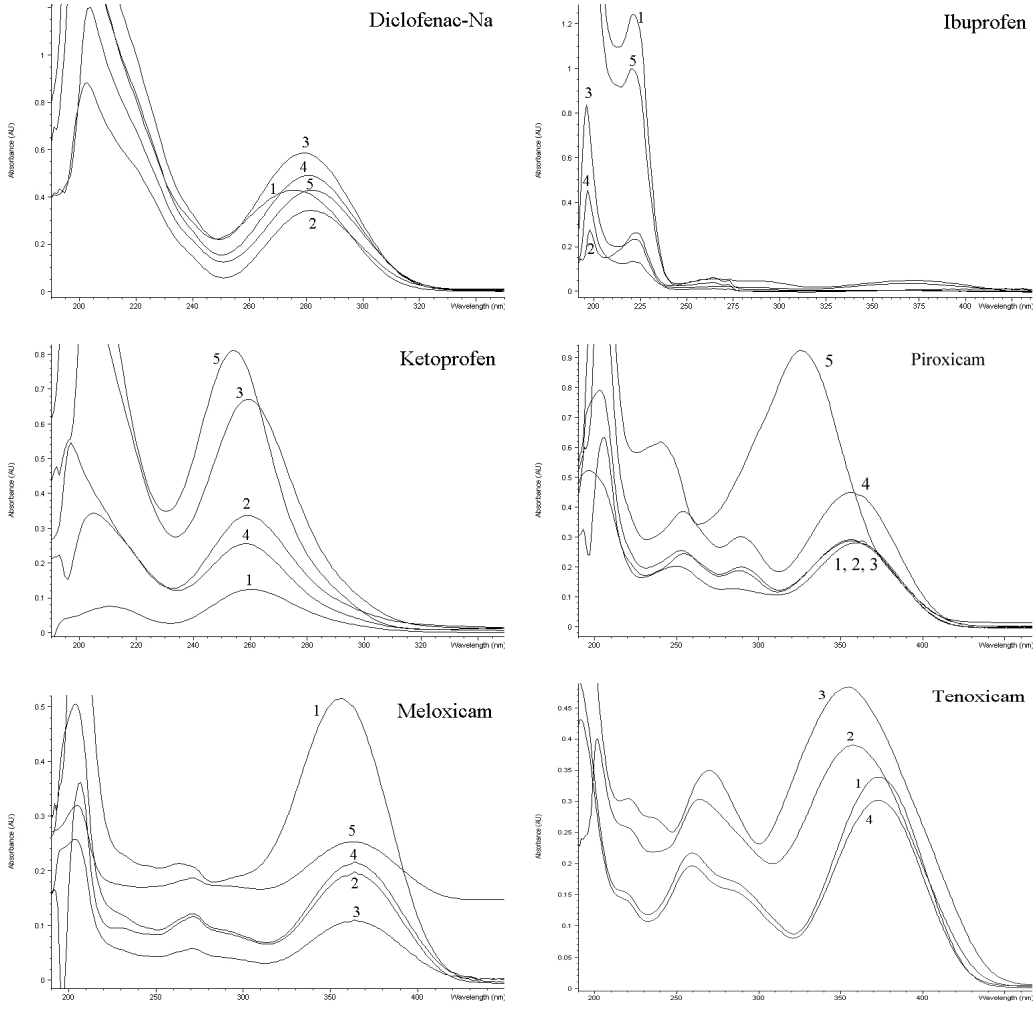

Fig. 2. Spectrele de absorbţie pentru diclofenac sodic, ibuprofen, ketoprofen, piroxicam, meloxicam şi tenoxicam în tampon pH = 5 / metanol (1 - 100/0 ; 2 - 70/30 ; 3 - 50/50 ; 4 - 30/70 şi respectiv 5 - 0/100)

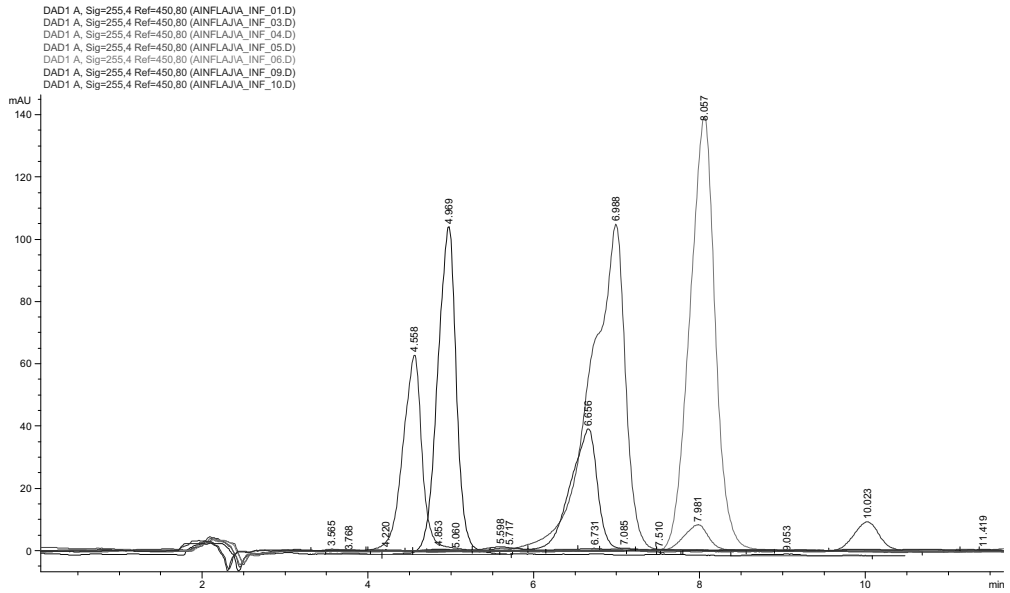

Fig. 3. Cromatogramele obţinute pentru diclofenac, piroxicam, ketoprofen, nimesulid, fenilbutazonă indometacin şi ibuprofen ; fază mobilă formată din acetonitril / metanol / tampon pH 3,6 în raportul 35 / 25 / 40

Având în vederea aceasta, dar şi faptul că aceşti compuşi prezintă o comportare asemănătoare faţă de metanol sau acetonitril, au fost înregistrate spectrele de absorbţie în soluţii metanolice de diferite concentraţii. Astfel, în figura 2 sunt redate spectrele de absorbţie obţinute în cazul în care ca solvent de dizolvare au fost folosite amestecuri tampon pH = 5 / metanol în proporţiile de 100 / 0, 70 / 30, 50 / 50, 30 / 70 şi respectiv 0 / 100.

Analizând spectrele din figura 2 se concluzionează faptul că aceşti compuşi prezintă modifiări semnificative ale absorbanţei în funcţie de raportul în care se află solvenţii (tampon pH = 5 / metanol) şi anume, valori mai mari ale absorbanţei pentru ketoprofen şi piroxicam la dizolvare în metanol 100%, mai mici pentru diclofenac (50%) şi tenoxicam (30%) şi respectiv pentru ibuprofen (0%). Ca şi o consecinţă, este de preferat să se folosească o valoare intermediară pentru raportul dintre faza apoasă şi cea organică astfel încât toţi compuşii să prezinte valori ale absorbanţelor suficient de mari pentru a putea fi decelate spectrofotometric după separarea prin HPLC. În acelaşi sens, având în vedere rezultatele experimentale obţinute, este de preferat să se folosească un amestec de solvenţi organici (metanol şi acetonitril).

Compuşii menţionaţi au fost studiaţi şi la dizolvare în soluţie tampon acetat 0,1M (pH = 3,6) observându-se o uşoară creştere a absorbanţei faţă de soluţii tampon acetat pH = 5, motiv pentru care în determinările ulterioare s-a folosit sistemul tampon acetat cu pH = 3,6.

Un studiu similar a fost realizat şi pentru alte AINS cum sunt nimesulidul, fenilbutazona şi indometacinul.

Având în vedere toate aceste aspecte s-a trecut la separarea cromatografică. Astfel, iniţial s-a folosit o coloană cromatografică de tipul StableBond SB C 18 (4,6 x 150 mm, mm) cu o fază mobilă formată din amestec acetonitril / metanol / tampon pH 3,6 în raportul 35 / 25 / 40 la un debit de 0,7 mL/min analizându-se mai întâi fiecare component în parte. În fig. 3 se prezintă

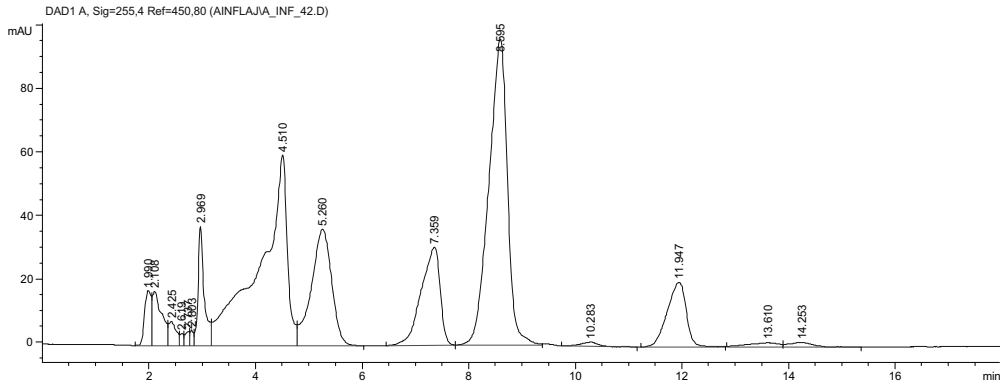

Fig. 4. Cromatogramele obţinute pentru diclofenac, piroxicam, ketoprofen, nimesulid, fenilbutazonă indometacin şi ibuprofen ; fază mobilă formată din acetonitril / metanol / tampon pH 3,6 în raportul 30/30/40 parametri cromatografici, valori prezentate în tab. III.

cromatogramele pentru diclofenac, piroxicam, ketoprofen, nimesulid, fenilbutazonă indometacin şi ibuprofen.

Analizând figura 3 se constată faptul că, în acest caz, compoziţia fazei mobile nu este optimă deoarece există suprapuneri între componenţii amestecului. Acest aspect a fost rezolvat modificând raportul între metanol şi acetonitril din compoziţia fazei mobile. Astfel, pentru o fază mobilă formată din acetonitril / metanol / tampon pH 3,6 în raportul 30 / 30 / 40 se obţine o cromatogramă ca şi cea din figura 4.

Pentru a creşte rezoluţia separării se poate micşora debitul fazei mobile, caz în care, evident, timpul de analiză total creşte. Comparând valorile experimentale obţinute pentru un debit al fazei mobile de 0,7 mL/min şi respectiv 0,5 mL/min se constată că alura spectrelor de absorbţie şi poziţia maximelor de absorbţie nu se modifică, în schimb absorbanţa scade odată cu scăderea debitului fazei mobile ; indiferent de valoarea debitului, înălţimea vârfului rămâne constantă ; odată cu scăderea debitului fazei mobile creşte aria şi înăţimea vârfului. Acest ultim fapt ar putea constitui un avantaj în situaţia în care în proba de analizat nu există toate antiinflamatoarele studiate, deci nu apar probleme în ceea ce priveşte calitatea separării, deoarece o creştere a ariei vârfurilor conduce la o creştere a sensibilităţii de detecţie, deci la o scădere a limitelor de detecţie şi de cuantificare. Trebuie avut însă în vedere şi alţi parametri cromatografici ca, de exemplu, simetria vârfurilor.

Pentru a studia influenţa compoziţiei fazei organice din faza mobilă asupra separării cromatografice a unui amestec de antiinflamatoare nesteroidiene, am ales ca fază mobilă un amestec format din acetonitril, metanol şi tampon acetat. În analizele efectuate, debitul a fost menţinut constant (0,7 mL/min), la fel şi proporţia între faza apoasă şi cea organică (40 / 60). Au fost modificate doar proporţiile de metanol şi acetonitril astfel încât conţinutul în metanol să crească în paşi de câte 10% de la 0 la 30%. Din cromatogramele obţinute se obţin o serie de informaţii referitoare la unii

Analizând aceste date, odată cu creştera conţinutului de metanol în faza mobilă în detrimentul acetonitrilului, se desprind următoarele observaţii :

1) se observă o creştere a timpilor de retenţie, fapt ce poate fi explicat printr-o solubilitate mai mică a acestor componente în metanol decât în acetonitril ;
2) pentru compuşii 1, 2 şi 3, odată cu creşterea conţinutului în metanol din faza mobilă în detrimentul acetonitrilului, se observă faptul că aria vârfurilor este rela-

TABELUL III
Modificarea unor parametri cromatografici în funcţie de raportul dintre metanol şi acetonitril din compoziţia fazei mobile

Component separat	CH_3CN / CH_3OH	Timp retenție (min)	Arie pic	Înălțime pic	Lățime pic
1	60 : 0	3,77	745,8	67,3	0,1613
	50 : 10	4,094	763,9	65	0,1691
	40 : 20	4,444	780,1	48,7	0,2317
	30 : 30	4,746	798,6	44,2	0,2588
2	60 : 0	4,261	902,8	101,1	0,1362
	50 : 10	5,056	884,5	76,6	0,1796
	40 : 20	5,65	903	56,8	0,2398
	30 : 30	6,464	913,8	43,8	0,3136
3	60 : 0	5,707	701,8	50,1	0,2306
	50 : 10	6,664	710,8	38,5	0,3016
	40 : 20	6,812	713,4	32	0,3529
	30 : 30	7,401	720,4	26,1	0,421
4	60 : 0	6,74	2372,1	190,7	0,1931
	50 : 10	9,375	2426,2	131,2	0,2894
	40 : 20	9,937	1798	45,1	0,5936
	30 : 30	10,815	1782,5	40,9	0,6522
5	60 : 0	7,889	496,5	36,3	0,2077
	50 : 10	10,234	1454,4	44,2	0,5247
	40 : 20	10,79	2304,9	89,2	0,404
	30 : 30	15,78	510,8	12,8	0,6203
6	60 : 0	8,666	1461,7	59,2	0,3807
	50 : 10	10,823	508,5	26,7	0,2924
	40 : 20	12,316	509	18,8	0,4145
	30 : 30	13,873	2397,8	67,4	0,5526

tiv constantă în timp ce înălţimea vârfurilor scade, iar lăţimea creşte ;

3) pentru compusul 4 aria şi înălţimea vârfurilor scad, în timp ce lăţimea creşte ;
4) pentru componenţii 5 şi 6 apare o anomalie, şi anume : aria, înălţimea, cât şi lăţimea vârfurilor nu prezintă o modificare uniformă.

Având în vedere faptul că în proba analizată există un număr de 7 componenţi şi tinand cont de observaţiile (3) şi (4) se poate afirma faptul că din ultimii patru compuşi care eluează, unul suferă o deplasare mai rapidă prin coloană decât ceilalţi trei, în fiecare situaţie în parte obţinându-se picuri suprapuse, deci impure lucru confirmat prin verificarea purităţii picului şi confirmare spectrală. În consecinţă, metoda în cauză nu poate fi aplicată în situaţia în care în proba de analizat se găsesc toţi aceşti patru compuşi.

Pentru justificarea alegerii tamponului acetat din compoziţia fazei mobile în loc de apă argumentul decisiv este că acesta este mai volatil decât alte soluţii tampon, lucru util în situaţia în care metoda de separare prin HPLC cu detecţie UV va fi transferată pentru o detecţie prin spectrometrie de masă.

Pe de altă parte, dacă se analizează parametrii cromatografici pentru două cromatgrame realizate pe faze mobile formate din acetonitril / metanol / apă şi respectiv acetonitril / metanol / tampon acetat în raportul 30 : 30 : 40 se obţin datele prezentate în tab. IV.

Analizând aceste date, dacă se utilizează soluţie tampon în loc de apă, se desprind următoarele concluzii :

TABELUL IV
Modificarea unor parametri cromatografici în funcţie de faza apoasă din compoziţia fazei mobile

Component separat	Faza apoasă	Timp retenţie (min)	Arie pic	Înălţime pic	Lăţime pic
1	apă	4,746	798,6	44,2	0,2588
	tampon acetat	2,971	350,2	37,5	0,1314
2	apă	6,464	913,8	43,8	0,3136
	tampon acetat	4,508	2145,2	60,1	0,4549
3	apă	7,401	720,4	26,1	0,421
	tampon acetat	5,26	975,9	36,7	0,4001
4	apă	10,815	1782,5	40,9	0,6522
	tampon acetat	7,356	781,1	31,3	0,349
5	apă	13,873	2397,8	67,4	0,5526
	tampon acetat	8,594	2387	96,8	0,358
6	apă	15,78	510,8	12,8	0,6203
	tampon acetat	11,946	501,3	20,3	0,3871

- timpii de retenţie scad, ceea ce înseamnă un timp de analiză per global mai mic ;
- pentru primii trei compuşi separaţi, calitatea separării scade, vârfurile obţinându-se suprapuse, din acest motiv un studiu al modificărilor ariei, sau înălţimii picurilor nefiind util ;
- pentru compusul 4 apare o scădere semnificativă a ariei, înălţimii şi lăţimii vârfului corespunzător ;
- pentru compuşii 5 şi 6 aria este relativ constantă, în timp ce se observă o creştere a înălţimii vârfurilor compensată de scăderea lăţimii acestora.
- pentru optimizarea metodei este indicat să se lucreze cu soluţie tampon acetat, dar de o concentraţia mai mică de 0,1M şi cu un pH mai mare de 3,6.

CONCLUZII

Pentru punerea la punct a unei metode de separare ce va fi utilizată la identificarea şi ulterior determinarea cantitativă prin HPLC a unor antiinflamatoare nesteroidiene, după studiul solubilităţii compuşilor ce urmează a fi analizaţi, se pleacă de la studiul proprietăţilor fizico – chimice ce constituie parametrii ce vor fi urmăriţi (în cazul de faţă, absorbanţa în diverşi solvenţi utilizaţi la solubilizarea probelor). Astfel, a fost studiat spectrul de absorbţie al unor medicamente antiinflamatoare nesteroidiene în apă, metanol, acetonitril şi soluţie tampon acetat pentru elucidarea lungimilor de undă optime de detecţie. Având în vedere faptul că în analiza cromatografică nu se utilizează ca fază mobilă solvenţi puri ci mixturi de solvenţi, s-au studiat şi spectrele de absorbţie ale unor amestecuri de solvenţi. După stabilirea unui raport optim între faza apoasă şi faza organică ce vor intra în compoziţia fazei mobile, se alege raportul optim între constituenţii fazei organice, respectiv a fazei apoase, având în vedere solubilitatea fiecărui component în parte, lungimea de undă corespunzătoare maximului de absorbţie, dar şi a parametrilor cromatografici esenţiali (timp de retenţie, aria, înălţimea şi lăţimea picului cromatografic etc.)

În urma tuturor acestor etape a fost elaborată o metodă de analiză prin HPLC a unui amestec de antiinflamatoare. Unul dintre avantajele acestui mod de abordare a problemei constă în costul relativ redus în ceea ce priveşte punerea la punct a metodei HPLC cu detecţie în UV în situaţia în care această metodă de separare va fi transferată pe un sistem ce permite detecţia prin spectrometrie de masă, modalitate de detecţie mai precisă în ceea ce priveşte puritatea picurilor cromatografice, sensibilitatea, evitarea utilizării de substanţe etalon etc.

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Rev. Med. Chir. Soc. Med. Nat., Iaşi – 2010 – vol. 114, nr. 4

FRECVENŢA ANOMALIILOR DENTO-MAXILARE CLASA A-II-A ANGLE – ANALIZĂ COMPARATĂ ÎN CADRUL ANOMALIILOR DENTO-MAXILARE ŞI ÎN INTERIORUL CLASEI A-II-A

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CLASS II ANGLE MALOCCLUSIONS FREQUENCY (Abstract): This study, based on a survey of 1018 children, aged between 6-13 years, from urban and rural areas, aims at estimating the frequency of malocclusions among those patients who required specialized orthodontic treatment. A comparison is made between Class II Angle malocclusions within malocclusions in general. The results show that malocclusions recorded in some specialized orthodontic services reveal significant differences function of the children's provenience, age and sex. **Key words**: CHILD, FREQUENCY OF MALOCCLUSION, CLASS II ANGLE

Anomalia dento-maxilară a devenit astăzi o problemă de sănătate publică datorită caracteristicelor pe care aceasta le înglobează, larga răspândire în populaţie şi tendinţa de creştere (1). Datele din literatură oferă o largă variabilitate a frecvenţei. În Europa frecvenţa variază între 40,8-99% (2,3,4,5, 6,7,8,9,10); pe continentul american este de 75% (11,12); în Asia între 19,6-90% (13); în România între 41-77,6% (14,15, 16,17). Prin urmare, este necesară stabilirea necesităţilor de tratament şi cunoaşterea indicelui de adresabilitate al populaţiei către serviciile de asistenţă medicală stomatologică în general şi cele de ortodonţie şi ortopedie dento-facială în special.

SCOPUL CERCETĂRII

Stabilirea frecvenţei anomaliilor dento--maxilare în funcţie de adresabilitatea către serviciul de asistenţă medicală ortodontică. Identificarea formelor clinice şi a reprezentativităţii malocluziei cls. a-II-a; analiza comparată în cadrul clasei a II-a Angle;

OBIECTIVE

Stabilirea frecvenţei şi a formelor clinice ale anomaliilor dento-maxilare în funcţie de prezentarea la cabinetul de stomatologie.

Cercetarea corelaţiei între frecvenţa tipului de anomalie dento-maxilară cu mediul de provenienţă, vârstă şi sex;

Analiza comparată a clasei II/1, II/2 din punct de vedere al frecvenţei şi gravităţii.

MATERIAL ŞI METODĂ

Studiul se bazează pe datele culese din foile de observaţie clinică întocmite la 1018 subiecţi, cu vârstele cuprinse între 6-13 ani, provenind din mediul rural şi urban, care s-au prezentat pentru prima dată în Clinica de Ortodonţie şi ortopedie dento-facială, UMF Iaşi şi la un cabinet privat, pentru evaluarea stării de sănătate şi tratament de specialitate. După excluderea a 77 de cazuri incomplet identificate, grupul de studiu s-a redus la 941 subiecţi - 486 fete (51,6%) şi 455 băieţi (48,4%). După mediul de provenienţă,

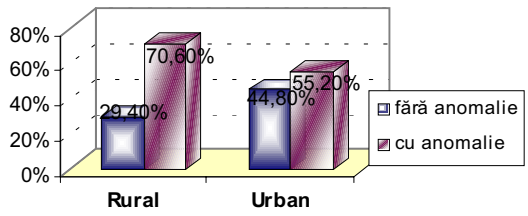

Fig. 1. Repartiţia cazurilor pe mediu de proveniență după prezenţa anomaliei

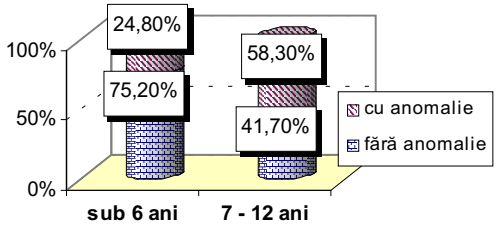

Fig. 2. Compararea frecvenţei relative de apariţie a anomaliei pe grupele 1 respectiv 2 de vârstă pentru sex masculin

73 (7,8%) au fost din mediul rural si 868 (92,2%) din mediul urban. Baza de date a fost constituită în sistem informatizat. Prelucrarea statistică s-a făcut utilizând programul SPSS 16.0 (Statistical Package for the Social Sciences).

Diviziunea lotului a avut în vedere cuprinderea grupelor cheie şi dentiţia/dentaţia: grupa de 6 ani: 222 subiecţi, adică 21,8%; grupa 7-12 ani: 684 subiecţi, adică 67,2%; grupa de 13 ani: 112 subiecţi, adică 11,0%. Analiza datelor s-a făcut pe fiecare din cele trei grupuri, pe lotul total de studiu şi comparaţia între ele. Am urmărit corelaţiile anomaliilor dento-maxilare în funcţie de sex, mediul de proveniență şi grupa de vârstă.

REZULTATE ŞI DISCUŢII

Analiza datelor cu privire la frecvenţa anomaliilor dento-maxilare în funcţie de adresabilitatea la serviciile de sănătate, relevă o frecvenţă a populaţiei infantile din rural de 7,8% şi 92,2% din urban şi o frecvenţă globală a anomaliilor dento-maxilare de 49,2%.

La grupa 7-12 ani frecvenţa cazurilor cu anomalii dento-maxilare din mediul rural este mai mare faţă de cei din mediul urban (fig. 1). Diferenţieri semnificative statistic $p=0,034$.

Analiza anomaliei dento-maxilare ca frecvenţă relativă de apariţie în grupa de 6 ani comparativ cu grupa 7-12 ani pentru băieţi demonstrează că aceasta este mai mare în grupa 7-12 ani (fig. 2), diferenţă semnificativă statistic p este sub 1‰. Acelaşi lucru este constatat şi în cazul fetelor (fig. 3), diferenţa este semnificativă statistic, p sub 1‰.

Compararea frecvenţei de apariţie a cazurilor cu anomalii dento-maxilare pe cele 3 clase de vârstă pe tot lotul cercetat (fig. 4) demonstrează că frecvenţa este mult mai mică la grupa de 6 ani faţă de celelalte 2 grupe de vârstă (fig. 5). Intervalul de variaţie al proporţiei prin metoda Wilson relevă că intervalul vârstei de 6 ani nu se suprapune cu niciun alt interval.

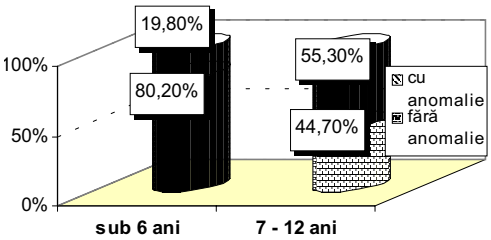

Fig. 3. Compararea frecvenţei relative de apariţie a anomaliei pe grupele 1 respectiv 2 de vârstă pentru sex feminin

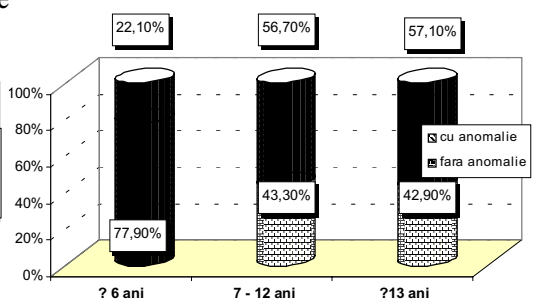

Fig. 4. Compararea frecvenţei relative de apariţie a anomaliei pe clasele de vârstă pentru tot lotul

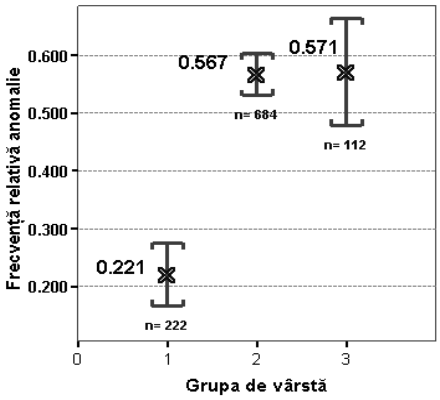

Fig. 5. Frecvenţa relativă a anomaliei pe grupe de vârstă

Eşalonarea tipurilor de anomalii dento--maxilare în funcţie de frecvenţă a fost: malocluzia clasa I (24,6%), clasa a II-a (11,3%) şi clasa a III-a (12,6%). Proporţia cea mai mare a tuturor tipurilor de anomalii se concentrează la grupa de vârstă 7-12 ani.

Cercetând frecvenţa tipurilor de anomalii din cadrul totalului de copii cu anomalii dento-maxilare constatăm diferenţe pe grupele de vârstă (fig. 6), diferenţe semnificative statistic- 0,008.

În grupa 7-12 ani ponderea cea mai mare este deţinută de clasa I Angle urmată de clasa a II-a şi a III-a, pentru ca la vârsta de 13 ani ordinea în funcţie de frecvenţă să fie clasa a I-a, clasa III şi clasa a II-a Angle.

Malocluzia clasa a II-a

Anomalia clasa a II-a Angle ca frecvenţă de apariţie deţine ponderea cea mai mare la grupa de vârstă 6 ani (32,70%), scade în

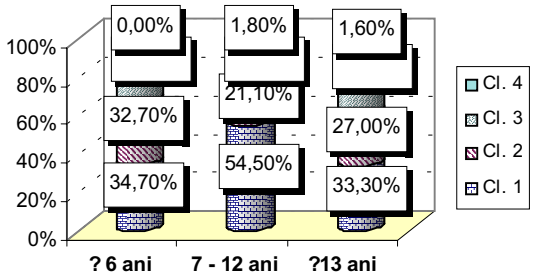

Fig. 6. Distribuţia frecvenţei de apariţie a tipului anomaliei pe clasele de vârstă pentru tot lotul

intervalul 7-12 ani (21,10%), crescând uşor la 13 ani (27,00%).

Distribuţia frecvenţei de apariţie a malocluziei clasa a II-a pe clase de vârstă si sex indică comportamente diferite între fete si băieţi, după cum urmează: băieţii au o frecvenţa de apariţie cea mai mare la grupa 13 ani (34,80%), urmată de grupa de 6 ani (19,00%) şi 16,80% în intervalul 7-12 ani (fig. 7).

Fetele marchează frecvenţa de apariţie cea mai mare la 6 ani (44,40%) şi apoi scade în grupa 7-12 ani (25,00%) şi ajunge la 13 ani la 23,70% (fig. 8).

Simetria

Analiza simetriei în cadrul malocluziei clasa a II-a evidenţiază diferenţieri corelate cu clasa de vârstă şi subdiviziunile clasei a II-a Angle, clasa II_1, II_2.

Vârsta de 6 ani se caracterizează în general prin simetrie pentru clasa II_2 şi într-o mare proporţie clasa II_1 (12% din subiecţi prezintă asimetrie).

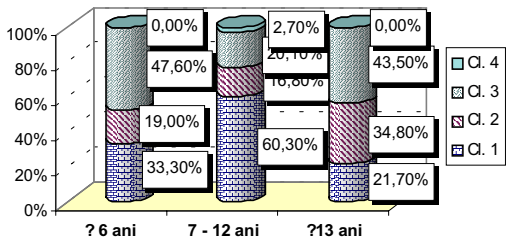

Fig. 7. Distribuţia frecvenţei de apariţie a tipului anomaliei pe clasele de vârstă pentru cazurile de sex masculin

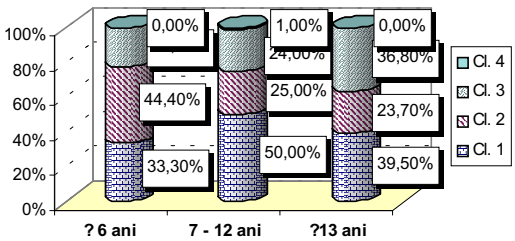

Fig. 8. Distribuţia frecvenţei de apariţie a tipului anomaliei pe clasele de vârstă pentru cazurile de sex feminin

Asimetria creşte pentru clasa II_1 în intervalul 7-12 ani atingând proporţia cea mai mare la 13 ani (29,4%).

Clasa II_2 este afectată de asimetrie în intervalul 7-12 ani, crescând la grupul de 13 ani la 17,6%.

Angrenajele inverse/ocluzia inversă apar în grupele de vârsta 7-12 şi 13 ani, atingând proporţia de 7%.

Ocluzia deschisă a fost identificată la grupele 7-12 şi 13 ani cu o frecvenţă de 2,6%.

Gradul de acoperire a fost la 38,50% din cazuri de 3-6 mm, iar la 3,80% mai mare de 6 mm.

Inocluzia sagitală cuprinsă între 3-6 mm s-a identificat la 20,50%, 57,70% mai mică de 3 mm si doar 1,30% au prezentat inocluzii sagitale mai mari de 6 mm.

Analiza datelor cu privire la frecvenţa anomaliilor dento-maxilare în funcţie de adresabilitate către serviciile medicale evidenţiază o proporţie îngrijorător de mică a populaţiei din mediul rural faţă de populaţia din mediul urban. La fel de neliniştitor este şi faptul că aproximativ jumătate din copiii cu anomalii dento-maxilare s-a adresat pentru consultanţă, dintr-o populaţie în care această patologie depăşeşte 75% şi tendinţa este de creştere. Aceasta înseamnă că o proporţie însemnată din copiii afectaţi de anomalii dento-maxilare nu au fost consultaţi sau nu au solicitat asistenţă medicală în intervalul de vârstă de la mai mic sau egal cu 6 ani până la 13 ani. Dacă o parte din anomalii beneficiază pe parcursul creşterii de autoreglare, fapt raportat în rezultatele unor studii în literatura de specialitate, marea majoritate se va agrava, complicând strategia de tratament, mărind durata acestuia şi preţul de cost.

Cauzele determinante ale precarităţii situaţiei descrise constau în reţeaua de stomatologie pediatrică inexistentă sau săracă în mediul rural şi în urban, nivelul scăzut de educaţie sanitară a populaţiei, absenţa deprinderilor de abordare interdisciplinară în patologia ortodontică cu caracter preventiv şi interceptiv al bolii; mulţi dintre confraţii medici: medici de familie, ORL-işti, pediatri consultă foarte de timpuriu copiii şi ar putea, în condiţia în care ar recunoaşte anomalia, să îi dirijeze către serviciile de specialitate şi poate nu în ultimul rând absenţa evaluării corecte şi la timp a nevoilor de tratament asigurată printr-un proces de dispensarizare corectă făcută de către medicii din sectorul de stat sau privat al copiilor.

Creşterea frecvenţei apariţiei copiilor cu anomalii dento-maxilare la vârsta 7-12 ani se datorează pe de o parte acumulării anomaliilor care s-au permanentizat şi agravat din prima perioadă a dentiţiei mixte cu cele apărute în a doua şi a treia perioadă a dentiţiei. Asistăm la agravarea incongruenţelor dento-alveolare prin înghesuire mai ales la erupţia caninilor, se măresc inocluziile sagitale, apar angrenaje inverse/ocluzii inverse în zona laterală. Tulburările funcţionale şi îndeosebi cele fizionomice devin evidente pentru copil şi membrii familiei ceea ce îi determină să solicite asistenţă medicală, fapt confirmat şi de alte lucrări în literatură.

Frecvenţa de apariţie a anomaliilor dento-maxilare mai mică la grupa de 6 ani se explică prin: simptomele reduse pe care le au anomaliile dento-maxilare la această etapă de vârstă pentru copil şi familie, posibilităţi reduse de a evalua anomaliile potenţiale care se dezvoltă pe baza zestrei ereditare sau sub influenţa unor practici vicioase, tratarea cu superficialitate a unor anomalii evidente sub pretextul rezolvării la apariţia dinţilor pemanenţi, fapt confirmat şi în alte studii. Absenţa dispensarizării din dentaţia temporară bazată pe un examen clinic riguros, dublat de explorări complementare minime necesare în unele cazuri.

Ierarhizarea tipurilor de anomalii dento-maxilare în funcţie de frecvenţă este aceeaşi ca şi în studiile populaţionale, atât la nivelul lotului global cât şi în grupul anomaliilor

dento-maxilare, respectiv clasa I, a II-a şi a III-a Angle, cu diferenţieri în ceea ce priveşte proporţia pe clase de vârstă, sex şi mediu, în sensul scăderii ei. Acest lucru indică nevoia politicilor în domeniul asistenţei stomatologice a copilului, dezvoltarea reţelei de stomatologie pediatrică, care sa vizeze în permanenţă starea de sănătate a copilului preşcolar, şcolar, prin introducerea strategiilor de tratament profilactic, interceptiv şi curativ, bazate pe evaluarea necesităţilor de tratament graduat. Diferenţele care se constată între sexe, în sensul că malocluzia clasa a II-a este mai mare la vârsta de 6 ani la fete faţă de băieţi şi creşte până la vârsta de 13 ani se explică prin procesele de creştere (inclusiv erupţia dentară) mai accelerate la fete, pubertatea mai târzie la băieţi şi interesul crescut al fetelor pentru estetic.

Asimetria, element de agravare a anomaliei dento-maxilare, mai timpurie şi cu tendinţă de creştere de la 6 la 13 ani în subdiviziunea II_1 este expresia dezechilibrelor care apar ca urmare a creşterii progresive a inocluziei sagitale care are drept consecinţă pierderea funcţiei ghidajului anterior în cinematica mandibulară. Dezechilibrele musculare apar pe acest fond al dezechilibrului scheletal şi dentar, apare incompetenţa labială, respiraţia mixtă sau orală, interpoziţia buzei inferioare. Aceste practici creează un impact major asupra echilibrului neuromuscular în ansamblul său. În esenţă anomalia se agravează. În subdiviziunea clasa II_2 Angle asimetria apare în grupa 7-12 ani, creşte către 13 ani, însă în proporţii mai mici decât în II_1 şi se asociază cu angrenajele inverse care apar în aceste perioade în zona laterală, precum şi dezechilibrelor musculare după cum arată şi alte lucrări în literatura.

Dacă prezenţa ocluziei deschise este într-o proporţie redusă ca şi cazurile foarte grave marcate de inocluzii sagitale de peste 1 centimetru sau a ocluziilor adânci cu trauma parodontală trebuie să reţinem necesitatea de tratament ortodontic la limită sau mare de tratament care este impusă de proporţia ridicată a inocluziilor sagitale mai mici ca şi a gradului de acoperire care pot evolua agravându-se în timp.

CONCLUZII

Adresabilitatea la serviciile medicale de specialitate, aşa cum era de aşteptat, ca şi în cazul altor afecţiuni, este mai mică a copiilor din mediul rural faţă de cei din mediul urban. Acelaşi lucru se poate afirma despre copii cu anomalii dento-maxilare faţă de prevalenţa îmbolnăvirilor la nivel populaţional ;

S-au stabilit, de asemenea, corelaţii semnificative statistic între frecvenţa anomaliilor dento-maxilare şi mediul de provenienţă, clasă de vârstă şi sex.

Frecvenţa malocluziei clasa II_1 este mai mare decât cea a malocluziei II_2.

Asimetria apare mai de timpuriu şi cu o frecvenţă mai mare în malocluzia clasei II_1.

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NOUTĂŢI

Hiponatremia Şi tratamentul vasoactiv cu Terlipresin

Standardul de tratament al hemoragiei digestive superioare prin efracţie variceală cuprinde în mod obligatoriu tratamentul vasoactiv cu Terlipresin sau Sandostatin. Un studiu realizat recent, pe 58 pacienţi cu hemoragie prin efracţie variceală trataţi iniţial cu Sandostatin, apoi trecuţi pe Terlipresin din cauza resângerării sau a lipsei de control a sângerării, a demonstrat că tratamentul cu Terlipresin determină hiponatremie, cu scăderea sodiului până în 5 mEq/l în 67% ($p=0.002$) din cazuri şi cu peste 10 mEq/L în 36% din cazuri ($p<0.001$). După analiza multivariată a rezultatelor, hiponatremia s-a corelat cu scorul MELD (Model for End-Stage Liver Disease), pacienţii care au avut cel mai mare risc de a dezvolta hiponatremie, fiind aceia care aveau valori ale sodiului la limita inferioară a normalului sau hiponatremie uşoară. În marea majoritate valorile natremiei au revenit la normal după întreruperea tratamentului cu Terlipresin. În unele cazuri hiponatremia a fost atât de severă, încât a determinat manifestări neurologice. Hiponatremia poate fi explicată prin acţiunea terlipresinei la nivelul receptorilor renali de vasopresină tip V2, ipoteză care nu a fost luată în calcul până în prezent. În concluzie studiul trage un semnal de alarmă asupra hiponatremiei induse de Terlipresin, dar şi a monitorizării cu atenţie a pacienţilor cu hemoragie variceală, ştiindu-se că hiponatremia este un factor favorizant al dezvoltării sau agravării encefalopatiei hepato-portale (Solà E, Lens S, et al. Hyponatremia in patients treated with terlipressin for severe gastrointestinal bleeding due to portal hypertension. *Hepatol* 2010 ; 52 (5) : 1783–1790).

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RELAŢIA VENITUL FAMILIEI – ALIMENTAŢIE LA UN LOT DE PREŞCOLARI DIN ORAŞUL NEGREŞTI JUDEŢUL VASLUI

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FAMILY INCOME – NUTRITION CORRELATION IN A GROUP OF NEGREŞTI VASLUI PRESCHOOL CHILDREN (Abstract): Growth and development are influenced by genetic and environmental factors. From the environmental factors, nutrition plays a key role and is income dependent. **Objectives**: Assessing food intake according to family financial status. **Material and method**: A study was conducted on 134 preschool children (ages from 3 to 7 years of age) from a Negreşti kindergarten. Questionnaires were filled by parents for weekly food intake and family financial situation. Statistical analysis used Pearson test. **Results**: According to income the group is structured in: very low income (32.8%), low income (28.3%), average income (26.8%) and above average income (12.1%). The majority of the families provides milk to their children (58.9%), varying from 54.5% (very low income) to 63.1% (low income). Poultry is present in the diet mainly for 2-3 times per week (45.5%) going from 36.8% in low income families to 55.5% in average income families. Daily fruit intake is present in 59.7% of families, varying from 45.4% (very low income) to 68.7% (above average income). Recorded differences have no statistic significance. **Conclusion**: Families income is different but the nutrition provided to children is similar, being strongly influenced by traditional nutritional habits. **Key words**: NUTRITION, PRESCHOOL CHILDREN, INCOME

În decursul vieţii organismul uman trece prin trei perioade reprezentate de: etapa de evoluţie, de stabilitate şi apoi de involuţie. Etapa de evoluţie cuprinde primii 18–19 ani de viaţă şi se particularizează printr-o creştere şi dezvoltare intensă. Aceste doua procese au o evoluţie adecvată doar atunci când copilul are condiţii normale de viaţă. În cadrul acestora includem, în mod deosebit, familia insistând asupra situaţiei sale economice. În familiile sărace calitatea îngrijirilor medicale acordate copiilor este precară, alimentaţia este insuficientă, iar locuinţa insalubră (1). În prezent alimentaţia poate fi în exces, ceea ce expune copilul la riscul apariţiei obezităţii sau insufucientă fiind asociată cu subnutriţia. Obiceiurile alimentare ale populaţiei diferă în variate culturi şi grupuri etnice, în funcţie de alimentele disponibile în zona geografică respectivă. Aceste obiceiuri alimentare se formează în familie în primii ani de viaţă, ulterior ele schimbându-se destul de greu. (2) În acest context, orice program de asigurare a unei alimentaţii sănătoase trebuie să pornească de la cunoaşterea aprofundată a acestor obiceiuri şi să se axeze pe perioadele în care ele se formează.

MATERIAL ŞI METODĂ

Studiul s-a realizat pe 134 preşcolari ce învaţă la Grădiniţa cu program normal nr.1 din Negreşti, jud. Vaslui. Aceşti copii au vărste cuprinse între 3 ani şi 6 luni şi 7 ani fiind incluşi în grupa mică (24 cazuri), mijlocie (33 copii), mare (19 cazuri) şi pregă-

titoare (58 copii). Părinţii au completat un chestionar legat de venitul pe membru de familie şi de alimentaţie. Venitul pe membru de familie a fost încadrat în patru categorii : între 50 – 190 roni (foarte mic) ; între 200 – 490 roni (mic) ; între 500 – 1000 roni (mediu) ; peste 1000 roni (mare). Alimentaţia a fost apreciată cu ajutorul unui chestionar de frecvenţă săptămânală a consumului alimentar (nu consumă – 0 ; 1 dată ; 2-3 ori ; 4-6 ori ; zilnic). Se insistă asupra consumului de lapte, brânzeturi, carne, ouă, grăsimi comerciale, produse cerialiere, legume, fructe şi produse zaharoase. (3,4)

Prelucrarea statistică s- a realizat cu ajutorul testului Pearson.

REZULTATE ŞI DISCUŢII

În general venitul familiei influenţează calitatea alimentaţiei, a îngrijirilor acordate copiilor, a locuinţei. În cadrul acestui studiu vom încerca să identificăm corelaţia ce există între venitul familiilor chestionate şi alimentaţia oferită copiilor. Venitul familiilor chestionate este dominant foarte mic (32,8%) şi mic (28,3%). Nivelul considerat de noi mediu (26,8%) şi mare (11,9%) apare doar în puţine cazuri. Practic în peste 60% din familii nivelul economic este modest, astfel că şi posibilităţile de asigurare a unei alimentaţii echilibrate sunt scăzute.

Consumul de lapte este dominant zilnic (58,9%) sau de 2-3 ori pe săptămână (17,9%) (fig. 1).

Pe tot lotul remarcăm un singur copil (0,7%) ce nu consumă lapte, acesta provenind dintr-o familie cu un venit mare. Indiferent de venitul familiei, în majoritatea cazurilor copii primesc lapte zilnic, astfel că diferenţele constatate sunt nesemnificative statistic ($p > 0,05$). Acest rezultat este surprinzător, el orientându-ne către obiceiuri alimentare asemănătoare, ce nu ţin cont de posibilităţile economice ale familiei.

Brânza este consumată mai ales de 2-3 ori pe săptămână (40,3%) sau 1 dată (26,1%). Există şi situaţii extreme de consum zilnic (15,7%) sau absent (8,9%). Diferenţele nesemnificative statistic ($p > 0,05$) ne orientează tot către existenţa unor obiceiuri alimentare asemănătoare.

Tipul de carne consumat cel mai des de preşcolari este de pui. Domină familiile cu un consum de 2-3 ori pe săptămână (45,5%) şi de 4-6 ori (22,4%). Surprinzător, dar apar şi răspunsuri gen nu consumă (3,7%) sau consumă 1 dată pe săptămână (12,7%). Astfel de răspunsuri apar la toate categoriile de venit (cu excepţia celui mare), diferenţele constatate fiind nesemnificative statistic ($p > 0,05$). Practic, chiar dacă venitul familiei este adecvat consumul alimentar nu cunoaşte o îmbunătăţire majoră fiind dominat de tradiţie, de ceea ce a fost învăţat de la părinţi (fig. 2).

Ouăle sunt consumate în majoritatea familiilor (56,7%) de 2-3 ori pe săptămână,

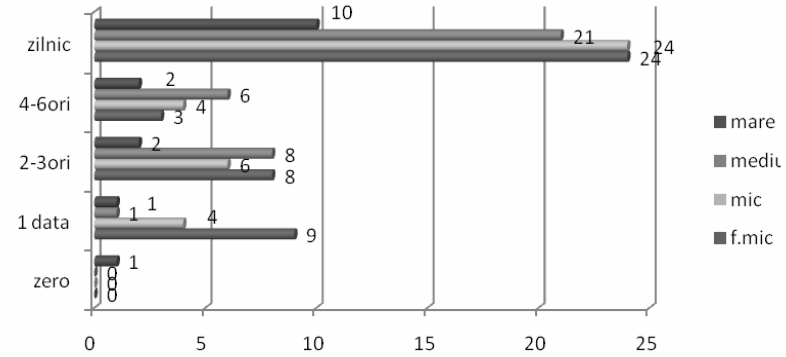

Fig. 1. Consumul de lapte în familiile chestionate în funcţie de venitul acestora

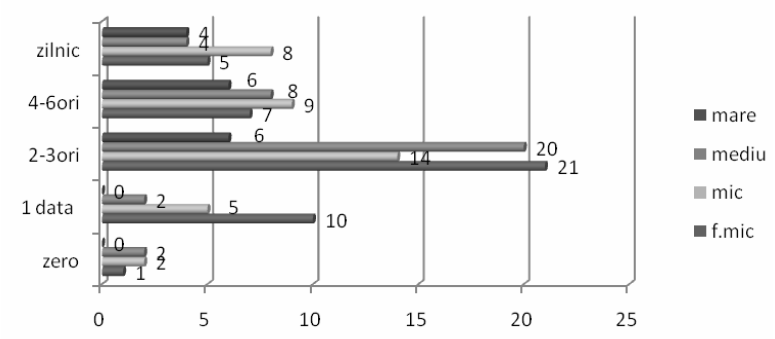

Fig. 2. Frecvenţa consumului de carne de pui

ceea ce corespunde normelor alimentaţiei raţionale pentru preşcolari (1 ou la 2 zile). Îngrijorătoare sunt situaţiile extreme reprezentate de consumul zilnic (3,7%) sau de lipsa aportului (4,5%). Ouăle sunt bogate în proteine şi lipide animale, în elemente minerale şi vitamine fiind un aliment de bază pentru preşcolar. De asemenea, ele sunt relativ ieftine şi uşor de gătit fiind produsele ideale pentru micul dejun. Pe tip de venit rezultatele sunt asemănătoare, diferenţele obţinute fiind tot nesemnificative statistic ($p > 0,05$) (fig. 3).

Grăsimile comerciale sunt de origine animală şi vegetală. Consumul de grăsimi comerciale animale este în majoritatea cazurilor de 2-3 ori pe săptămână (38,05%), dar apar şi situaţii de consum zilnic (6,7%) sau absent (19,4%). Grăsimile comerciale vegetale sunt consumate mai ales zilnic (26,8%) sau de 2-3 ori pe săptămână (26,1%). Sunt îngrijorătoare cele 14,9% răspunsuri de zero ce indică absenţa uleiului/margarinei din alimentaţia copilului. Diferenţele nesemnificative statistic ne orientează către obiceiuri alimentare tradiţionale stabile.

Produsele cerealiere sunt ieftine, dar bogate în proteine vegetale şi glucide, asigurând un aport caloric crescut. Consumul lor în cantităţi prea mari se asociază cu riscul apariţiei obezităţii, îngrijorătoare în acest sens fiind 5,2% cazuri cu aport zilnic. Cel mai frecvent consumul este 1 dată (41,7%) sau de 2-3 ori pe săptămână. Studiul distribuţiei cazurilor în funcţie de venit evidenţiază diferenţe nesemnificative statistic.

Legumele sunt consumate în majoritatea cazurilor (35,8%) de 2-3 ori pe săptămână. Atrag atenţia 8,2% cazuri cu răspuns zero ce indică absenţa legumelor din alimentaţia copiilor (fig. 4).

Total surprinzător răspunsurile negative apar la toate categoriile de venit, diferenţele obţinute fiind nesemnificative statistic. Cumpărarea legumelor nu este o problemă în anumite familii, dar consumul alimentar

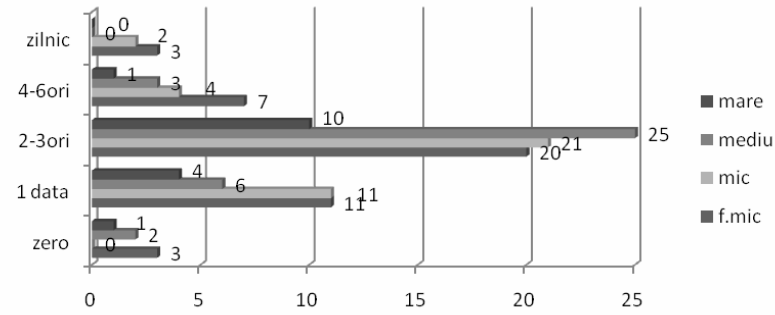

Fig. 3. Consumul săptămânal de ouă în funcţie de venitul familiei

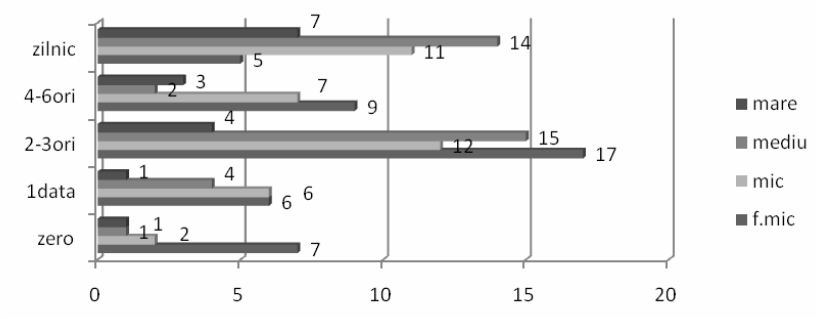

Fig. 4. Corelaţia venit/consum de legume la lotul de studiu

este puternic legat de tradiţia familiei. De asemenea, aceste răspunsuri negative sunt ciudate în condiţiile în care oraşul Negreşti este o colectivitate urbanizată în anii '60, majoritatea familiilor având teren agricol în apropierea oraşului.

Fructele sunt prezente zilnic în alimentaţia a 59,7% din copiii luaţi în studiu. Asigurarea necesarului de fructe nu este o problemă pentru familii, în condiţiile legăturii permanente cu mediul rural. Doar în 0,7% din cazuri consumul este absent.

Aportul de fructe este asemănător în funcţie de venitul familiei ceea ce ne orientează către obiceiuri alimentare stabile. Chiar dacă familia are posibilităţi financiare consumul alimentar respectă strict tradiţia. În condiţiile unor venituri medii/ mari şi a unor informaţii adecvate legate de alimentaţie ar fi de aşteptat ca aportul zilnic de fructe să fie prezent la toţi copii, situaţie ce nu apare în practică.

Produsele zaharoase sunt consumate mai ales zilnic (37,3%) sau de 2-3 ori pe săptămână (29,9%), diferenţele constatate în funcţie de venit fiind tot nesemnificative statistic ($p > 0,05$).

CONCLUZII

Din prezentarea rezultatelor obţinute pe acest lot de studiu putem trage două concluzii foarte importante. În primul rând nivelul economic al familiilor chestionate este precar, remarcându-se 61,1% situaţii de venit foarte mic sau mic pe membru de familie. A doua concluzie de bază este reprezentată de marea stabilitate a obiceiurilor alimentare. Familiile sunt puternic ancorate în tradiţii, astfel că obiceiurile alimentare se schimbă puţin, chiar dacă situaţia economică o permite. În acest context este necesară o intervenţie educativă susţinută pentru a reuşi schimbarea acestora. Înterdicţiile legiferate şi intens mediatizate nu reuşesc să rezolve această problemă.

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EVALUAREA LEZIUNILOR DENTARE NECARIOGENE ÎN FUNCŢIE DE INDICII SMITH ŞI KNIGHT

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ASSESSMENT OF TOOTH WEAR DEPENDING ON SMITH AND KNIGHT INDEX (Abstract): For the clinical diagnosis of tooth wear Smith and Knight have been suggested the index with the same name, the criteria for the quantification of the hard tissue loss being the size of the area affected and the depth of the defect. The aim of this study was to assess tooth wear depending on Smith and Knight index, on a group of 614 patients. **Material and method**: The criteria for the selection of the patients were the age more than 18 years old and the Smith and Knight index >0. **Results**: The biggest proportion of tooth wear (47.3%) presented the value 2 of the Smith and Knight index. Most of the tooth wear with Smith and Knight index 3 (54.4%) are abrasions, 71.4% of the tooth wear with Smith and Knight index 1 are attritions, and 20.3% of the tooth wear with Smith and Knight index 3 are erosions. High alcohol consumption is associated with deep tooth wear, exposing dentine (value 2 and 3 of the Smith and Knight index). Currently, various indices are used, making the comparison of the results of prevalence studies difficult. **Key words**: SMITH AND KNIGHT INDEX, TOOTH WEAR

FAST RP-HPLC METHOD FOR THE DETERMINATION OF BISOPROLOL

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FAST HPLC METHOD FOR THE DETERMINATION OF BISOPROLOL (Abstract): **Aim:** To develop and validate a fast, robust, isocratic reversed-phase high performance liquid chromatographic method for the determination of bisoprolol in bulk and pharmaceutical formulations. **Materials and method:** Optimum separation of bisoprolol was achieved using as stationary phase a Zorbax SB-C18 column (100×3 mm; 5µm). The mobile phase was a mixture of phosphate buffer (pH = 3.5) and acetonitrile (70:30) with a flow rate of 1mL/min. The UV detection was performed at 225nm. The temperature of the column and autosampler was 25°C. The specificity was assessed by using metoprolol as internal standard. The method was validated in accordance with the current ICH guidelines in terms of linearity, limit of detection, limit of quantification, precision, accuracy, recovery and system suitability. **Results:** The retention time for bisoprolol was 1.158 minute. The calibration graph was linear in the concentration range 5-90 µg/mL. The LOD and LOQ of bisoprolol were 1.63 µg/mL and 4.94 µg/mL, respectively. The intra and interday precision of measurements were lower than the accepted criteria (RSD ≤ 2%). The recovery values of HPLC determination of bisoprolol from tablets proved that none of the excipients influenced the results of the analysis. **Conclusions:** The assay it was found to be accurate, reproducible, sensitive and less time consuming. The proposed method can be successfully applied to quality control studies of pharmaceutical products. **Keywords:** BISOPROLOL, HPLC METHOD VALIDATION, UV-DETECTION.

Arterial hypertension affects many people and is a major risk factor for stroke, myocardial infarction, vascular disease, and chronic kidney disease. Numerous classes of drugs are available to help lower blood pressure: diuretics, beta-blockers, calcium-channel blockers, central agonists, peripheral adrenergic inhibitors, vasodilators, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers. Beta-blockers, also known as beta antagonists, beta-adrenergic blocking agents, or beta-adrenergic antagonists, are drugs that are prescribed to treat several different types of conditions, including hypertension (high blood pressure), angina, some abnormal heart rhythms, heart attack (myocardial infarction), anxiety, migraine, glaucoma, and overactive thyroid symptoms.

There are various types of beta-blockers (1). Bisoprolol fumarate is one of the most widely used beta-blockers in the treatment of cardiovascular diseases such as hypertension, cardiac ischemia and congestive

heart failure. Bisoprolol has been determined in tablets and biological fluids using numerous analytical methods such as high performance liquid chromatography (2, 3), UV-VIS spectrophotometry (4, 5), electrophoresis (6, 7).

The main objective of this work was developing and validating a simple, rapid, accurate and economical method for the determination of bisoprolol using high performance liquid chromatography with ultraviolet detection (HPLC-UV).

MATERIAL AND METHODS

The solvents and other materials were HPLC grade provided by Sigma Aldrich. The bisoprolol fumarate reference substance was provided by Unichem Laboratories Ltd., India. Pharmaceutical formulations containing bisoprolol were purchased from local pharmacies.

An Agilent Technologies 1100 High Performance Liquid Chromatograph with Diode Array Detector, a Kern 770 analytical balance and a Cole Parmer ultrasonic bath were used. The chromatographic separation of bisoprolol was achieved using isocratic elution on a reverse phase stainless steel column Zorbax SB-C18 (100×3 mm; 5µm). The temperature of the column and auto sampler was 25°C. A 50µL sample loop was used. The detector was set at 225 nm.

Preparation of calibration standard. Five miligrams of bisoprolol were weighed and dissolved in distilled water to produce a 100 µg/mL stock solution. Working solutions were prepared by suitable dilutions of the stock solution with the mobile phase. The stock solution of the internal standard (metoprolol) was similarly prepared and then added to the analyzed sample (8).

Determination of bisoprolol in tablets. Two types of bisoprolol tablets produced by two manufacturers were analyzed. The amount of powder equivalent to 5 mg bisoprolol was dissolved into distilled water. The solutions were then filtered through a 0.45 µm Millipore filter in order to obtain a clear solution before injections.

Method validation. Validation of the method was carried out according to The International Conference on Harmonization (ICH) guidelines (9). The linearity was investigated in the 2.5.-120 µg/mL concentration range and the calibration curve was obtained by plotting the peak area values against the bisoprolol concentration using linear regression. The sensitivity of the analytical method was evaluated by determining the detection limit (LOD) and quantitation limit (LOQ) using the calibration curve. The precision and accuracy were assessed in the same day and in different days. The precision of the method was evaluated through standard deviation (SD) and relative standard deviation (RSD) (%), respectively. The accuracy was evaluated through recovery (10). To evaluate the robustness of the developed RP-HPLC method, some small deliberate variations in the optimized method parameters were made. The stability of the bisoprolol solution was assessed by comparing the area for the standard solution preserved for various time intervals with the freshly prepared standard and for sample and standard preparations.

RESULTS AND DISCUSSION

Several parameters were examined for the optimization of HPLC analysis of bisoprolol fumarate. The first step was establishing the composition of the mobile phase and retention time. Various proportions of phosphate buffer: acetonitrile were tested. The most appropriate chromatographic

peak was obtained with a phosphate buffer: acetonitrile 70:30 (v/v) mobile phase. Then the influence of the mobile phase flow rate and pH of buffer on peak normalization were studied. The optimum flow rate was 1 mL/min. The detection was performed at a wavelength of 225 nm as it was determined from the absorption spectra of bisoprolol (fig. 1). The retention time was established at 1.158 min (fig. 2).

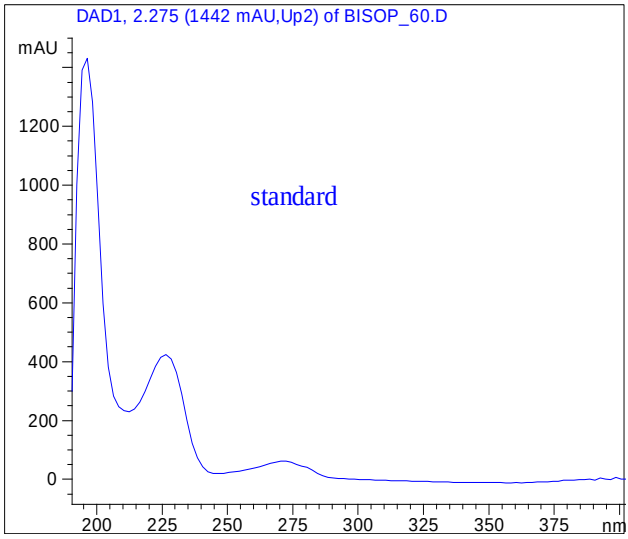

Fig. 1. The absorption spectra of bisoprolol

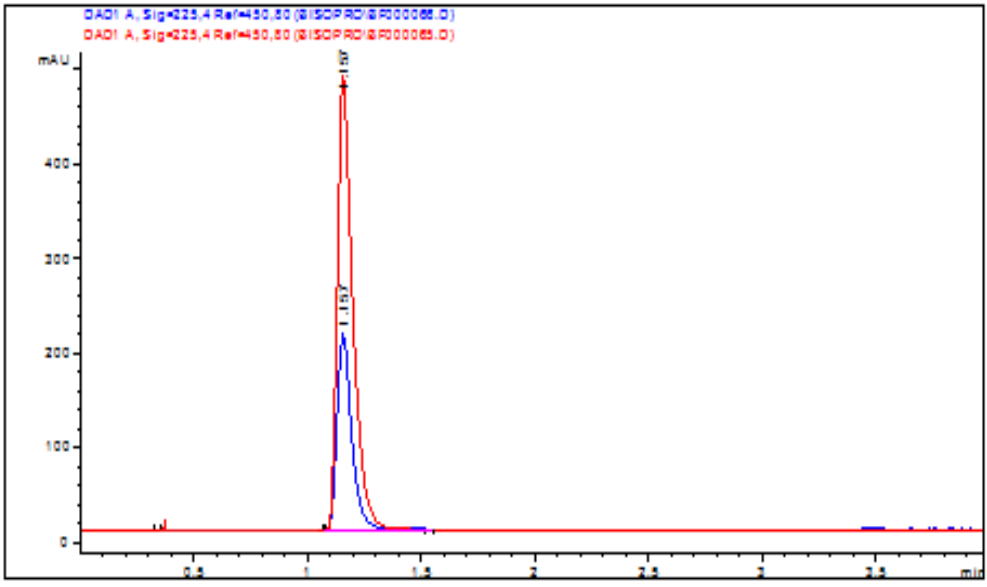

Fig. 2. Retention time of bisoprolol

The specificity was assessed by using metoprolol as internal standard. Chromatogram showed that the method was selective because it had the ability to separate the signal corresponding to bisoprolol from that of metoprolol. For the linearity study, a calibration graph (fig. 3) plotted the concentration against bisoprolol peak area; that graph showed a good linearity in the 0.50-90.0 µg/mL concentration range. Statistical parameters are presented in table I. The LOD and LOQ of bisoprolol were 1.63 µg/mL and 4.94 µg/mL, respectively. Because RSD area ≤ 2% and RSD retention time ≤ 1,5%, we concluded that the chromatographic system was robust (tab. II). The intra and interday precision (tab. III) of measurements were lower than the accepted criteria (RSD ≤ 2%). The proposed method was accurate because maximum value for RSD% was lower than 2% and recovery yield was in the range 97.34-101.04% (tab. IV). The robustness of the method was evaluated by deliberate variation in flow rate (±0.2 mL) and temperature of the column (±5ºC). It was revealed that there were no significant variations in the results of the assay (tab. V).

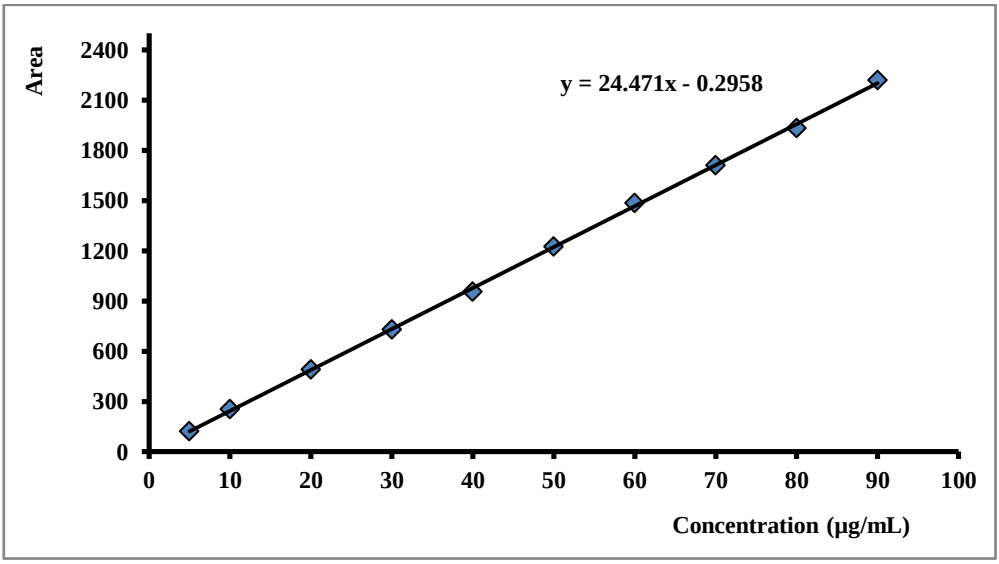

Fig. 3. Calibration curve

TABLE I

Statistical parameters

Regression coefficient (r^2)	0.9996
Correlation coefficient (r)	0.9998
Standard Error (SE)	12.09
Intercept	-0.29
Slope	24.47

TABLE II

System suitability

Nº	Pick area	Statistical Data	Retention time (min)	Statistical Data
1	1204.25	Mean = 1198.77 SD = 16.49 RSD% = 1.37	1.55	Mean = 1.54 SD = 0.02 RSD% = 0.02
2	1189.87		1.58	
3	1201.75		1.53	
4	1212.96		1.52	
5	1197.25		1.56	
6	1213.25		1.54	
7	1159.87		1.57	
8	1211.75		1.51	
9	1209.25		1.54	
10	1187.56		1.52	

TABLE III

Method precision

Repeatability		Intermediate precision	
Theoretical concentration (µg/mL)	Concentration found (µg/mL) ± SD ; RSD%	Theoretical concentration (µg/mL)	Concentration found (µg/mL) ± SD ; RSD%
40	39.70±0.9; 0.91	40	39.21±0.55; 0.58
50	49.62± 1.1; 1.2	50	49.87±0.88; 0.9
60	59.96±0.85; 0.87	60	59.66±1.1; 1.2

TABLE IV

Accuracy

Nº	Theoretical concentration (µg/mL)	Pick area	Concentration found (µg/mL)	Recovery (%)
1	40	984.17	40.23	100.58
2		972.83	39.77	100.73
3		985.05	40.27	99.25
4	50	1277.68	52.22	99.74
5		1293.41	52.87	100.20
6		1234.23	50.45	99.24
7	60	1483.26	60.63	101.04
8		1428.82	58.40	97.34
9		1167.45	59.98	99.97
Statistical data			Mean recovery	99.79
			Minimum	97.34%
			Maximum	101.04%
			RSD%	1.05%

TABLE V

Robustness of chromatographic parameters

Nº	Bisoprolol concentration (µg/mL)	Flow rate (mL/min)	Column temperature (ºC)	Average area (n = 3)	Recovery (%)
1	40	0.8	24	998.87	101.06
2		1.0	29	991.68	100.73
3		1.2	34	983.62	99.25
4	50	0.8	24	1213.21	99.74
5		1.0	29	1214.23	100.20
6		1.2	34	1220.02	99.24
7	60	0.8	24	1481.66	100.93
8		1.0	29	1490.28	101.52
9		1.2	34	1487.15	101.31

The influence of interferers such as formulation excipients was evaluated. The recovery values proved that none of the excipients had influenced the results of the analysis.

The experimental results of HPLC determination of bisoprolol in tablets are summarized in table VI.

TABLE VI

Bisoprolol analysis from tablets

Nº	Label claim (mg/tablet)	Concentration found (mg/tablet) (n=3)	Recovery (%)	RSD (%)
1.	P 1 (5)	4.97	99.40	0.77
2.	P 2 (10)	9.95	99.50	0.62

The results of analysis proved that the amount of bisoprolol was in good agreement with the label claim of the formulation, and the mean recovery of bisoprolol as mg/tablet was in agreement with the requirements of the 10th Romanian Pharmacopoeia.

The experimental results of this study showed that the stability of the solution containing bisoprolol was not affected after being brought to the mobile phase and thus the samples can be kept at room temperature for 8 hours; the samples could be submitted to further analysis without suffering degradation, the peak area values for bisoprolol being relatively constant, covariance (%) (CV) in this case having a value of 1.31 and 1.06%, respectively (CV% ≤ 15 %).

CONCLUSIONS

The present study described the development and validation of a novel fast and accurate HPLC method with UV detection for the quantitative determination of bisoprolol in bulk and pharmaceutical formulations. The recovery percentage of bisoprolol was higher than those obtained using other methods. The newly developed method had a much shorted analysis time. It was a simple method that eliminated the need for mass spectrometry. The proposed method may be used in quality control studies of pharmaceutical products.

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NEWS

NEXT GENERATION SEQUENCING (NGS) AS A PROMISING TOOL IN FORENSIC GENETICS

The biggest challenge for a forensic geneticist is to obtain sufficient information from trace samples, and thus being able to sequence every DNA molecule in a sample is a very intriguing idea. At the present, the forensic markers are typed using PCR-CE and there are individual assays for autosomal STRs, Y-chromosome STRs, X-chromosome STRs, indels, mtDNA SNPs, autosomal SNPs, Y-chromosome SNPs etc. The most important advantage of NGS is that all these assays could be combined into a single NGS assay. This will save time when dealing with cases where supplementary investigations are necessary and reduce the overall time needed for processing a sample. Another advantage is that the DNA fragments do not need to be separated by lengths in CE, so all the analyzed fragments can be designed to be as short as possible, increasing the chance of typing degraded DNA (Claus Børsting, Niels Morling. Next generation sequencing and its applications in forensic genetics. Forensic Sci Int Genet. 2015 Sep;18:78-89. doi: 10.1016/j.fsigen.2015.02.002).

Sabina Bunescu

IN VITRO MEASUREMENT OF TOTAL ANTIOXIDANT CAPACITY OF *CRATAEGUS MACRACANTHA LODD* LEAVES

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IN VITRO MEASUREMENT OF TOTAL ANTIOXIDANT CAPACITY OF CRATAEGUS MACRACANTHA LODD LEAVES (Abstract): *Crataegus macracantha Lodd*, family *Rosaceae,* is a very rare species in Europe, and unlike *Crataegus monogyna* is less investigated for pharmacologic activity**. Aim:** To analyze the ability of the lyophilisate of extract obtained from leaves of *Crataegus macracantha Lodd* (single plant at the Iaşi Botanical Garden) to capture free radicals *in vitro*. **Material and methods:** The lyophilisate obtained in Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iaşi. The decreased absorbance of chromophore chlorpromazine radical cation in the presence of the lyophilized solutions was studied spectrophotometrically. The indicator radical cation, obtained by oxidation of chlorpromazine by potassium persulfate, has the maximum absorbance at 525 nm. Ascorbic acid was used as a standard antioxidant. **Results:** The absorbance of radical solution was determined after the addition of a certain amount of lyophilisate at different time intervals. The antioxidant activity was calculated using the calibration curve obtained by plotting the variation in radical solution absorbance depending on ascorbic acid concentration. For each ascorbic acid concentration the area under the curve was calculated from plotting the percentage inhibition of the absorbance at two pre-established time intervals. **Conclusions:** The results confirm the antioxidant activity of the leaves of *Crataegus Macracantha Lodd* and by optimizing the proposed analytical methods the antiradical activity can be quickly evaluated with minimal reagent consumption. **Keywords:** ANTIOXIDANT ACTIVITY, CHLORPROMAZINE RADICAL CATION, SPECTROPHOTOMETRIC ANALYSE.

The interest in natural antioxidants, aimed at reducing the harmful action of free radicals on the human body has been steadily increasing. In this context, various methods for assessing the antioxidant activity of plant products in view of their use in pharmaceutical, food, cosmetics industries etc. have been developed (1-4). *Crataegus macracantha Lodd*, *Rosaceae* family, is a very rare species in Europe, and unlike *Crataegus monogyna*, is less studied pharmacologically.

The aim of this study was to analyze the ability of the lyophilisate obtained from leaves of *Crataegus macracantha Lodd* (single plant at the Iaşi Botanical Garden) to capture free radical cations resulting from oxidation of chlorpromazine by potassium persulfate ($CP^{\bullet+}$). These chromophore radicals show maximum absorbance at 525 nm. The color of radical solution is diminished by the presence of antioxidants. Ascorbic acid was used as a standard antioxidant.

MATERIAL AND METHODS

The lyophilisate was obtained at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa" - Iaşi as follows: 10 g of plant product (leaves) was treated with 100 mL of 70% methanol (v/v); the mixture was stirred for one hour (FALC F-30 ST magnetic stirrer), filtered, concentrated (Rotavapor, BUCHI R-210) and lyophilized (UNICRYO TFD 5505 freeze-dryer).

Of the 10g of plant product 2.62g of crude extract were obtained.

To determine the antioxidant activity the decrease in absorbance of pre-formed radical cation solution ($CP^{\bullet+}$) depending on the amount of lyophilisate was measured spectrophotometrically.

The spectrophotometric measurements were made with a Hewlett Packard 8453 UV-VIS spectrophotometer equipped with multi-diode detector.

Preparing the chlorpromazine radical cation. Chlorpromazine (CP) was oxidized in acidic environment (HCl) by potassium persulfate ($K_2S_2O_8$), forming a radical cation ($CP^{\bullet+}$) with maximum absorbance at λ = 525 nm. For the preparation of the radical 1 mL of 0.01 M chlorpromazine maleate solution was treated with 8.5 mL of 1 M hydrochloric acid and 0.5 mL of 0.001M potassium persulfate solution.

The optimum time interval for the use of radical solution was 20-40 minutes after the addition of potassium persulfate. During this time interval the absorbance of radical solution remained constant.

Sample preparation. About 0.05 g lyophilisate (a) was weighted and dissolved in 10 mL of distilled water. After 30 minutes the resulting suspension was filtered and the obtained solution was diluted with distilled water to final volume of 25 mL.

Working method. To 2 mL radical solution was added a certain amount of lyophilisate solution (v) diluted to 1 mL with distilled water. The absorbance of the solution was measured 10 and 20 minutes after the start of reaction, at λ = 525 nm, 1 cm cuvettes, against water.

The antioxidant activity of the analyzed samples was expressed as molar equivalents (micromolar) of ascorbic acid/gram dry plant product. For this, we used the calibration curve obtained by plotting the variation in absorbance of radical solution depending on the concentration of the standard antioxidant - ascorbic acid (fig. 1).

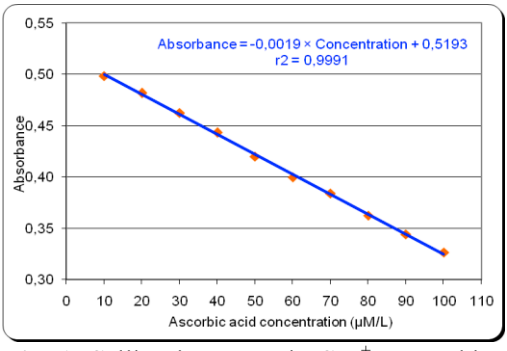

Fig. 1. Calibration curve in $CP^{\bullet+}$ – ascorbic acid system

The method was validated for the linear range of 10-100 μM/L ascorbic acid (5).

RESULTS

For the analyzed samples, the concentration in ascorbic acid was calculated using the straight line equation of calibration depending on the absorbance of freeze-dried solution ($C_{calculated}$). As the final volume of freeze-dried solutions was 1 mL,the concentration of each sample was C = $C_{calculated}$/1000.

Then the result was related to 1 gram dried plant product, taking into account the dilutions made. Measurements were made at 10 minutes (tab. I) and 20 minutes after the start of reaction (tab. II).

TABLE I

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (10 minutes after the reaction)

No. det.	a (g) raw extract	v (mL) lyophilisate	Absorbance	AAE (μM/L lyophilisate)	AAE (μM/ 1 g leaves)
1	0.0552	0.1	0.44543	37.97	45.05
2	0.0552	0.3	0.27479	125.73	49.73
3	0.0324	0.2	0.43309	44.31	44.79
4	0.0324	0.6	0.26442	131.06	44.16
5	0.0264	0.5	0.32683	98.96	49.11
6	0.0264	0.7	0.26485	130.84	46.37
Statistical data				**Mean**	**46.54**
				SD	**2.3549**
				RSD	**5.0605%**

TABLE II

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (20 minutes after the reaction)

No. det.	a (g) raw extract	v (mL) lyophilisate	Absorbance	AAE (μM/L lyophilisate)	AAE (μM/ 1 g leaves)
1	0.0552	0.1	0.42615	47.88	56.81
2	0.0552	0.3	0.25721	134.77	53.31
3	0.0324	0.2	0.41397	54.15	54.73
4	0.0324	0.6	0.22287	152.43	51.36
5	0.0264	0.5	0.29325	116.23	57.67
6	0.0264	0.7	0.21944	154.19	54.65
Statistical data				**Mean**	**54.76**
				SD	**2.2983**
				RSD	**4.1972%**

The values obtained at the two fixed time intervals demonstrate that quenching of radical reaction by antioxidants from natural product is not completed 10

minutes after the start of reaction (fig. 2).

For this reason, measurements at 20 minutes have RSD values within the acceptable range given while measurements at 10 minutes show a slight deviation from the acceptable range.

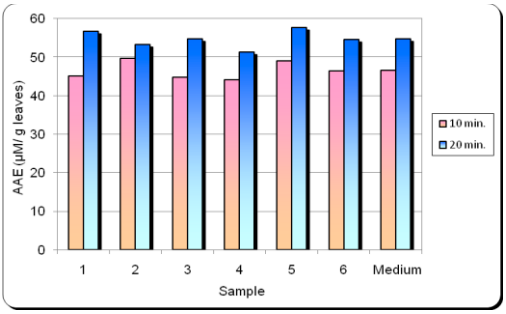

Fig. 2. Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves(10 and 20 minutes after the start of reaction)

In order to eliminate the fluctuations due to changes in absorbance during testing time we used the calibration curve obtained by plotting the area under the curve (AUC) versus ascorbic acid concentration (fig. 3).

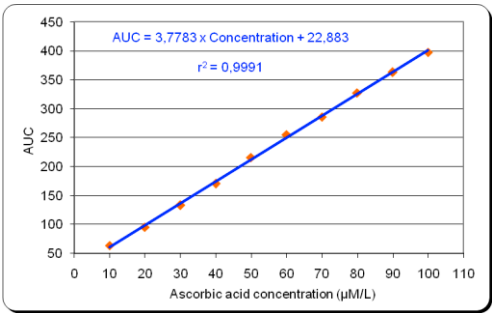

Fig. 3. Area under the curve (AUC) dependent on ascorbic acid concentration

The area under the curve was calculated for each concentration in ascorbic acid (10-100 µM/L) in the graphical representation of the variation of percent inhibition during the fixed time interval (10 minutes and 20 minutes). % Inh = Ar - As / Ar.100, where As–sample absorbance; A_r - radical absorbance (6)

The values obtained by this calculation method for the analyzed samples are given in Table III. The percent inhibition was calculated with Ar = 0.53103.

TABLE III

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (values calculated from AUC)

No. det.	Absorbance		Inhibition (%)		AUC	µM / L	µM / g
	10 min.	20 min.	10 min.	20 min.			
1	0.44543	0.42615	16.12	19.75	179.35	41.41	49.14
2	0.27479	0.25721	48.25	51.56	499.09	126.04	49.85
3	0.43309	0.41397	18.44	22.04	202.44	47.52	48.04
4	0.26442	0.22287	50.21	58.03	541.18	137.18	46.22
5	0.32683	0.29325	38.45	44.78	416.15	104.09	51.65
6	0.26485	0.21944	50.13	58.68	544.01	137.93	48.89
Date statistice						**Medie**	**48.96**
						SD	**1.8124**
						RSD %	**3.7016**

DISCUSSION

It has been demonstrated that the radical quenching reactions are influenced by the activity of the antioxidant (its reducing

power), its concentration and the time point selected for carrying out the determinations (7-9).

Thus, antioxidants with a higher reducing power will react faster with the radicals, while in case of a decreased reducing power the reaction rate is slower and the time required to complete the reaction of radical quenching will be longer.

The variations in the antioxidant activity of the leaves of *Crataegus macracantha Lodd* within 10-20 minutes after the start of reaction is explained by the presence in the lyophilisate of some compounds with a weaker reducing power, acting on radicals with lower speed.

The values obtained when evaluating the antioxidant activity from the calibration curves AUC- standard antioxidant concentration are situated between the two limit values (from 10 and 20 minutes respectively), the error of determinations being also below the RSD value of the method.

Determination of AUC is a more accurate criterion for the assessment of total antioxidant activity, because it considers both the antioxidant concentration and reaction time, being a parameter for measuring the ability of the antioxidant to capture free radicals, compared to a standard antioxidant, during a fixed time interval.

CONCLUSIONS

Our results confirm the antioxidant activity of the leaves of *Crataegus macracantha Lodd* and by optimizing the proposed analysis methods the antiradical ability of this less studied species can be measured with minimum reagent consumption and in a short period of time. Its antioxidant properties justify a more thorough research aimed at making full use of this plant in therapy.

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EPIDEMIOLOGICAL STUDY ON URTICARIA IN CHILDREN

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EPIDEMIOLOGICAL STUDY ON URTICARIA IN CHILDREN (Abstract). **Aim:** The conducted study was focused on identifying children with an atopic background diagnosed with urticaria and also to increase parental responsibility regarding child alimentation, given the impact of allergic reactions (especially those caused by food factors) on the pediatric patient. **Material and methods:** This retrospective study was conducted in 246 patients diagnosed with urticaria admitted to "Sf. Maria" Clinical Pediatric Hospital, Iaşi between January 2012 and December 2013. **Results:** Regarding the etiology of urticaria, the corroboration of the history, clinical and biological data revealed a predominance of urticaria triggered by food factors (54%, 132 cases). Urticaria caused by drugs was identified in 27 (12%) cases, mostly triggered by antibiotics. The analysis of Immunoglobulin E (IgE) blood levels showed elevated levels in 93.75% of the subjects in the age group 0-2 years and 44.44% in children over 6 years old had. Specific IgE analysis for food allergens revealed that β-lactoglobulin, casein and egg white were the most frequently involved allergens for which positive results were obtained. Skin tests (prick tests) showed sensitivity to airborne allergens more frequently than sensitivity to food allergens. **Conclusions:** Our study showed a predominance of urticaria triggered by food factors, given the fact that the prevalence of food allergies increased in the recent decades. History of food allergy is a risk factor in the development of other allergic conditions. **Keywords:** URTICARIA, ATOPIC BACKGROUND, FOOD ALLERGY.

Urticaria is a multifactorial condition with a dermal or hypodermal localization and which appears more frequently in children and youngsters and less frequently in the female population. Data in the literature estimate that approximately 20% of urticaria cases are triggered by the immune-allergic mechanism (activation of mast cells by immunoglobulin E binding, with the release of vasoactive mediators which activate the cutaneous receptors) (1).

In the past decades, statistics have shown an increase in the prevalence of allergic diseases triggered by environmental agents and eating habit modifications (2, 3). Allergic conditions are prevalent amongst the issues of medico-social nature due to the increase in their incidence over the past decades, as well as to their increasing severity, prognostic severity, and high costs implied by the related diagnosis and treatment (4, 5). The involved pathogenic mechanisms are immunological (IgE or non-IgE mediated) or non-immunological

(non-specific activation of mast cells as well as the release of histamine).

Urticaria has a strong impact on the quality of life, especially in children, as it represents an important cause of morbidity, which requires the mobilization of resources to establish an early diagnosis as well as an appropriate treatment.

MATERIAL AND METHODS

A transversal analytical study was conducted in 246 patients admitted to the Iaşi "Sf. Maria" Children Hospital between January 2012 and December 2013. These patients were diagnosed with various forms of urticaria. The patients met the diagnostic criteria (suggestive anamnesis, patient history, general clinical interview and atopic family background).

The patients were assessed based on history data, general physical examination and laboratory investigations: total Immunoglobulin E (IgE), Immunoglobulin E specific to food allergens and airborne allergens and by skin tests (prick test). The anamnesis focused on patient's family history, physiological and pathological history, as well as on dietary habits and history of present illness.

The general physical examination focused on the typical aspect of the urticarial eruption (pruritic papular erythematous plaques of migratory nature) as well as on the associated clinical manifestations: angioedema, gastrointestinal and respiratory symptoms; at the same time, the differential diagnosis included the major pathological entities which may give rise to confusion (erythema multiforma, mastocytosis, urticarial stage bullous pemphigoid).

Statistical analysis was performed by using ANOVA method.

RESULTS

Gender distribution of patients showed a slightly higher frequency of urticaria in males, 129 (52%) patients, compared to females 117 (48%) patients. In terms of area of origin, 136 (55%) patients came from an urban background, while only 110 (45%) came from a rural background. Age-group distribution revealed that the disease was most frequent in the age group 1-5 years, 105 (42.68%) cases, followed by age group 6-10 years, 61 (24.79%) cases.

The distribution of cases according to clinical manifestations revealed that angioedema is the most common symptom associated with urticaria, 78 (31.7%) cases. Regarding the etiology of urticaria (fig.1), the corroboration of history, clinical and biological data revealed a predominance of urticaria triggered by food factors, 132 (54%) cases.

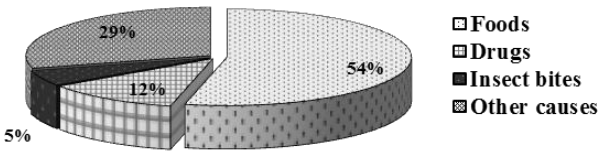

Fig. 1. Cases distribution in terms of urticaria etiology

Drug-induced urticaria was diagnosed in 27 (12%) cases, most frequently caused by antibiotics (14 cases). The most frequently involved were the β-lactam antibi-

otics (penicillins and cephalosporins) followed by the nonsteroidal anti-inflammatory drugs (7 cases). Sensitivity was proved to be prevalent in the case of propionic acid derivatives (ibuprofen, ketoprofen).

In 13 (5%) cases which presented associated angioedema, the cause was insect bites (bees, wasps); the allergen was the hymenoptera venom (Apidae family: Apis mellifera and Vespula).

Given that total serum IgE levels differ according to age, the cases were analyzed by age groups: 0-2 years, 2-6 years, 6 years or more. The distribution of total IgE levels in the whole study group showed that 81 (65%) cases had elevated IgE levels.

A statistical analysis of total IgE showed that the obtained Skewness asymmetry coefficient was 9.719, indicating a tilt to the left of the frequency distribution curve at an early age. The coefficient of vaulting (kurtosis) of 112.471 indicates a distribution suggesting that the sample is not homogeneously distributed around the central value, as there are significant differences between the values of the analyzed interval – their grouping takes place around small values (tab. I, fig. 2).

TABLE I

Statistical analysis of total IgE levels

N	Valid	244
	Missing	0
Mean		122.173
Median		0.000
Std. Deviation		483.572
Skewness		9.719
Std. Error of Skewness		0.156
Kurtosis		112.471
Std. Error of Kurtosis		0.310
Minimum		0.000
Maximum		6244.100

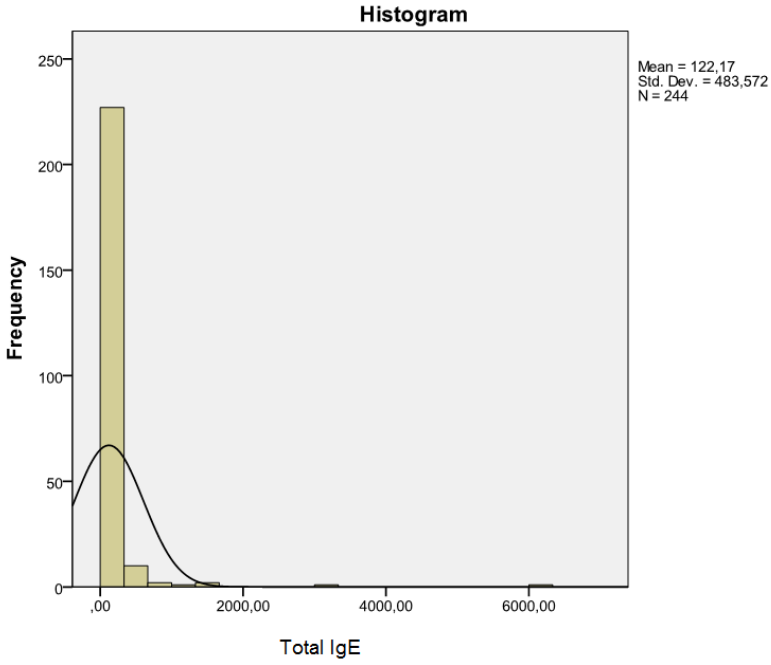

Fig. 2. Distribution of total Ig E level in the study group

The analysis of total IgE levels showed that 93.75% of the children in the age group 0-2 years, 69.23% of the children aged 2-6 years and 44.44% of the children over 6 years had elevated IgE levels.

ANOVA, used to analyze the differences between age group total IgE mean levels indicated a low level (0.896) for age group 2-6 years and normal levels for the other two age groups (6.165 for age group 0-2 years and 8.077 for age group 6-18, respectively), for p values = 0.01, <0.05 for groups 0-2 years and 6-18 years, respectively, p = 0.896 for age group 2-6 years (tab. II). The null hypothesis was rejected, thus there were statistically significant differences between the study groups for a 95% confidence interval (CI).

TABLE II

Statistical correlations by age groups (ANOVA)

ANOVA				Sum of Squares	df	Mean Square	F	Sig.
Age group 0-2 years	Between Groups	(Combined)		8979167.352	16	561197.960	6.165	0.001
		Linear Term	Weighted	25186.841	1	25186.841	0.277	0.607
			Deviation	8953980.511	15	596932.034	6.557	0.000
	Within Groups			1365470.469	15	91031.365		
	Total			10344637.822	31			
Age group 2-6 years	Between Groups	(Combined)		735562.492	20	36778.125	0.896	0.597
		Linear Term	Weighted	236149.035	1	236149.035	5.752	0.028
			Deviation	499413.457	19	26284.919	0.640	0.828
	Within Groups			739053.277	18	41058.515		
	Total			1474615.769	38			
Age group 6-18 years	Between Groups	(Combined)		4429141.535	24	184547.564	8.077	0.000
		Linear Term	Weighted	6452.726	1	6452.726	0.282	0.601
			Deviation	4422688.809	23	192290.818	8.415	0.000
	Within Groups			456993.006	20	22849.650		
	Total			4886134.541	44			

The diagnosis of food allergy was confirmed by specific IgE and skin tests (prick test). Specific IgE analysis for food allergens revealed that β-lactoglobulin, casein and egg white were the most frequently involved allergens for which positive results were obtained. Skin tests (prick tests) showed sensitivity to airborne allergens more frequently than sensitivity to food allergens.

DISCUSSION

Regarding the etiology of urticaria in the study patients, it was revealed that urticaria symptoms were due to food factors in more than half of the cases, 132 (54%). In children aged less than three years the most common food allergen was cow milk, while in older children hypersensitivity reactions occurred due to consumption of cocoa (chocolate), eggs, peanuts, fish or strawberries. History of food allergy is a risk factor in the development of other allergic conditions, and specialized studies show that food allergy increases the risk of asthma or allergic rhinitis (6).

In the study cases the analysis of family history revealed the presence of family

aggregation, as 15% of cases had at least one family member suffering from polymorphic allergies (asthma, allergic rhinitis, angioedema and drug or food allergy). The clinical events have the tendency of getting worse from one generation to another. Assessment of personal pathology and allergic history revealed that 92 (38%) cases presented at least one previous allergic event. In patients with a history of pathological antecedents, the cases of recurrent urticaria were analyzed; the findings showed that 57 of them had at least one previous urticarial episode: 1 episode (17 cases), two episodes (24 cases) and more than two episodes (16 cases).

Correlation analysis of clinical manifestations and total IgE levels showed that both angioedema and asthma can be considered factors that are associated with a higher degree of severity, assessed by the elevated IgE levels (7). Tracking the dynamics of specific IgE levels can give information on the development of the allergic condition. In children with cow milk allergy, decreased levels of specific IgE compared to allergenic proteins precede the induction of immunological tolerance, and high specific IgE levels are associated with persistent allergic phenomena (8,9). The phenomenon of immunological tolerance occurs at age 7, when the immune mucus barrier becomes mature (secretory IgA) and the antigen-antibody complexes are formed (10, 11).

The analysis of IgE levels in different age groups (0-2 years, 2-6 years and over 6 years) showed that total IgE levels are lower in older children compared to those in young patients. This was also inferred from the dynamic tracking of total IgE and specific IgE in patients with allergic history, since the values decrease after a period as a result of allergen eviction and of specific treatment (12, 13).

CONCLUSIONS

Our study showed a predominance of urticaria triggered by food factors, given the fact that the prevalence of food allergies increased in the recent decades. History of food allergy is a risk factor in the development of other allergic conditions.

ACKNOWLEDGMENTS

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NEWS

POLIO THIS SUMMER

In June, an outbreak response assessment took place in the Horn of Africa. The team concluded that transmission in Kenya and Ethiopia has been interrupted, however, undetected low level transmission cannot be ruled out in Somalia. They outlined key measures to continue to strengthen immunity and surveillance, and to put risk mitigation plans in place. Subnational Immunization Days (SNIDs) are planned in Ethiopia from 1 – 3 July and in Somalia for August, with dates to be confirmed. In September, National Immunization Days (NIDs) are planned in Eritrea and Somalia, and SNIDs in Ethiopia, Kenya and Uganda. Five cases of circulating vaccine derived poliovirus type 1 (cVDPV1) have been reported in Madagascar, with dates of onset of paralysis in April and May 2015. The emergency outbreak response is now being intensified to build immunity against the virus in the country, as 25% of children across Madagascar remain un- or under- immunized. One new polio case was reported in the past week, in Afghanistan on the 21 April, when the most recent case had onset of paralysis on 05 May. The total number of WPV1 cases for 2015 is now four. The Technical Advisory Group met in early June and identified that southern and eastern Afghanistan remain at particular risk to polio. The group identified reasons for missed children, which to a great extent are due to remaining operational challenges during campaign implementation. The meeting put forward key recommendations to urgently address these remaining gaps. National Immunization Days in Afghanistan are scheduled on 16 to 18 August using bivalent oral polio vaccine. In other countries, SNIDs are planned across northern Nigeria from 25 – 28 July using tOPV, NIDs are underway in the Central African Republic that are being staggered between end-May and August. NIDs are also taking place in Cameroon on 26 – 28 June. In July, NIDs will take place in Angola and Congo with SNIDs in Central African Republic. National Immunization Days (NIDs) are planned in Sierra Leone for 10 - 13 July. (polioeradication.org, accessed on 30.06.2015)

Alina Manole

SECOND MOLAR UPRIGHTING AFTER PREMATURE LOSS OF MANDIBULAR FIRST PERMANENT MOLAR- CASE REPORT

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SECOND MOLAR UPRIGHTING AFTER PREMATURE LOSS OF MANDIBULAR FIRST PERMANENT MOLAR- CASE REPORT (Abstract): **Introduction:** Uprighting a tipped molar by using an uprighting spring is a fundamental orthodontic treatment technique. Following a weak anchorage done only by the anterior teeth the permanent lower second molar will rotate around its center of resistance, and besides the distalizing movement will occur its extrusion. Following the reaction, the mandibular anterior teeth will have a movement of intrusion. All these elements will lead to anterior open bite. Correction of vertical problems has become easier with the advent of mini-implants. **Case presentation:** U.I patient aged 24 years presenting for aesthetic and functional disorders. Clinical examination reveals intraoral Class I molar malocclusion on the right sight, upper narrowing arch with a slight overbite, and the both permanent first molars on the left side lost prematurely. The tooth 37 is inclined at 60^0 degrees to the plane of occlusion. To avoid front opening occlusion we used an orthodontic miniscrew inserted between the canine and the first premolar on the same side to achieve the second molar up righting. Lower second molar uprighting was achieved without unwanted movements in anterior mandibular region and without molar extrusion. **Conclusions:** Using mini implants in uprighting a tipped molar will achieve the desired tooth movement and will reduce the number of unwanted side effects and eventually to improve patient's esthetics. **Keywords:** SECOND MOLAR UPRIGHTING, PREMATURE LOSS, MANDIBULAR FIRST MOLAR, ORTHODONTIC MINISCREW

The first permanent molar has been reported to be the most caries prone tooth in the permanent dentition. More than 50% of children over the age of 11 years have some caries experience in this tooth (1, 2, 3). The early loss of permanent first molars is a routine problem in the orthodontic clinic and results in mesial inclination of the second molars. Especially the early loss of the lower first permanent molar should be immediately addressed by prosthetic replacement or orthodontic space closure as it may lead to functional and anatomical disturbances. The sequelae of events include distal migration of premolars, extrusion of the antagonist molar, premature contacts in centric relation, occlusal interferences during lateral and protrusive movements. In addition there is a difficulty in prosthetic adaptation when the inclina-

tion is excessive. Mesial inclination favors the appearance of vertical bone defects and infra-osseous pockets in the mesial region of molars (4, 5).

Molar uprighting into its correct position leads to the normalization of functional and periodontal occlusion situation, enabling the roots to be positioned perpendicular to the occlusal plane so that they are better able to resist to occlusal forces, in addition to facilitating the plane of insertion of the prosthesis parallel to the long axis of the tooth (6). Various conventional orthodontic approaches were used to achieve such a movement, but all have been plagued to a greater or lesser extent by the following issues: extrusion of the molar to be up righted, unwanted movement of the anchorage unit, the need for bulky appliances, the frequent need of accessories to strengthen anchorage, and long treatment time (7).

With the advent of the use of miniscrews as orthodontic anchorage, molar uprighting procedures have become simpler, with more predictable results, and since there is no need to use other teeth as anchorage, there is better control of undesirable effects (6).

The aim of this study is to describe a method for molar uprighting with the use of a titanol spring supported on a messially placed miniscrew.

CASE REPORT

Therefore we want to present a case that presented in the clinic of Orthodontics and dento-facial orthopedics in the Ambulatory Pediatric Dentistry from Iasi, Romania. U. I. patient aged 24 years presenting for functional disorders. The extra-oral examination (fig. 1) revealed an oval shape of the face, a mesocephalic head shape, a mesoprosopic face, and a mild convex profile. Facial changes occur hyperdivergent profile and an acute naso-labial angle. The patient showed a good range of mandibular movements and no temporomandibular-joint symptoms.

Fig. 1. Extraoral aspect-before treatment

Clinical examination revealed intraoral Class I molar malocclusion on the right sight, upper narrowing arch with a slight overbite, and the both permanent first molars on the left side lost prematurely. The space for 26 and 36 decreased due to mesial inclination and migration of the second molars. Tooth 16 was covered by a metal-ceramic crown (fig. 2).

Orthopantomogram (OPG) examination revealed good root canal treatment on tooth 16. All wisdom teeth were present.

The OPG was processed by tracking the following structures (fig. 3):

-*the occlusion plane* – line tangent to the vestibular cusps tips of premolars and the canine tip on the same side;

-*the axis of lower second permanent*

molar – the line passing through the center of the crown and the center of radicular bifurcation.

The tooth 37 was tipped at approximate 60^0 degrees to the plane of occlusion (fig. 3). Second permanent molar from the four quadrants had an inclination of 78 ° to the plane of occlusion.

Cephalometric analysis using *Onyx Ceph*^{R} software revealed a Class I skeletal (∠ ANB=2.2^0) with a hyperdivergent profile ∠GoGn/SN=35^0.

Fig. 2. Intraoral aspect-before treatment

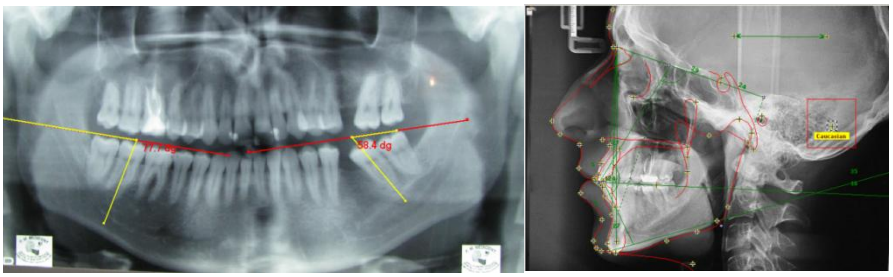

Fig. 3. OPG and Cephalometry - before treatment

The patient's chief concern was the difficulty of achieving chewing on the left side. There were two treatment alternatives for this case:

(1) Opening the space, uprighting and distalization of upper and lower left molars and implant/prosthetic replacement of the left first permanent molars or (2) orthodontic space closure by uprighting the second and third molars and translating them into mesial space.

Because the patient did not want a prosthetic/implant replacement he accepted an orthodontic treatment. The treatment plan included a surgical palatal expansion (fig.4), extraction of 18 and 48, uprighting the second permanent molar and a full fixed appliance therapy with space closure.

We use a prefabricated superlastic Sander spring (Memory-Titanol-Fefer; Forestadent,

Bernard Förster GmBH, Pforzheim, Germany) to achieve the second molar uprighting. Because the patient had a slightly hiperdivergent profile (∠GoGn/SN=35^0) and to avoid front opening occlusion we used a titan orthodontic miniscrew (8 mm, Ortho-Easy®Pin Forestadent®) inserted between the canine and the first premolar on the lower left side as for anterior anchorage (fig. 5).

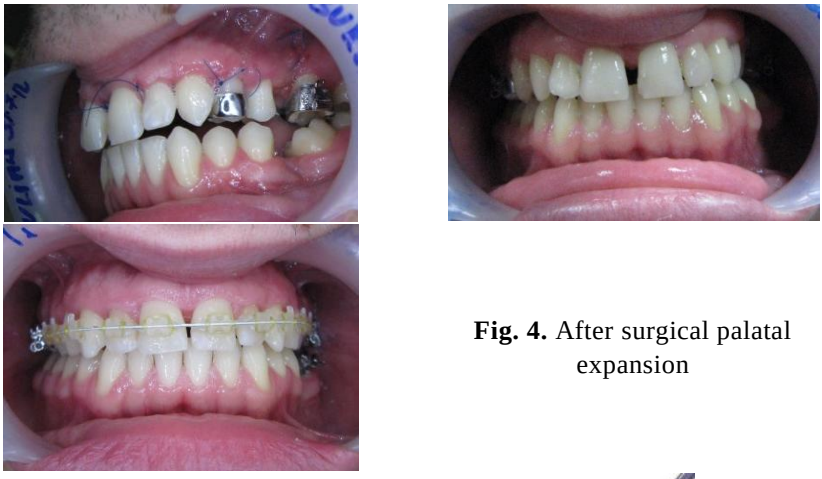

Fig. 4. After surgical palatal expansion

Fig. 5. Achieving uprighting of 37: superlastic Sander spring and mini implant insertion

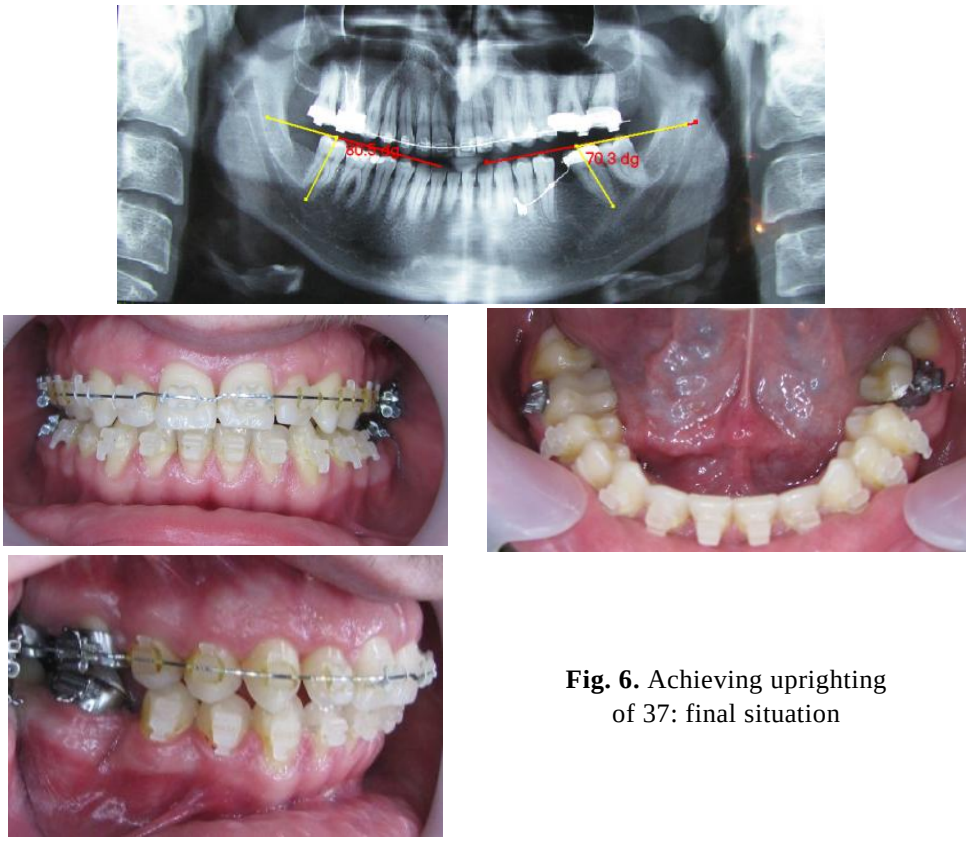

Fig. 6. Achieving uprighting of 37: final situation

DISCUSSION

Permanent first molars are the first permanent teeth to erupt into the oral cavity. They have been quoted as the most caries-prone tooth in the permanent dentition leading to their early loss, probably as a result of their early exposure to the oral environment (1, 2). Loss of a first permanent molar should be immediately addressed by prosthetic replacement or orthodontic space closure as it may lead to functional and anatomical disturbances (4). The second and third molars from that side will incline and rotate, canine and premolars will move distally into the molar space, and the opposing first molar will extrude. Correction of these problems is the biggest challenge to the orthodontist (5).

The ideal solution of six years molar syndrome is protraction of the second and the third molars and toothless gap closure without biological sacrifice. The procedure is not simple, as these strong roots and a significant path that follows the apex, if we take into account initial second molar inclination. In a second molar inclination of 30^0-40^0 correspond to 11 mm travel only apical-uprighting recovery (8) (fig. 7).

The optimal molar uprighting technique for most situations in adults should thus apply an intrusive force to the molar crown, within an inflammatory- free environment. A safe and simple method to achieve such conditions is to use the prefabricated superlastic Sander spring (Memory-Titanol-Fefer; Forestadent, Ber-

nard Förster GmBH, Pforzheim, Germany). Although the prefabricated Sander spring is a simple and effective method for mandibular molar up righting, the method can involve unwanted movements in anterior mandibular region (9) (fig. 8).

Lee et al. have promoted the use of miniscrew as direct anchorage changing the mini implant position in order to obtain two different types of force. The miniscrew can be positioned in the retromolar area, where it acts as a fulcrum for the traction by pulling distally to the second molar (10). This position of the miniimplant can be used in mild tipped molars cases with mesial inclination of the crown, because it generates a sufficient effective uprighting moment to allow the complete straightening of the molar. Instead in moderate to severe tipped cases, the generated moment is too small to produce a sufficient straightening of the tooth (11). For this reason it is essential to consider the possibility of placing the mini implant mesially to the tipped tooth so as a thrusting action force from mesial to distal in generated (12). To avoid front opening occlusion we used in this case an orthodontic miniscrew inserted between the canine and the first premolar on the same side to achieve the second molar uprighting. Achieving uprighting of 37 the slightly hiperdivergent profile remained constant ($\angle GoGn/SN=35^0$).

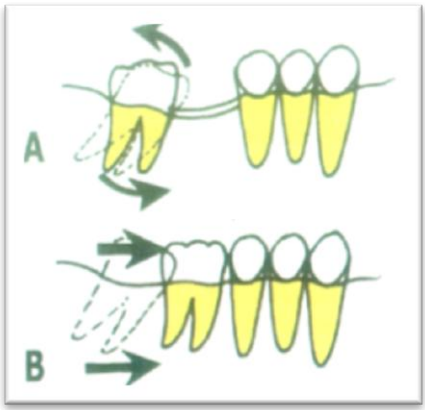

Fig. 7. Closing gaps steps left by the absence of a first lower molar

Fig. 8. Consequences of molar uprighting with poor anterior anchorage and molar extrusion

CONCLUSIONS

In this article we used a miniimplant in uprighting a tipped molar to achieve a desired tooth movement and to reduce the number of unwanted side effects and eventually to improve patient's esthetics. It is necessary a proper clinical, radiological, and biomechanical evaluation and a thoughtful selection of the best orthodontic devices to grant successful treatment results.

ACKNOWLEDGMENTS

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PINUS CEMBRA L.: HISTO-ANATOMICAL FEATURES, ANTIOXIDANT ENZYME ACTIVITIES AND HEAVY METAL CONTENTS OF LEAVES AND LONG SHOOTS

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PINUS CEMBRA L.: HISTO-ANATOMICAL FEATURES, ANTIOXIDANT ENZYME ACTIVITIES AND HEAVY METAL CONTENTS OF LEAVES AND LONG SHOOTS (Abstract) **Aim**: This study aimed to investigate the histo-anatomical features of the long shoots and leaves (young and mature) of *Pinus cembra* L.. The activity of antioxidant enzymatic systems and the content of heavy metals were also evaluated. **Material and methods**: For the histo-anatomical study, the cross-sections were performed by usual techniques. The activity of antioxidant enzymatic systems (superoxide dismutase, catalase and peroxidase) was evaluated by spectrophotometric methods. The content of heavy metals was determined by atomic absorption spectroscopy. **Results**: The cross-section through the long shoots shows many resiniferous canals and a periderm of variable thickness. The leaf has a triangular shape and only two vascular bundles in the inferior and upper levels. The highest level of superoxide dismutase activity (344.90 U/mg protein) was determined in the long shoots collected from a cembran pine in Vatra Dornei, while the highest level of peroxidase activity (7611.11 U/mg protein) was found in the leaves collected in Calimani Mountains. Cd level in all samples was under the quantification limit. Higher levels of Pb were determined in the long shoots (3 µg/g dry weight for the vegetal material collected in Vatra Dornei and 2.86 µg/g dry weight for the vegetal material collected in Calimani Mountains). **Conclusions**: *Pinus cembra* L. leaves show specific elements of subgenus *Strobus* (a triangular shape of the cross section, one single vascular bundle and two resiniferous canals). The results obtained for the superoxide dismutase and peroxidase activities corroborated with those obtained for the heavy metal contents indicate that antioxidant enzymes play an important role in the protection of *Pinus cembra* L. against exogenous stress factors. **Keywords**: *PINUS CEMBRA* L., LONG SHOOTS, LEAVES, HEAVY METALS, ANTIOXIDANT ENZYMES

Pinus cembra L. (cembran pine) is a resinous conifer which belongs to subgenus *Strobus* (*Pinaceae*). It is a species that grows in the alpine and subalpine regions,

at the upper limit of forest area (1350 - 1880 m altitude in the Northern Carpathian Mountains and 1350 - 1986 m altitude in the Southern Carpathians). In these regions, the climate is characterized by a high difference between winter and summer and also between day and night temperatures. Cembran pine is well adapted to high altitude continental climate, frosts and ample temperature oscillations (1).

Environmental factors such as high or low temperatures, drought, radiation, air and soil pollution generate reactive oxygen species (ROS) in plant tissues. The plants outspread in the mountain area are often exposed to large variations of both temperature and radiation (2). Antioxidant enzymatic and non-enzymatic systems protect plants against ROS. The most studied enzymes involved in the protection against oxidative stress are superoxide dismutase, catalase and peroxidase.

Soil and water contamination with heavy metals has become a severe environmental problem regarding the potential ecological consequences. Heavy metals from different sources (industry, mining, agriculture) have an increased tendency of accumulation leading to negative consequences on plants, animals and humans. In plant kingdom, they affect physiological mechanisms (photosynthesis, transpiration), cause protein denaturation and affect the permeability of cell membranes. It is known that some metals (Fe, Mn, Mo) are micronutrients and play an important role in the normal cell development. On the other hand, As, Ag, Cd, Pb and Sb are toxic for plants and affect cell metabolism (3).

As *Pinus cembra* L. is frequently exposed to different stress factors in mountain and urban areas, the aim of this study was to evaluate the activity of antioxidant enzymatic systems (superoxide dismutase, catalase and peroxidase) and quantify some heavy metals in leaves and long shoots. A histo-anatomical study on the leaves and long shoots of cembran pine was also performed.

MATERIAL AND METHODS

Histo-anatomical study involved the microscopic examination of cross-sections through the long shoots and leaves (young and mature). The plant material was collected from a cembran pine in Calimani Mountains in September 2009 and identified by specialists from *Anastasie Fatu* Botanical Garden Iasi. The vegetal material was preserved in 70% (v/v) ethanol. Manual sections were made by using a botanical razor and a hand microtome (4). The cross sections were colored with ruthenium red and iodine green and analyzed using a Novex (Holland) microscope with a Canon A540 camera.

The evaluation of ***antioxidant enzyme activities*** was performed on leaves and long shoots collected from two different locations (Calimani Mountains at approx. 1650 m altitude – codified CM and Vatra Dornei City, Suceava County – codified VD) in June 2011. The plant material was kept at -20°C until biochemical studies. ***Superoxide dismutase (SOD) activity*** was measured according to the method of Winterbourn *et al.* with minor modifications (5). ***Catalase (CAT) activity*** was evaluated using the method of Sinha, cited by Artenie *et al.* (5). ***Peroxidase (POD) activity*** was measured according to the method of Gudkova and Degtiari, cited by Artenie *et al.* (5). The specific activity of all enzymes was calculated using protein content determined by Bradford method (6). All determinations were made in triplicate. The results were

expressed as mean ± standard deviation.

For ***quantification of heavy metals*** in leaves and long shoots, the samples were analyzed using an atomic absorption spectrometer (burner length of 100 mm) (ContrAA 300, Analytic Jena, Germany). The wavelengths used for each metal were: 213.85 nm (Zn), 324.75 nm (Cu), 217 nm (Pb), 228.8 nm (Cd). The limits of detection and quantification were calculated for 0.5 g of sample and a final volume of 25 mL.

RESULTS AND DISCUSSION

Histo-anatomical study

The cross section through the long shoot is circular (fig. 1A). The periderm is thick and persists only in some areas, in others being exfoliated together with a part of bark (fig. 1B). Many resiniferous canals are visible in the bark and wood rings (fig. 1C). The pith is star-shaped due to numerous groups of cells that penetrate the wood. Some cells have cellulosic walls, and others have lignified ones (fig. 1D).

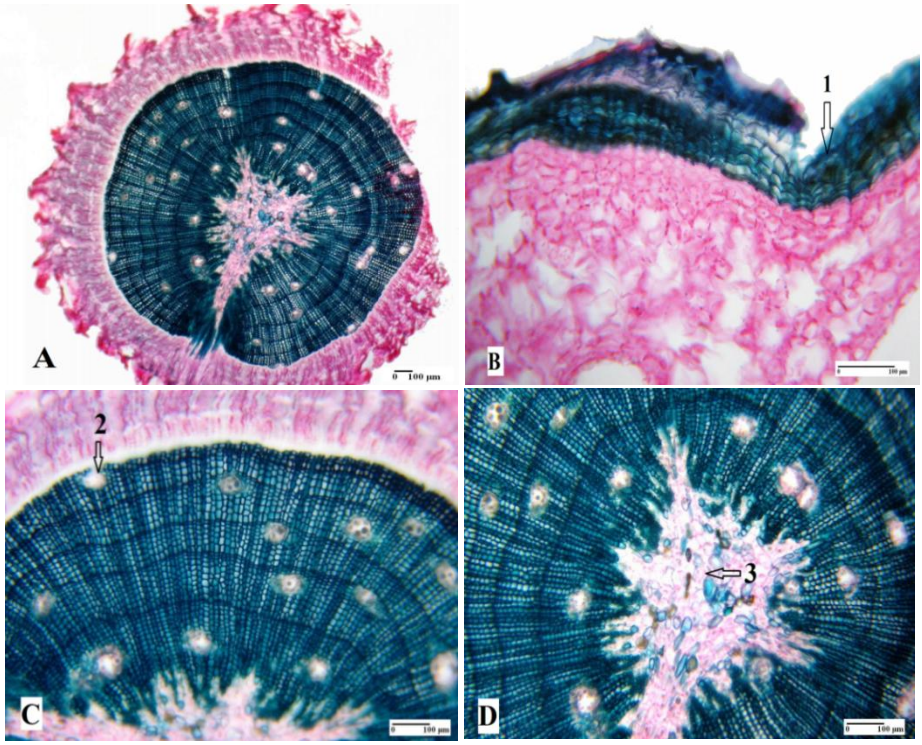

Fig. 1. The long shoot of *Pinus cembra* L.: cross section
A-overview; B-periderm; C-resiniferous canals; D-pith
(1-periderm; 2-resiniferous canal; 3-pith)

The leaves were cross sectioned on different levels from the top to the basis. In cross section, the leaves of *Pinus cembra* L. show a triangular profile.

In the cross section through the inferior level of the young leaf, the epidermis with isodiametric cells and thin walls is observed (fig. 2A). The homogenous meso-

phyll is represented by cells with sinous walls. In the transfusion tissue a vascular bundle with phloem and a very extended xylem is visible (fig. 2B).

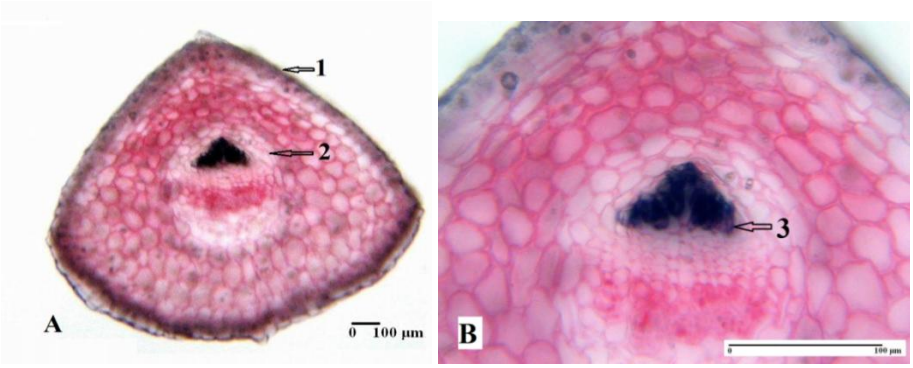

Fig. 2. The young leaf of *Pinus cembra* L.: cross section through the inferior level
A-overview; B-vascular bundle
(1-epidermis; 2-median vein; 3-vascular bundle)

In the cross section through the middle level of the young leaf, the epidermis is unilayered and consists of isodiametric cells having all walls thickened, while the lumina is almost circular; the external walls are covered by a thin cuticle and epicuticular wax (fig. 3A). Under the epidermis, there is a hypodermis represented by one-two layers of fibrous cells with polygonal shape having uniformly thickened and lignified walls. This hypodermis is continuous, interrupted only by stomata (fig. 3B). The stomata in the epidermis of *Pinus cembra* L. leaves (placed deeper than the surrounding epidermic cells) have a typical xeromorphic structure (fig. 3A).

The mesophyll is a homogenous tissue consisting of large septate cells (especially those placed near the hypodermis) with not too deep septums (fig. 3B).

In the mesophyll there are three resiniferous canals situated in the corners of the leaf. The central cylinder consists of a colorless parenchyma where there can be distinguished a pericycle, two collateral vascular bundles, fibers and a transfusion parenchyma. The pericycle consists of large parenchymatic cells, adjacent to the endodermis. The xylem of the vascular bundles shows numerous vessels with strongly thickened and lignified walls. The phloem has a few layers of sieved tubes, surrounded by large cells of phloem parenchyma. The vascular bundles are closed one to the other forming a single conductive unit (fig. 3C). The external part of the phloem is surrounded by a region of cellulose fibers similar to those in the hypodermis. The transfusion tissue represents an anatomic feature of the Gymnosperms' leaves. This tissue is represented by the parenchymatic cells endowed with punctuations (fig. 3C).

In contrast to the previous level, in the upper third of the young leaf, the epidermal and hypodermal cells have more thickened and lignified walls. In addition, a great number of mesophyll cells show deep septums (fig. 4A).

The secretory cells of the three resinifer-

ous canals are surrounded by schlerenchyma cells with thick, lignified walls (fig. 4B).

Regarding the cross sections performed in the mature leaves of *Pinus cembra* L., there are differences between the superior and inferior levels (fig. 5A, 5C). In the inferior level, there are only two resiniferous canals (fig. 5A); the assimilatory parenchyma with characteristic ridge-like invagination into the cells lacks (fig. 5B). The cross-section in the middle level is similar to that of a young leaf (fig. 6A, 6B).

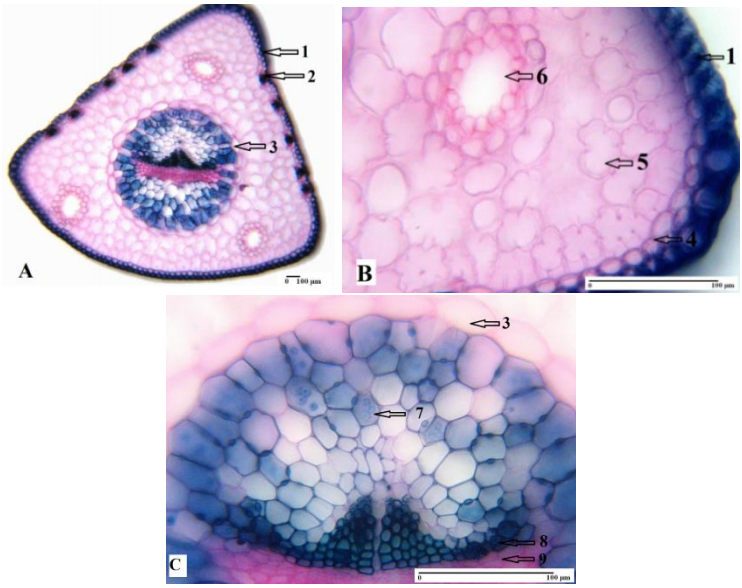

Fig. 3. The young leaf of *Pinus cembra* L.: cross section through the median level
A-overview; B-detail of mesophyll; C-detail of median vein
(1-epidermis; 2-stomata; 3-endodermis; 4-hypodermis; 5-assimilatory tissue; 6-resiniferous canal; 7-transfusion tissue; 8-xylem; 9-phloem)

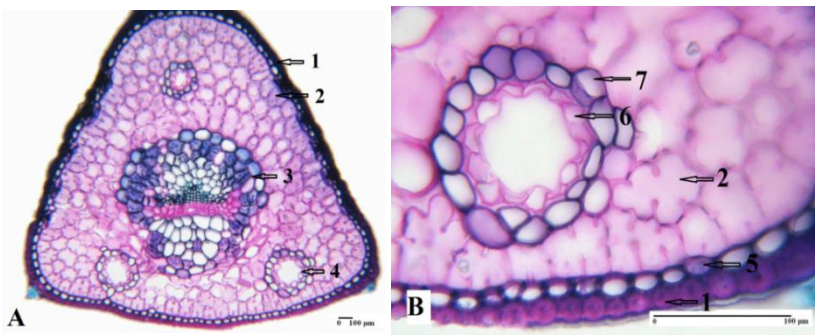

Fig. 4. The young leaf of *Pinus cembra* L.: cross section through the upper level
A-overview; B-detail of resiniferous canal and mesophyll
(1-epidermis; 2-septate assimilatory tissue; 3-median vein; 4-resiniferous canal; 5-hypodermis; 6-secretory cells; 7-schlerenchyma cells)

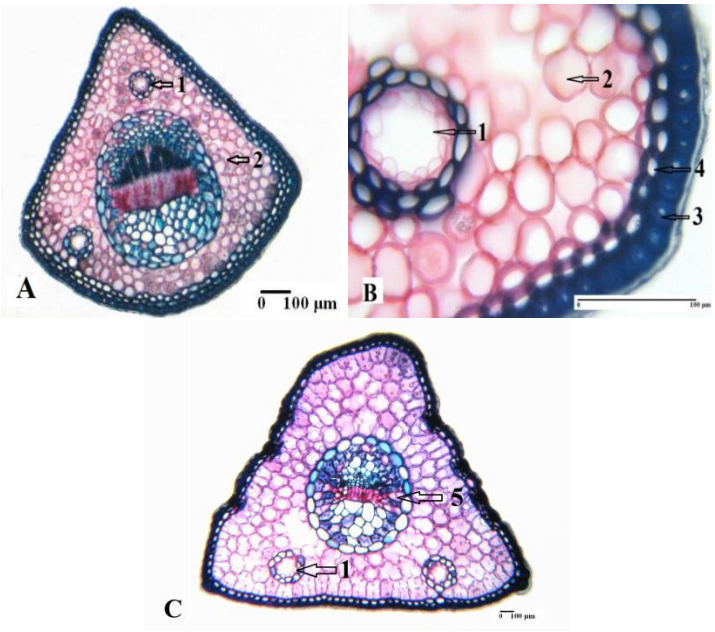

Fig. 5. The mature leaf of *Pinus cembra* L.:
A, B-cross section through the inferior level; C-cross section through the upper level
(1-resiniferous canal; 2-assimilatory tissue; 3-epidermis; 4-hypodermis; 5-median vein)

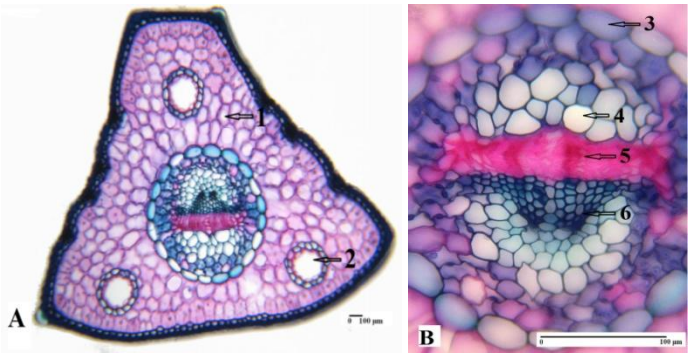

Fig. 6. The mature leaf of *Pinus cembra* L.: cross section through the median level
A-overview; B-detail of median vein
(1-assimilatory tissue; 2-resiniferous canal; 3-endodermis; 4-transfusion tissue;
5-phloem; 6-xylem)

Toma *et al.* (7) showed the similarities and differences between the species of the same subgenus in a comparative histo-anatomical study on the leaves collected from different pine species, in different locations (Ceahlau Mountains, Calimani Mountains, *Anastasie Fatu* Botanical Garden Iasi). The triangular shape, the presence of a single vascular bundle and two resiniferous canals are characteristic for

Strobus subgenus. The number of resiniferous canals varies from a species to another. Thus, the lowest number of secretory canals (two) is found in the leaves of *Pinus monticola* Dougl., *Pinus strobus* L., *Pinus ponderosa* Dougl. ex Laws. In addition, *Pinus nigra* J.F. Arnold and *Pinus sylvestris* L. have the highest number (between eight and twelve) of secretory canals.

Antioxidant enzyme activities

The activity of antioxidant enzymatic systems was studied in samples collected from two different locations (Vatra Dornei - VD and Calimani Mountains - CM). In the samples collected from Calimani Mountains, SOD activities were almost similar (224.86 ± 15.38 U/mg protein in leaves and 230.97 ± 33.52 U/mg protein in the long shoots). The highest SOD activity (344.90 ± 12.89 U/mg protein) was determined in the long shoots collected in Vatra Dornei (tab. I).

POD activity varied significantly, especially in the leaves. In CM samples, the activity was almost six times higher than the one in VD leaves (7611.11 ± 53.57 in comparison to 1411.49 ± 7.07 U/mg protein). CAT activity was not detectable showing that hydrogen peroxide produced in *Pinus cembra* L. is not decomposed by catalase pathway.

TABLE I

Superoxide dismutase and peroxidase activity in *Pinus cembra* L. leaves and long shoots

	SOD activity U/mg protein±SD	**POD activity U/mg protein±SD**
VD leaves	206.03 ± 7.28	1411.49 ± 7.07
VD long shoots	344.90 ± 12.89	3384.99 ± 24.39
CM leaves	224.86 ± 15.38	7611.11 ± 53.57
CM long shoots	230.97 ± 33.52	3228.77 ± 4.94

VD-collected in Vatra Dornei; **CM**-collected in Calimani Mountains

There is no correlation between SOD and POD activities cembran pine samples. There are many studies concerning the metabolism of hydrogen peroxide in plant cells, in normal and also in stress conditions. For example, the overproduction of hydrogen peroxide in chloroplasts is related to excess light stress. This finding can explain the high level of POD activity determined in CM leaves. Hydrogen peroxide can also accumulate by other mechanisms. Hydrogen peroxide can also be generated by some oxidases that release hydrogen peroxide after oxidation of their substrates. Literature data also mentions that oxidative stress in polluted regions causes an increase of SOD and POD levels in pine needles (8).

Studies on *Pinus halepensis* Mill. showed that Cu/Zn-SOD isoform had a low activity in the full-grown leaves exposed to ozone. The authors suggested that it is possible to correlate these results with the lack of involvement of SOD isoform in the defence mechanisms against ozone-induced stress. The study showed a decrease in Cu/Zn-SOD activity in the full-grown leaves in comparison to the young leaves. This result was attributed to the premature leaf senescence as a response to environmental stress factors (9).

Regarding the environmental pollution, SOD activity was increased in *Pinus sylvestris* L. samples next to industrial areas

(a phosphate fertilizer factory and a copper foundry) in comparison to samples collected from unpolluted areas (8). This finding shows the impact of environmental pollution on the level and activity of plant cell antioxidants. In our study, the cembran pine leaves and long shoots were collected from an area next to a former sulphur quarry in the Calimani Mountains. The green belt of pines made the transition between the mining area and the alpine vegetation. It is possible that, besides other stress factors (low temperatures, increased light and abundant snow), the pollution generated by the sulphur quarry affected the stress enzyme activities. Although the sulphur quarry was closed in 1997, the environmental works have not been finished yet.

CAT, an enzyme involved in the photorespiratory mechanisms, showed no activity in all cembran pine samples. This lack of activity may suggest that the enzyme does not play a key role in antioxidant protection of *Pinus cembra* L.. Intense light exposure led to catalase photoinactivation.

Higher POD activity in samples from Calimani Mountains can also be explained by the exposure to UV-B radiations. Literature data show that this exposure can be correlated with an increase in flavonoid content and antioxidant enzyme activities, SOD and POD having a key role in the prevention of photooxidative damage (8, 9).

Heavy metal contents

The contents of Zn, Cu, Pb and Cd in the leaves and long shoots of cembran pine are shown in tab. II.

TABLE II

Zn, Cu, Pb and Cd levels in *Pinus cembra* L. leaves and long shoots

Samples	Level (µg/g dry weight)			
	Cd	**Pb**	**Zn**	**Cu**
VD leaves	< 0.32	< 1.5	83	15.78
VD long shoots	< 0.32	3	62.8	17.11
CM leaves	< 0.32	< 1.5	92.9	13.84
CM long shoots	< 0.32	2.86	75.6	19.1

Cd quantification limit = 0.32 µg/g, Pb quantification limit = 1.5 µg/g;.
Zn quantification limit = 0.74 µg/g; Cu quantification limit = 0.07 µg/g.
VD-collected in Vatra Dornei; **CM**-collected in Calimani Mountains

Studies concerning the influence of heavy metals on the plant development showed that plant exposure to high concentrations of Cd affected photosynthesis, transpiration and stomatal opening mechanisms, inhibited different enzymes, induced root browning and decreased plasma membrane permeability. Cd is a non-essential metal for plant growth (10). For all cembran pine samples, Cd concentration was lower than the quantification limit (< 0.32 µg/g) suggesting that Cd is found only as traces in the cells and does not affect the plant life cycle.

Pb, the most spread toxic metal in soil, alters membrane permeability in plants, induces and increases ROS generation. Generally, in plants, the highest Pb concentration is found in roots, but significant quantities may be transported to all other plant parts. Pb can also be absorbed from the atmosphere. In the lignified tissues, the concentration seems to be more important than in the non-lignified ones. In cembran

pine samples, Pb levels were lower than the quantification limit in leaves, but two times higher than the quantification limit in the long shoots (3 µg/g in VD samples and 2.86 µg/g in CM samples). This result may be associated with a slight increase in SOD activity in the long shoots in comparison to the leaves. Other studies reported an increase in POD activity proportional to Pb content (11).

Zn is considered an important micronutrient for plant development. Zn is used by plants in many metabolic pathways such as chlorophyll synthesis. Zn is also vital for the activity of more than 300 enzymes (dehydrogenases, isomerases, peptidases, polymerases, superoxide dismutase, anhydrase, aldolase, enolase). Zn excess generates phytotoxicity. The main effects of Zn accumulation are delays in growth and development, chlorosis in the young leaves and alteration of physiological processes. An increase in Zn concentration can lead to Mn and Cu deficiency in plant branches. Our results showed high Zn levels and lower Cu levels in all samples. Literature reports that a content of 30 to 100 µg Zn/g of dried plant material is sufficient to support adequate plant growth (12). Our research showed a concentration between 62.8 and 92.9 µg Zn/g in all samples which suggests that Zn was not a stress factor in the samples tested in our study.

Cu is also known as a micronutrient for plant development. It is an important component for different biological constituents such as proteins. It also contributes to carbon dioxide assimilation and ATP synthesis. Increased Cu levels lead to cytotoxicity and oxidative stress. Baker and Senef (3) concluded that the average content of Cu in plant tissue is 10 µg/g dry weight. Values below and over this limit generate plant disorders. Our study determined Cu concentrations between 13.84 and 19.1 µg/g dry weight which are higher in comparison to the normal values.

CONCLUSIONS

In our study, the histo-anatomical cross sections revealed specific elements of *Strobus* subgenus, *Pinus cembra* L. belongs to: a triangular shape of leaf cross section, one single vascular bundle and two resiniferous canals in the mature leaf (in the inferior and superior level, respectively). The values determined for the antioxidant enzyme activities correlated with those obtained for the heavy metal contents suggest the importance of SOD and POD for the protection of this species against different biotic and abiotic stress factors.

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NEWS

ANTICANCER COMPOUND ISOLATED FROM SEEDS OF *MAESA LANCEOLATA*

Maesa lanceolata (*Myrsinaceae* family) is an ethno-medicinal shrub growing in countries in Central Africa, especially in Ethiopia and Kenya, where it has been used against bacterial and viral infections but also in the treatment of rheumatic arthritis. Studies performed to date on *M. lanceolata* concentrated on saponins which showed the capacity to affect angiogenesis, flavonol glycosides and alkylated benzoquinones. Present studies focus on identifying anti-cancer agents in the seeds. The methanolic extract obtained from seeds of *M. lanceolata* was fractionated with *n*-hexane, chloroform, ethyl-acetate, *n*-butanol and water. Based on the results of activity-guided assay, the ethyl acetate fraction showed the highest biological activity and was selected for further analysis. Preparative HPLC led to the isolation of quercetin-3-O-rhamnoside (quercitrin). The compound was tested for anti-tumor activity on HCT116 human colon cancer cells: it decreased cell viability in a dose-dependent manner. Moreover, in a long-term survival clonogenic assay, quercitrin determined dose-dependent inhibition of the colony-forming activity of HCT116 cells. Its GI_{50} (50% growth inhibition) value was 72.3 μM, suggesting that the compound has intrinsic antitumor activity (Shin SI, Dekebo A, Terfa A et al. Identification of an anticancer compound contained in seeds of *Maesa lanceolata*, a medicinal plant in Ethiopia. *J Korean Soc Appl Biol Chem* 2014; 57(4): 519−522).

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ASSESSMENT OF THE CONSEQUENCES IN PREMATURE LOSS OF THE TEMPORARY LOWER MOLARS

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ASSESSING OF THE CONSEQUENCES IN PREMATURE LOSS OF THE PRIMARY LOWER MOLARS (Abstract): The consequences of premature loss of temporary teeth are complex, both of functional and morphological order and the clinical presentation depends on multiple factors: the temporary tooth loss rate as compared with permanent tooth eruption sequence and the number and topography of teeth extracted, so the clinical form of edentulous, which can be frontal or lateral, symmetric or asymmetric, isolated or continuous. **Keywords:** PREMATURE LOSS, TEMPORARY MOLAR, D+E SPACE, SPACE MAINTAINER.

From all the assessment and monitoring methods of the consequences of premature loss of deciduous molars, examining the casts remains an additional examination of undeniable value, providing an overview of the addressed issues and especially of the therapeutic solutions that can be applied.

Popovich and Thompson argued that premature loss of temporary molars may result in reducing the length of the arch, which in turn will cause crowding, rotations and will influence the harmonious alignment of the permanent dentition (1).

Regarding the premature loss of temporary molars, evaluating of the consequences that can appear and how it can intervene therapeutically may be crucial to prevent permanent damage to dentition.

MATERIAL AND METHODS

The study group was constituted by the selection of 50 models of child patients, between 6 and 10 years old, which were presented in the Pediatric Dentistry Clinic of Iasi in period May 2010 - September 2013, with diverse diagnosed pathologies.

Among them, the control group consisted of 6 children who did not have any temporary molar loss, the remaining 44 children represents the study group with loss of one or more temporary molars in the mandibular arch. Measurement and interpretation of the casts was performed by a single physician.

Interpretation of the casts involved evaluating and measuring the following data:

- recording molars lost prematurely and proximal caries in the molars present on the lower arch;
- extractional space, arch width, arch length, arch perimeter, distance between the canines.

The database was created using MS Excel software and statistical analysis was performed in *SPSS 16.0.* We first applied the Kolmogorov - Smirnov normality test

to see which of the variables that we will continue to analyze normal and do not respect the law - to decide what significance tests will still apply. Finally, tests were used for statistical interpretation: Levene test for equality of opportunity, equality t test environments, nonparametric Mann -Whitney test, ANOVA and Pearson correlation coefficient.

RESULTS

As a first step of statistical processing we assessed whether the length, width, perimeter and distance between the canines lower arch decrease in children with premature loss of temporary lower molars. Lower arch width ranged from cases without premature loss of temporary molars between 40.57 and 46.22 mm, and in cases of premature loss of temporary molars between 42.65 and 44.45 mm. Lower arch length ranged from cases without premature loss of temporary molars between 24.53 and 27.00 mm, and in cases of premature loss of temporary molars between 24.96 and 27.27 mm. Arch perimeter ranged from cases without premature loss of temporary molars between 69.17 and 73.76 mm, and in cases of premature loss of temporary molars between 24.74 and 26.29 mm.

In the third quadrant, the space D without loss of temporary molars varied between 7.43 and 7.84 mm, the average being 7.64 mm, while in the cases with loss of first temporary molars varied between 5.12 and 8.21 mm, average being 6.66 mm. Space is varied in the cases without second molar temporary loss between 8.98 and 9.82 mm, average being 9.40 mm, while in cases with temporary second molar loss it ranged between 4.49 and 7.76 mm, average of 6.13 mm. Area D + E in the cases without loss of temporary molars ranged between 16.06 and 17.51 mm, with an average of 16.79 mm (standard deviation = 1.94066), while in cases with loss of both temporary molars, it ranged between 13.56 and 16.63 mm, with an average of 15.10 mm (standard deviation = 1.83848).

Applying non-parametric Mann - Whitney test showed that there was a statistically significant difference in cases with loss of temporary molar of space D (p = 0.047) and in cases of temporary second molar loss of space E (p = 0.042) (tab. I).

TABLE I

Mann -Whitney test (quadrant III – premature loss of first temporary molar / second temporary molar - region D + E)

	C III – Premature loss	N	Average rank	Some of ranks	Statistic	Value
C III - Space D	no	29	19.48	565.00	Mann-Whitney U	44.000
	m I	6	10.83	65.00	Wilcoxon W	65.000
					Z	-1.991
	Total	35			Semnif. asimpt. p	.047, SS
C III - Space D	no	29	16.59	481.00	Mann-Whitney U	46.000
	m II	6	24.83	149.00	Wilcoxon W	481.000
					Z	-2.035
	Total	35			Semnif. asimpt. p	.042, SS

T test for equality of averages showed a statistical significance in the comparison of the space E and that D + E in cases of temporary second molar loss (p=0.000, p= 0.005) (tab. II)

In the quadrant IV, the space D without loss of first temporary molar varied between 7.47 and 7.94 mm, the average being 7.71 mm, while in the cases with loss of first temporary molar varied between 4.44 and 8.55 mm, average being 6.50 mm. Space E is varied in cases without second molar temporary loss between 9.00 and 9.92 mm, averaging 9.46 mm , while in cases of second temporary molar loss it ranged between 5.53 and 6.77 mm, average being 6.05 mm. Area D + E in cases without loss ranged between 15.22 and 17.94 mm, an average of 16.58 mm (standard deviation = 3.57239), while in cases with loss of both temporary molars, it ranged between 13.12 and 17.20 mm, with an average of 15.16 mm (standard deviation = 1.94079).

TABLE II

Independent Samples Test (quadrant III - Area D + E – premature loss of second temporary molar)

		Levene test equality of variants		T test for equality of averages						
		F	Sig.	t	df	Semnif. P	The average difference	Std error difference	95 % confidence interval for the difference	
									Lower limit	Upper limit
C III - Space E	equal variances	.840	.366, NS	6.090	34	.000, SS	3.27333	.53749	2.18102	4.36564
	unequal variances			4.896	6.095	.003	3.27333	.66854	1.64360	4.90307
C III - SpaceD+ E	equal variances	.026	.872, NS	3.026	34	.005, SS	2.57667	.85139	.84643	4.30690
	unequal variances			3.347	7.951	.010	2.57667	.76973	.79976	4.35358

Nonparametric Mann -Whitney test showed that there was a statistically significant difference in cases of first temporary molar loss of the space D (p = 0.024) and in cases of second temporary molar loss of the space E (p = 0.000).

T test for equality of averages showed a statistical significance in the comparison of the space D + E and temporary second molar loss cases (p = 0.034) (tab. III)

In the quadrant III, D space where no decay on temporary molars approximated varied between 7.17 and 7.79 mm, an average of 7.48 mm, while in case of the presence of proximal caries it ranged between 7.12 and 8.07 mm, averaging 7.60 mm. E space is varied in cases without proximal caries in the molars between 8.27 and 9.62 mm, averaging 8.95 mm, while in cases with presence of proximal caries it ranged between 8.65 and 9.87 mm, averaging 9.26 mm. Levene test of equality of variants showed a significant difference statistically between the amount of space is where the temporary molars with proximal cavities and where they were present (p=0.027) (tab. IV).

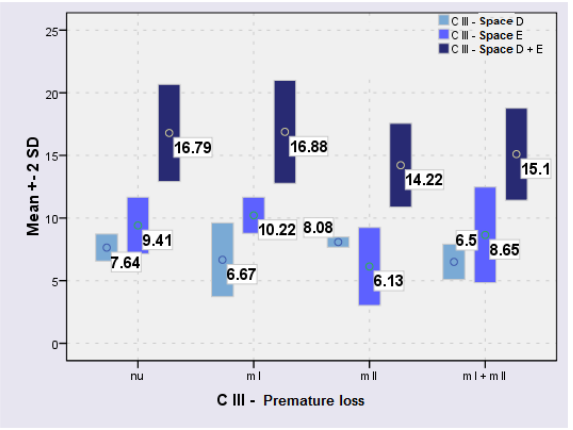

Fig. 1. Changes in spatial dimensions D + E, quadrant three

TABLE III

Independent Samples Test (quadrant IV - space D + E – premature loss of first temporary molar/ second temporary molar)

		Levene test equality of variants		T test for equality of averages						
		F	Sig.	t	df	Semnif. P	The average difference	Std error . difference	95 % confidence interval for the difference	
									Lower limit	Upper limit
C IV - Space D+E (mt I)	equal variances	.244	.625, NS	-.252	32	.802, NS	-.41379	1.64063	-3.75565	2.92807
	unequal variances			-.427	12.731	.677	-.41379	.96957	-2.51293	1.68535
C IV - Space D+E (mt II)	equal variances	1.139	.293, NS	2.200	37	.034, SS	2.53621	1.15269	.20064	4.87177
	unequal variances			3.479	36.006	.001	2.53621	.72901	1.05771	4.01470

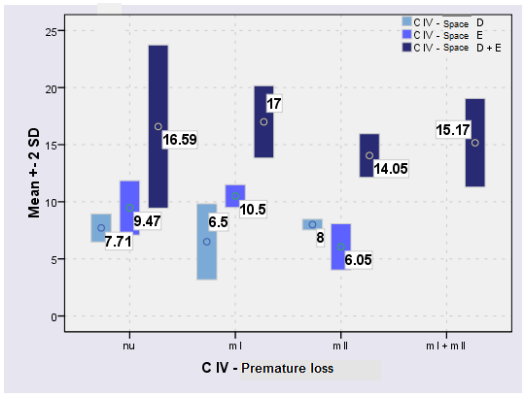

Fig. 2. Changes in spatial dimensions D + E in quadrant four

TABLE IV

Independent Samples Test (quadrant III - Space - decay approximated)

		Levene test equality of variants		T test for equality of averages						
		F	Sig.	t	Df	Semnif. p	The average difference	Std error . difference	95 % confidence interval for the difference	
									Lower limit	Upper limit
C III - Space E	equal variances	5.284	,027, SS	-.559	42	.579	-.31667	.56697	-1.46085	.82752
	unequal variances			-.736	37.564	.466, NS	-.31667	.43014	-1.18778	.55444

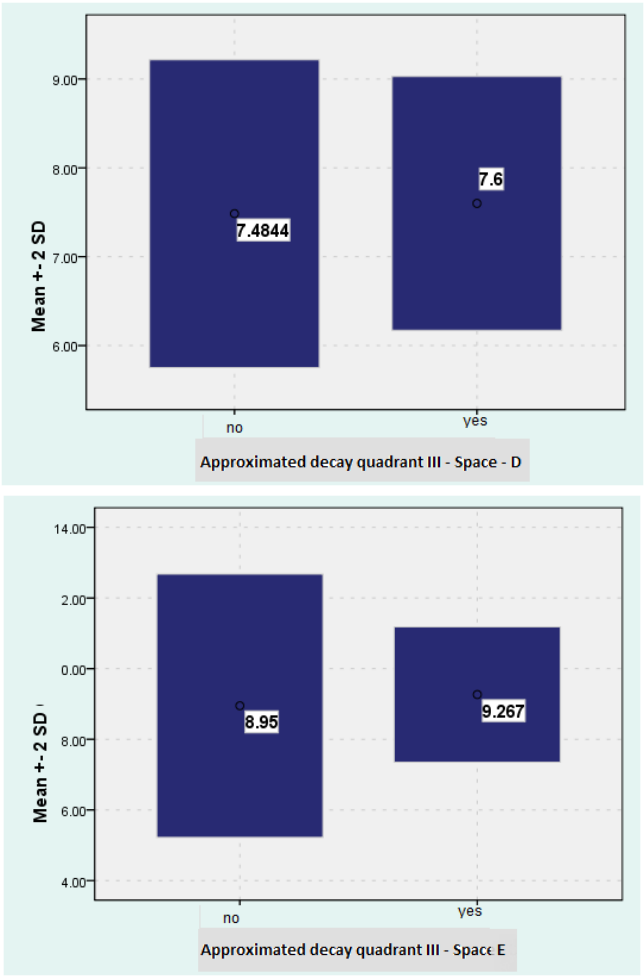

Fig. 3. Changes in spatial dimensions D and E in quadrant III

In the quadrant IV, the space D in cases without tooth proximal loss varied between 7.39 and 8.01 mm, the average being 7.70 mm, while in case of the presence of prox-

imal caries it ranged between 6.96 and 7.78 mm, averaging 7.37. Space E is varied in cases without proximal caries in temporary molars between 7.85 and 9.34 mm, averaging 8.60 mm, while in cases with presence of proximal caries it ranged between 8.38 and 10, 19 mm, averaging 9.29 mm.

Nonparametric Mann -Whitney test showed no statistically significant difference between cases with and without the presence of proximal tooth decay of temporary molars on the size of the space E, instead showed a statistically significant difference for the space D (p = 0.027) .

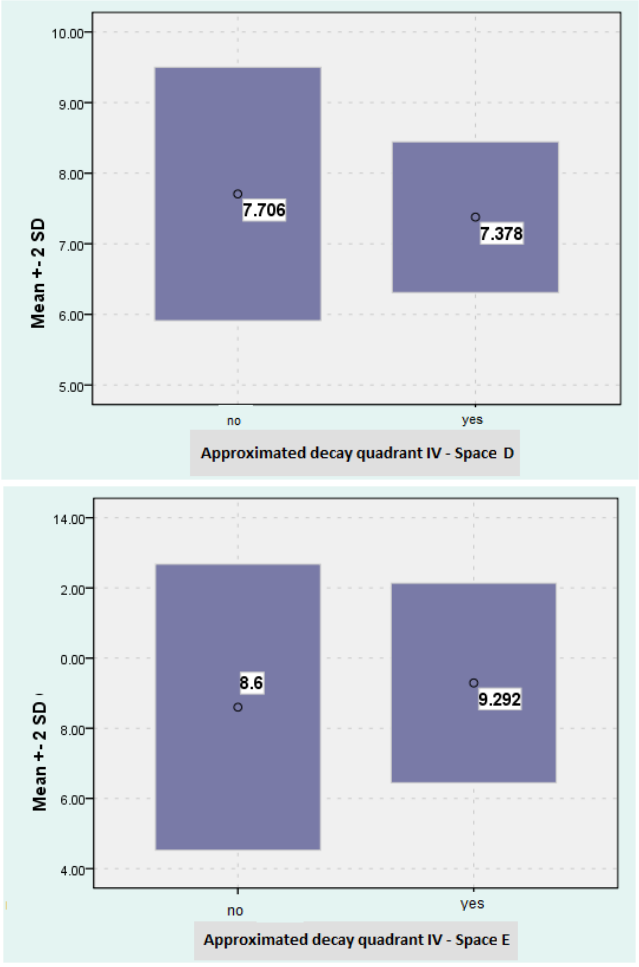

Fig. 4 .Changes in spatial dimensions D and E in quadrant IV

DISCUSSION

The research of space changes in dental and alveolar arches is difficult to accurately due to multifactorial variants that influence the results. Among the factors that influence these evaluations are: age in moment of temporary molar premature loss, facial and dental growth potential, occlusion status, vicious habits and study methodology. Another factor related to the patient was the

time while since the premature loss. Largest amount of space loss was observed within 6 months of tooth loss (Beierl & Hune, Stratford, and Wright & Kennedy) (2).

Our study showed that impaired molars with proximal cavities in the mandible determine a statistically significant reduction of leeway space. Cavities located on mesial face of second temporary molar have largest impact. Proximal caries and their sequelae are considered potential factors in creating changes in occlusal relationship (Pedersen et al., 1978) (3). Helm and Ronnerman have shown that in particular the cavities of the lower jaw have a great effect on crowding. Crowding distribution in mixed dentition occupies a greater percentage at the children affected by caries (4, 5).

In this study, statistical processing of data showed a statistically significant difference in cases of premature loss of first temporary molar on space D and in cases of premature loss of temporary second molar on the space E. Loss of two temporary molars appear to cause a decrease in space greater than the loss of only one temporary molar. The loss of area was significantly higher on mandible than in the maxillary jaw. These results are comparable to those reported in the literature (6).

The Northway et al. studied the effects of premature loss of temporarily teeth upon 107 children and they have shown that at the lower jaw was observed a severe reduction of space of 3,7mm in association with the premature loss of second temporary molar compare to the control group (7).On a group of 104 children, Ronnerman & Thilander found that children with early loss of temporary molars showed significantly less space in both jaws relative to the control group. Seipel compared the loss of space on the extraction side in 50 cases with another side, where there is not extraction. The average difference between these two sites was 1.9 ± 0.3 mm, with the majority of cases, about 70 %, less than 3 mm. Kronfeld found that 51 % of first temporary molars lost prematurely and 70% of second temporary molars lost prematurely have resulting a loss of space and consequent tooth malposition of succession of permanent tooth.

Our study revealed that premature second temporary molar extraction causes a greater reduction of leeway space than premature first temporary molar extraction, leading to the idea that premature loss of deciduous second molar is expected to have a more pronounced effect on malocclusions with crowding in the permanent dentition, because medium and great crowding cannot be solved on the basis of drift space of Nance. The mandibular arch, premature loss of first deciduous molar active during active eruption of first permanent molar can cause migration to mesial both temporary second molar and first permanent molar. Similarly, if second temporary molar fall off prematurely, mandibular arch length is reduced so that that the normal leeway space will reduce and crowding will occur (8).

Our results confirm studies by Helm, Owen, Lin and Li - Ching (9). In premature loss of first temporary lower molar, our results are consistent with those of Lin, who showed that the space D + E on the extraction side to 6 months after extraction was $15,62\text{mm} \pm 1,13\text{mm}$ significantly lower than in the control space, 16.88 ± 1.12mm.

A space maintainer is appropriate to prevent loss of space; however, it can also increase carious susceptibility due to poor oral hygiene and causes discomfort to long-

term use. Also, the failure of a space maintainer can cause parents to question the effectiveness and cost between such devices. The decision to use space maintainers should be based on good judgment and knowledge of the principles of oro-facial development and growth (10, 11).

CONCLUSIONS

Premature loss of temporary molars in mixed dentition caused a loss of space statistically significant in the lower arch.

Early space changes after premature loss of temporary molars is realized by moving to temporary canine distal to extraction space and especially by moving of second temporary and first permanent molar to the mesial.

There are changes significant statistically upon arch width, arch length and arch perimeter after premature loss of second deciduous molars.

This study shows the need for a space maintainer in the circumstances of premature loss of temporary molars to prevent loss of space and crowding.

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SCREENING OF ANTIBACTERIAL EFFECTS OF ANISE ESSENTIAL OIL ALONE AND IN COMBINATION WITH CONVENTIONAL ANTIBIOTICS AGAINST *STREPTOCOCCUS PNEUMONIAE* CLINICAL ISOLATES

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SCREENING OF ANTIBACTERIAL EFFECTS OF ANISE ESSENTIAL OIL ALONE AND IN COMBINATION WITH CONVENTIONAL ANTIBIOTICS AGAINST *STREPTOCOCCUS PNEUMONIAE* CLINICAL ISOLATES (Abstract): **Aim**: This study aimed to investigate the effects of anise essential oil alone and in combination with conventional antibiotics (amoxicillin, ciprofloxacin) against *Streptococcus pneumoniae* clinical isolates. **Material and methods**: Anise essential oil (AEO) was isolated by hydrodistillation from dried powdered fruits. Its chemical composition was investigated by GC-MS and GC-FID. Broth dilution assay was used to evaluate the antibacterial effects of anise essential oil. The interactions with antibiotics were studied by the checkerboard assay. **Results**: *Trans*-anethole (90.18%) was identified as major constituent in anise essential oil. Almost all combinations AEO-amoxicillin and AEO-ciprofloxacin showed indifferent interactions ($1 < \text{FIC index} \leq 2$). Positive interactions (addition and weak synergism) were found only for four combinations AEO-amoxicillin (FIC index=1, 0.62, 0.75 and 0.5) and one combination AEO-ciprofloxacin (FIC index=0.62). **Conclusions**: Herbal products containing anise essential oil may be used as expectorants in combination with amoxicillin or ciprofloxacin in *Streptococcus pneumoniae* infections without diminishing antibiotic efficacy. **Keywords**: *PIMPINELLA ANISUM* L., AMOXICILLIN, CIPROFLOXACIN, *STREPTOCOCCUS PNEUMONIAE* CLINICAL ISOLATES.

Pimpinella anisum L. (Apiaceae, anise) is an herbaceous annual plant native to the eastern Mediterranean region and western Asia. The fruit, also called aniseed, has a sweet, aromatic taste and a characteristic flavor due to a high content of essential oil (1.5-5%) containing *trans*-anethole as major constituent (80-90%). The fruits have been used to flavor foods and alcoholic beverages since Ancient times. Nowadays both the anise fruits and anise essential oil are used as carminative, galactagogue, spasmolytic and expectorant agents. Crushed or powdered dried fruits, alone or in combination with other herbal drugs and herbal products containing anise essential oil are recommended for their expectorant properties in lower respiratory tract infec-

tions. The efficacy is mainly due to *trans*-anethole. *Trans*-anethole activates the ciliary movements in the bronchial epithelium thus facilitating the removal of bronchial secretions (1-3). In addition, both *trans*-anethole and anise essential oil showed antimicrobial effects against different pathogens responsible for lower respiratory tract infections. Anethole was found to be effective against *Escherichia coli* 35218 (MIC = 16 μg/mL), *Pseudomonas aeruginosa* ATCC 10145 (MIC=4 μg/mL), *Klebsiella pneumoniae* RSKK 574 (MIC=8 μg/mL) and *Staphylococcus aureus* ATCC 25923 (MIC=4 μg/mL). *Escherichia coli* and *Klebsiella pneumoniae* isolates with extended spectrum β-lactamase (MIC=64 and 128 μg/mL, respectively), gentamicin-resistant *Pseudomonas aeruginosa* isolate (MIC=64 μg/mL), methicillin-resistant *Staphylococcus aureus* isolate (MIC>128 μg/mL) had a lower susceptibility to anethole (4). Anise essential oil showed antibacterial activity against *Escherichia coli* (MIC=0.5% v/v) and *Staphylococcus aureus* (MIC=0.25% v/v) (1). The efficacy of anise essential oil against isolates of lower respiratory tract infections has been poorly investigated. A literature survey revealed only one study reporting a weak activity of anise essential oil against methicillin resistant *Staphylococcus aureus* isolates: 10 μL of anise essential oil developed inhibition zones of 8-11 mm (5). Possible interactions between anise essential oil and conventional antibiotics against pathogens responsible for lower respiratory tract infections have not been investigated yet.

Penicillin-resistant *Streptococcus pneumoniae* is the major pathogen leading to community acquired infections including pneumonia (6). Amoxicillin is a first-choice antibiotic in lower respiratory tract infections. Ciprofloxacin is also an important treatment option; it has a good antibacterial activity against most of the respiratory pathogens and a high penetration rate into the bronchial tissue (7, 8). As anise preparations are often recommended in lower respiratory tract infections, the aim of the present study was to investigate possible interactions between anise essential oil and conventional antibiotics (amoxicillin, ciprofloxacin) against *Streptococcus pneumoniae* clinical isolates.

MATERIAL AND METHODS

Chemicals. Alkane standard solution C_8-C_{20} and dimethylsulphoxide (DMSO) were purchased from Sigma-Aldrich (Steinhem, Germany). Mueller Hinton agar and broth were obtained from Biolab Zrt. (Budapest, Hungary). Lysed horse blood was from Oxoid (Basingstoke, UK).

Plant material. Dried anise fruits were purchased from a local supermarket and were identified in the Discipline of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa"-Iasi where a voucher specimen has been deposited.

Bacterial isolates. Several *Streptococcus pneumoniae* clinical isolates (*S. pneumoniae* 4409; *S. pneumoniae* 4546 and 4566–resistant to penicillin; *S. pneumoniae* 4423 and 4723 – resistant to penicillin, streptomycin and erythromycin) were obtained from the Culture Collection of the Microbiology Laboratory, Lung Clinical Hospital, Iasi.

Essential oil isolation. The powdered dried fruits were hydrodistilled for 3 h in a Clevenger type apparatus. Anise essential oil (AEO) was dried over anhydrous sodium sulphate and stored in a sealed glass tube at 4°C until analysis.

Gas-chromatography analysis. GC-FID analysis of AEO was carried out on an Agilent 6890 gas-chromatograph equipped with a flame ionization detector and a DB-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). A volume of 0.3 μL of AEO was injected in the split mode (split ratio 1:30). Helium was used as carrier gas at a flow rate of 1.5 mL/min. The injector and detector temperatures were maintained at 250°C. The analysis was performed using the following temperature program: 3°C/min from 40 to 250°C, 4 min at 250°C, 10°C/min from 250 to 280°C, 2 min at 280°C. GC-MS analysis was carried out on an Agilent type 7890A gas-cromatograph equipped with an Agilent 5975C inert mass selective detector with electron impact ionization. The column and GC-MS conditions were the same as the ones described in GC-FID analysis. The MS source and quadrupole temperatures were set at 230 and 150°C, respectively. Mass spectra were acquired in the scan mode (mass range 15–450 *m*/*z*) (9). The retention indices were determined by analysing a standard solution of *n*-alkanes (C_8–C_{20}) under the same chromatographic conditions. The compounds were identified by comparing their recorded mass spectra with those stored in the GC-MS database (Wiley 275 mass spectral library) and with those of pure standards. In addition, the retention indices relative to *n*-alkanes were compared with those mentioned in literature and with those of pure standards (10, 11). The relative percentages of the essential oil constituents were obtained from the FID peak areas without using correction factors.

Broth dilution assay. The minimum inhibitory concentration (MIC) values were assessed by a broth dilution assay (12). A 200 mg/mL stock solution of AEO was prepared in a mixture of ultrapure water and DMSO. Serial two-fold dilutions of AEO, ranging from 100 to 0.05 mg/mL, were prepared in Mueller Hinton broth supplemented with 5% (v/v) lysed horse blood. Amoxicillin and ciprofloxacin were used as positive controls in dilutions ranging from 16 to 0.007 and 8 to 0.003 μg/mL, respectively. 10 μL of *inoculum*, adjusted to 0.5 McFarland turbidity standard (10^5 UFC/mL), were dispensed in 0.5 mL dilution. Negative controls (bacterial growth control, sterility control, solvents used to dilute AEO and antibiotics) were also included in the assay. The incubation was performed at 37°C for 24 h. The experiment was carried out in triplicate. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of sample that completely inhibited the visible growth of tested microorganisms.

Checkerboard assay. The checkerboard method was performed to evaluate the effects of the combinations AEO-antibiotics (12, 13). Twelve serial two-fold dilutions of AEO and antibiotics were prepared using the same procedure as described for assessment of MIC values and dispensed into 96-well plates in a checkerboard manner: AEO (50 μL) was diluted two-fold along the X-axis while the antibiotic (50 μL) was diluted two-fold along the Y axis. The *inoculum* (5 μL), adjusted to 0.5 McFarland standard of bacterial concentration, was added into each well of the plate. The plates were incubated at 37°C for 24 h. The experiment was performed in triplicate. The interaction of AEO with the antibiotics was interpreted according to the fractional inhibitory concentration index (FIC index) values. FIC index of the combination was determined by the following formula: FIC index = FIC_{AEO} + $FIC_{antibi-}$

$_{otic}$, where: FIC_{AEO} = MIC of AEO in combination/MIC of AEO alone and $FIC_{antibiotic}$ = MIC of antibiotic in combination/MIC of antibiotic alone. An interaction is defined as synergistic if FIC index ≤ 0.5, additive if 0.5 < FIC index ≤ 1, indifferent if 1 < FIC index ≤ 2 and antagonistic if FIC index > 2 (14).

RESULTS AND DISCUSSION

Anise essential oil analysis. The hydrodistillation of anise fruits gave a yellowish essential oil with a yield of 2.63% (v/w). GC-FID and GC-MS analyses of AEO revealed the presence of twelve compounds accounting for 97.34% of the total essential oil. These compounds, in order of their elution on DB-5MS column, their retention indices and percentages are listed in tab. I. *Trans*-anethole (90.18%) was the most abundant.

TABLE I
Chemical composition of anise essential oil

Compound	RI[a]	RI[b]	%
limonene	1022	1024	0.05
γ-terpinene	1050	1054	0.02
estragole	1193	1197	0.75
trans-anethole	1280	1282	90.18
γ-elemene	1335	1335	0.18
anisyl methyl ketone	1376	1380	0.11
α-himachalene	1446	1449	0.41
γ-himachalene	1476	1478	4.60
α-zingiberene	1493	1493	0.49
β-himachalene	1500	1500	0.24
β-bisabolene	1510	1506	0.31
Total			**97.34**

[a] retention indices relative to C8–C20 n-alkanes calculated on DB-5MS capillary column; [b] retention indices reported in literature.

Antibacterial activity. MIC values showed different susceptibilities of *Streptococcus pneumoniae* isolates to AEO and antibiotics (tab. II). Almost all combinations AEO-amoxicillin showed indifferent effects (FIC index=1.015-2). Additive effects on *S. pneumoniae 4566* (FIC index=1) and *4723* (FIC index=0.62 and 0.75) isolates were noticed for one and two combinations, respectively. It is worthy to note that one combination determined a weak synergistic effect on *S. pneumoniae 4723* isolate (FIC index=0.5) (tab. III). In case of combinations AEO-ciprofloxacin, only one showed an additive effect (FIC index=0.62); all the others developed indifferent effects (FIC index=1.03-2). In this study no antagonistic interactions were noticed suggesting that the co-administration of herbal products containing AEO with amoxicillin or ciprofloxacin will not affect the activity of these antibiotics in infections with *Streptococcus pneumoniae* strains.

Antagonistic interactions between plant extracts, essential oils or even phytochemicals and antibiotics have already been men-

tioned in literature. The combination *Coriandrum sativum* essential oil-cephalotin showed antagonistic effects on a large number of bacteria (*Escherichia coli*, *Staphylococcus aures*, *Pseudomonas pyocyaneus*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Yersinia enterocolitica*) (15). Essential oils of *Melaleuca alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis* in combination with amphotericin B showed mainly antagonistic effects on *Candida albicans* (13).

TABLE II

Antibacterial activity of anise essential oil, amoxicillin and ciprofloxacin against *Streptococcus pneumoniae* clinical isolates

Clinical isolate	MIC (mg/mL)		
	AEO	Amox	Cipro
S. pneumoniae 4409	12.5	0.12×10^{-3}	1×10^{-3}
S. pneumoniae 4546	6.25	0.03×10^{-3}	0.5×10^{-3}
S. pneumoniae 4566	3.12	2×10^{-3}	0.5×10^{-3}
S. pneumoniae 4423	3.12	4×10^{-3}	2×10^{-3}
S. pneumoniae 4723	25	2×10^{-3}	2×10^{-3}

AEO = Anise Essential Oil; Amox = Amoxicillin; Cipro = Ciprofloxacin.

TABLE III

Antibacterial activity of anise essential oil in combination with amoxicillin against *Streptococcus pneumoniae* clinical isolates

Clinical isolate	MIC in combination (mg/mL)		FIC		FIC index	Effect
	AEO	Amox	AEO	Amox		
S. pneumoniae 4409	12.5	0.12×10^{-3}	1	1	2	I
	12.5	0.007×10^{-3}	1	0.06	1.06	I
S. pneumoniae 4546	6.25	0.03×10^{-3}	1	1	2	I
	6.25	0.007×10^{-3}	1	0.23	1.23	I
S. pneumoniae 4566	1.56	2×10^{-3}	0.5	1	1.5	I
	1.56	1×10^{-3}	0.5	0.5	1	A
	3.12	1×10^{-3}	1	0.5	1.5	I
	3.12	0.06×10^{-3}	1	0.03	1.03	I
S. pneumoniae 4423	3.12	4×10^{-3}	1	1	2	I
	3.12	0.06×10^{-3}	1	0.015	1.015	I
S. pneumoniae 4723	3.12	2×10^{-3}	0.12	1	1.12	I
	3.12	1×10^{-3}	0.12	0.5	0.62	A
	6.25	1×10^{-3}	0.25	0.5	0.75	A
	6.25	0.5×10^{-3}	0.25	0.25	0.5	S
	25	0.5×10^{-3}	1	0.25	1.25	I
	25	0.06×10^{-3}	1	0.03	1.03	I

AEO = Anise Essential Oil; Amox = Amoxicillin; A = Addition; I = Indifference;S = Synergism.

TABLE IV

Antibacterial activity of anise essential oil in combination with ciprofloxacin against *Streptococcus pneumoniae* clinical isolates

Clinical isolate	MIC in combination (mg/mL)		FIC		FIC index	Effect
	AEO	Cipro	AEO	Cipro		
S. pneumoniae 4409	12.5	1×10^{-3}	1	1	2	I
	12.5	0.06×10^{-3}	1	0.06	1.06	I
S. pneumoniae 4546	6.25	0.5×10^{-3}	1	1	2	I
	6.25	0.06×10^{-3}	1	0.12	1.12	I
S. pneumoniae 4566	0.39	0.5×10^{-3}	0.12	1	1.12	I
	0.39	0.25×10^{-3}	0.12	0.5	0.62	A
	3.12	0.25×10^{-3}	1	0.5	1.5	I
	3.12	0.06×10^{-3}	1	0.12	1.12	I
S. pneumoniae 4423	3.12	2×10^{-3}	1	1	2	I
	3.12	0.06×10^{-3}	1	0.03	1.03	I
S. pneumoniae 4723	25	2×10^{-3}	1	1	2	I
	25	0.06×10^{-3}	1	0.03	1.03	I

AEO = Anise Essential Oil; Cipro = Ciprofloxacin; A = Addition; I = Indifference.

CONCLUSIONS

The results of this study suggest that herbal products containing anise essential oil may be used as expectorants in combination with amoxicillin or ciprofloxacin in *Streptococcus pneumoniae* infections without diminishing antibiotic efficacy.

It is obvious that an extensive investigation on a large number of clinical isolates is needed to confirm the results reported in the present study.

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NEWS

NEW TARGET FOR THE OLD ASPIRIN

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, a classical and authentic cyclooxygenase (COX) inhibitor, reduces the risk of developing several types of cancer. On site of inflammation, induction of COX-2 quickly results in the biosynthesis of prostaglandins leading to inflammatory response. Overexpression of inducible COX-2 and an increased prostaglandin biosynthesis play a significant role in carcinogenesis. Ogawa and coworkers investigated the effect of aspirin on regional lymph node metastasis during the development of lung cancer in mice. The carcinoma model was obtained by injecting a solution containing Lewis lung carcinoma (LLC) cells overexpressing green fluorescent protein (GFP) and BD Matrigel in the male mice lungs. Operative mortality was 10%. Single pulmonary nodules developed at the implanted site in 95% of animals, and regional mediastinal lymph node metastasis were observed 14 days after LLC-GFP cell injection in mice. Aspirin was orally administered (100 mg/kg, twice a day) after LLC-GFP cell injection. The reducing of percentage metastasis to regional lymph nodes was observed beginning with day 12 (63%), with no significant suppression of primary tumor growth in the lungs. Aspirin treatment led to a significant reduction in mortality (survival rate 70%; $P < 0.0001$). The authors suggested that COX inhibitors have potential to prevent lymph node metastasis (Ogawa F, Amano H, Ito Y, Matsui Y et al. Aspirin reduces lung cancer metastasis to regional lymph nodes. *Biomed Pharmacother* 2014; 68:79–86).

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NONCONVENTIONAL TECHNIQUES FOR SEPARATION OF BIOSYNTHETIC AMINO ACIDS

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NONCONVENTIONAL TECHNIQUES FOR SEPARATION OF BIOSYNTHETIC AMINO ACIDS (Abstract): Amino acids can be obtained by biosynthesis, by protein hydrolysis or by extraction from natural sources. The most efficient methods are the first two, but the separation of amino acids from fermentation broths or protein hydrolysates is rather difficult. Amino acids dissociate in aqueous solutions, forming characteristic ionic species depending on the solution pH-value. These properties make amino acids to be hydrophilic at any pH-value. This paper presents a review of the separation studies of some amino acids by nonconventional methods, namely individual or selective reactive extraction. Separation of some amino acids from their mixture obtained either by fermentation or protein hydrolysis by reactive extraction with different extractants indicated the possibility of the amino acids selective separation as a function of the pH-value of aqueous solution correlated with the acidic or basic character of each amino acid. **Keywords:** AMINO ACIDS, REACTIVE EXTRACTION, EXTRACTANT, DISSOCIATION CONSTANT, SELECTIVITY FACTOR

Amino acids represent one of the most important classes of biosynthetic compounds, being the main structural components of protein and enzymes. The growing interest for amino acids production is supported by their numerous applications in biology, biochemistry, food, pharmaceutical and chemical industry. They can be obtained by chemical synthesis, isolation from natural materials, biosynthesis and chemo-enzymatic methods (1, 2). The most promising methods for amino acids production are based on biosynthesis and protein hydrolysates, but the separation step is difficult and sometimes represents the main drawback of the biotechnological process (1-5).

The downstream processing of amino acids includes ion exchange, crystallization at the isoelectric point, chromatography, liquid-liquid extraction, aqueous two phase systems and liquid membrane (1, 4, 6). Recent studies approached the separation of amino acids by reactive extraction as an alternative for individual separation but most important for achieving selective separation of amino acids from various mixtures. Compared with solvent extrac-

tion, the reactive extraction implies the presence of an extractant added in solvent, compound that is capable to react with the solvent resulting a compound miscible only with the solvent and though increasing either the compound solubility or extraction selectivity (6-9).

Mechanism of amino acids separation by reactive extraction

Reactive extraction method have been used for the recovery of biosynthesis products (organic acids, antibiotics, vitamins, amino acids etc.) from aqueous solutions using an organic phase in which different extractants were added (10-14).

Amino acids are compounds that possess both amino and carboxyl groups (fig.1) and, depending on the functional side chain groups, they can have basic properties like arginine, acidic like glutamic acid, or neutral like glycine (2).

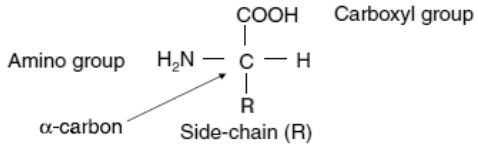

Fig. 1. General structure of amino acids

In aqueous solution, amino acids present amphoteric properties according to the following equilibrium (10):

$$^{-}H_3N-RCOOH \overset{K_{a1}}{\rightleftharpoons} H_2N-RCOOH \overset{K_{a2}}{\rightleftharpoons} H_2N-RCOO^{-}$$

If the pH of aqueous solution is lower than their corresponding dissociation constant (pKa) the amino groups are protonated, and ionized if the pH is above the respective pKa value.

The solubility of amino acids in nonpolar solvents is very low, the extraction using nonpolar solvent being very difficult. However, the extraction efficiency can be increased by the addition of cationic or anionic extractants which, at certain pH-values, below or above the isoelectric point of an amino acid can form complexes with amino acids, which are preferentially soluble in the organic phase (7, 15-17).

Separation of amino acids by reactive extraction is possible by using an extractant in the organic phase, like phosphoric acid derivates, high molecular weight quaternary aliphatic amines or crown ethers (7,17-21).

The reactive extraction of amino acids with an organophosphoric extractant (HP) occurs by means of an interfacial chemical reaction of the ion exchange type and implies the presence of amino acids in the cationic form (17):

$$R_1^{+}\text{-}CH(NH_3^{+})\text{-}COOH_{(aq)} + 2HP_{(o)} \rightleftharpoons R_1^{+}\text{-}CH(NH_3^{+})\text{-}COOH.P^{-}{}_{2\,(o)} + 2H^{+}_{(aq)}$$

The extraction of amino acids depends on the pKa values of the amino acids and the cationic extractant. A predicting model for extraction behavior of amino acids by cationic extractants was developed by Lukhezo et al. (23) and correlated well with the experimental data from extraction systems of individual amino acids with di(2-ethylhexyl)phosphoric acid(D2EHPA), di(2-ethylhexyl) monothio phosphoric acid (D2EHPA(S)), dinonyl naphthalene-sulphonic acid (DNNSA) and Versatic 10

(decanoic acid) in toluene. The main disadvantage of the extraction at low pH-value of aqueous phase is the co extraction of other cations existing in solution.

Extraction of amino acids with amine occur with the formation at the interface of hydrophobic ion pairs with the participation of the protonated amino acid (pH of the aqueous phase lower than the pH of isoelectric point of the amino acid), of an anion present in the aqueous solution and an extractant added in the organic solvent (17):

$$R\text{-}CH(NH3+)\text{-}COOH(aq) + A\text{-}(aq) + Q(o) \leftrightarrow R\text{-}CH(NH3+A\text{-})COOH.Q(o)$$

The reactive extraction with the amine salts requires the amino acid in the anionic form which occurs for pH-value of the aqueous phase greater than the pH of the amino acid isoelectric point. In the extraction system can be established the following equilibriums:

- dissociation of amino acid in the aqueous phase

$R\text{-}CH(NH_2)COOH_{(aq)} + HO^-_{(aq)} \leftrightarrow H_2O + R\text{-}CH(NH_2)COO^-_{(aq)}$

- reactive extraction of amino acid in the anionic form by an interfacial reaction of ion exchange type

$R\text{-}CH(NH_2)COOH^-_{(aq)} + QCl_{(o)} \leftrightarrow R\text{-}CH(NH_2)COO^-Q^+_{(o)} + Cl^-_{(aq)}$

- coextraction of hydroxyl group or of another anion present in aqueous phase

$HO^-_{(aq)} + QCl_{(o)} \leftrightarrow Q^+HO^-_{(o)} + Cl^-_{(aq)}$

Besides pH-value, the extraction of amino acids is influenced by the extractant concentration, surface-active substances in the system, the intensity of mixing, etc. One of the most important challenges in the separation of amino acids is the coextraction of other compounds present in the medium, like secondary amino acids, hydroxyl ions, affecting the extraction of the main solute. Some of them may exist in an ionic form, at the optimum pH of extraction, which can compete with primary amino acid and so decreasing the separation efficiency (24).

Separation of individual and mixture of amino acids

In order to achieve a high selectivity for extraction of different compounds from mixtures it is necessary to understand the mechanism and conditions for extraction process which is usually performed for every compound by studying the behavior of this in individual extraction systems.

Optimum conditions for recovery of amino acids are usually achieved by use of hydrophobic counter-ions. For example, Cascaval et. al (1996), Kelly et. al (1998), Smirnova et al. (2004) studied the extraction of amino acids with cationic extractants like D2EHPA and DNNSA. The cationic carrier di(2-ethylhexyl)phosphoric acid (D2EHPA) was widely used for extraction of amino acids because it is one of the most promising candidates for its chemical stability, high complexing ability, stripping characteristics, and extremely low solubility in aqueous acidic solutions (5,17,25).

Didi et al. (2009) studied the extraction of two aminoacids, phenylalanine and tyrosine, with different extractants: D2EHPA, TOPO (trioctylphosphine oxide) and TBP (tri n-butyl phosphate) dissolved in chloroform and Aliquat 336 (n-methyl-n,n-dioctyloctan-1-ammonium chloride) dissolved in kerosene (26). The experimental data indicated that the best extraction system for phenylalanine was the one with D2EHPA, the extraction yield being

82.12%, followed by D2EHPA + TBP (73.48%) and Aliquat 336 + kerosene (30.1%). Reactive extraction of tyrosine with D2EHPA or Aliquat 336 showed the highest yield of 23.4% and 22.4% respectively. Once more, the authors highlighted that the separation yield is controlled by the pH-value of the aqueous phase, which is due to the acidic or basic character of each amino acid (26).

The extraction of phenylglycine in alkaline solution using TOMAC (tri-n-octylmethylammonium chloride) as extractant dissolved in1-decanol was studied by Munoz et al. (10). The authors quantified the extraction yield as a function of the chloride/phenylglycine initial molar ratio and the influence of water coextraction on the extraction efficiency. As shown in figure 2, the extraction yield increases with the chloride/phenylglycine initial molar ratio, for the same initial hydroxide ion concentration, decreases when the initial hydroxide ion concentration is increased for the same chloride/phenylglycine initial molar ratio, and reaches an asymptotic value due to the limited amount of phenylglycine and hidroxyl ions to be extracted.

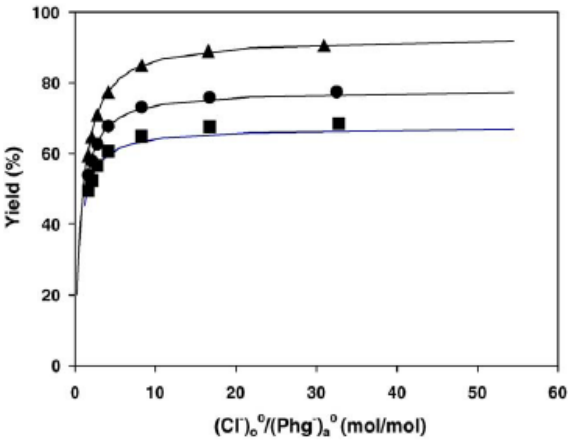

Fig. 2. Extraction yield of phenylglycine as function of chloride/phenylglycine initial molar ratio. $[Phg^-]^0_a$ = 3.3–66.1 mM, *Va*/*Vo* = 2.
(▲) $[OH^-]^0_a$ = 10 mM; (●) $[OH^-]^0_a$ = 50 mM; (■) $[OH^-]^0_a$ = 100 mM.

There are some studies where the separation of phenylalanine was performed for high values of aqueous phase pH, using TOMAC, Adogen 464 or Amberlite LA-2 dissolved in xylene (26,27). Thus, Cameron et al (2001) studied the recovery of amino acids from aqueous solutions by reactive extraction using a mixture of extractants: D2EHPA and Aliquat 336 dissolved in methyl isobutyl ketone (26). By using this extraction system it was possible to extract phenylalanine for large range of aqueous phase pH from 1.8-9.2. When using only the Aliquat 336 in methyl isobutyl ketone for extraction of phenylalanine, the extraction mechanism involves an ion exchange reaction between chloride ion of extractant and the amino acid anion. The formation of 2:1 complexes form was explained by the possibility of formation of hydrogen bonds

between the amino acid zwitterion form and a complex amino acid / extractant. If the extraction is carried out using only D2EHPA, at the formation of the interfacial complex participates one molecule of amino acid and one molecule of extractant (27).

Extraction of tryptophan and tyrosine from basic solutions using TOMAC, as extractant, dissolved in xylene requires the amino acids to be in anionic form, a pH-value with at least two units greater than the corresponding value of pK_{NH2} = 9.39. Tyrosine extraction occurs by formation of dianions by proteolyses of the phenolic proton, pH_{OH} = 10.07 at pH greater than 12. At the end of the extraction process, tryptophan remains in the organic phase, whereas tyrosine is transferred into the aqueous phase thus the selective separation can be achieved (28).

Caşcaval et al. (1996) studied the extraction of glutamic acid using Amberlite LA-2, dissolved in butyl acetate. It was observed that the pH of the aqueous phase or of the emulsion has a very important influence on the yield of extraction. Increasing the pH-value from 0.5 to pH = 3.2, corresponding to the isoelectric point, leads to a decrease of the extraction efficiency from 98% to 0%. At low pH-values corresponding to the first stage of glutamic acid ionization, pK_1, the amino acid is found only in the protonated form. Consequently, the reactive extraction processes involve the protonated glutamic acid and the anion derived from sulfuric acid (used to change the pH) in the aqueous phase and extractant in the organic phase. The complex formed is of ion pair type and it is soluble only in the organic phase. The coefficients for individual mass transfer (tab. I) were determined for three values of pH and three concentrations of the extractant (29).

TABLE I

Values of the individual mass transfer coefficients

Extractant concentration, g/l	**pH**	**$k \cdot 10^2$, s^{-1}**
64	1.5	2.66
92	0.5	4.60
92	1.5	4.00
92	2.0	0.67
166	1.5	5.00

In another study, Cascaval et al. analyzed the possibility to extract individual amino acids with basic, acidic or neutral character by reactive extraction systems with D2EHPA as extractant dissolved in butyl acetate. In all cases, the extraction degree increases with the increase of pH value, reaches a maximum for a pH extraction domain of about 2-3 pH units, followed by a sharp decrease. The maximum is the result of the two opposite phenomena which occur as pH-value increases: increase of the concentration of the extractant active form which is able to react with the amino acid and decrease of the total amount of amino acid present in cationic form (17).

Reactive extraction of the acidic amino acids: aspartic acid, glutamic acid occurs with high efficiency at pH = 2.5, the extraction degree being comparable: 77% for aspartic acid and 80% for glutamic acid (figure 3).

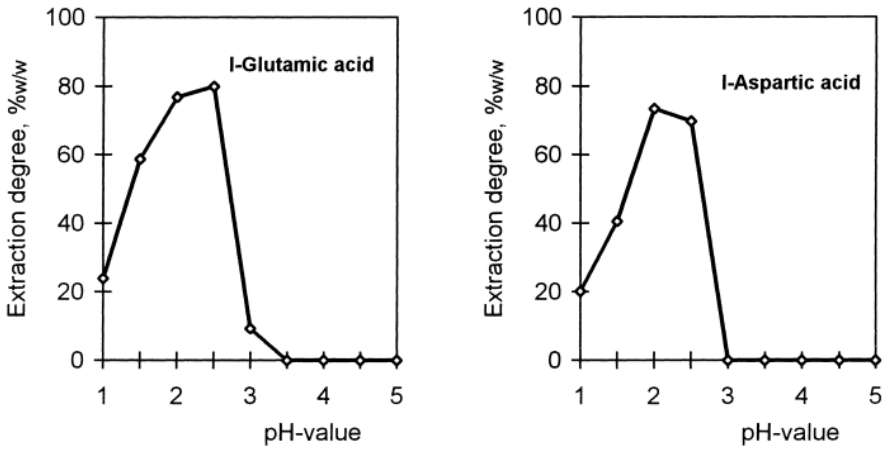

Fig. 3. Effect of aqueous solution pH-value on the reactive extraction degrees of acidic amino acids with D2EHPA dissolved in butyl acetate.

Due to the increasing concentration of the zwitterions of both amino acids in the aqueous phase, any further increase of pH leads to strong decrease of the extraction degree. At the isoelectric point of each amino acid reactive extraction becomes ineffective (17). For extraction system of neutral amino acids (l-glycine, l-alanine, l-tryptophan, l-cysteine), the maximum extraction yields were obtained at pH = 3 and depend on the amino acid hydrophobicity, the values varying between 78.4% for l-glycine and 90% for tryptophan. For pH-values higher than 3, the separation efficiency decreases, as a result of the amino acid carboxyl group dissociation and reaches zero at the corresponding value of isoelectric point. The maximum separation yield for the basic amino acids studied (l-histidine, l-lysine, l-arginine) varies between 57.8% w/w for l-histidine and 68% w/w for l-lysine and l-arginine, being reached at pH-value of 2.5–3. The difference is due to the more pronounced dissociation of l-histidine at this pH-value and, thus, to the formation of dication-anionic species in aqueous solution. The increase of pH-value leads to a significant decrease of the extraction degrees, which become 0 at pH 4 for l-histidine, and pH = 5 for the other two amino acids (17).

Selective separation of amino acids from fermentation broths is currently an intensively studied problem. Analysis of the correlation between the extraction constant the pH at isoelectric point and the hydrophobicity of amino acids is a precondition to achieve selectivity for extraction of these compounds.

Kelly et al. (1998) studied the extraction and fractionation of mixtures of amino acids by some cationic extractants with varying acid dissociation constants (D2EHPA, DNNSA, D2EHPA(S) and Versatic 10) dissolved in toluene. The authors evaluated the separation factors, as the ratio of partition coefficients, between amino acids with similar chemical structures. The amino acid mixture included arginine, phenylalanine, glycine, alanine and aspartic acid. It was observed that the extraction capacities for the amino acids, subject to the extractants,

were generally found to decrease in the order arginine < phenylalanine < alanine < glycine < aspartic acid. In order to achieve high efficiency of selective extraction (over 80%) the experiments were performed using an excess of cationic extractants, their concentrations being: 4 times for DNNSA, 400 times D2EHPA and D2EHPA(S) and 1000 times Versatic that of amino acid concentrations. The separation factor of amino acids with similar chemical structure, defined as the ratio of the distribution coefficient between two amino acids, were calculated for different pH-values and for used extractant, except Versatic 10 (in which extraction was poor). The highest values for extraction efficiency and the separation factor were obtained for the extraction system with DNNSA (tab. II). Extractive loading capabilities of the extractants were found to decrease in the order DNNSA < D2EHPA(s) < D2EHPA < Versatic 10.

TABLE II

Separation factor of amino acids at different pH-values by DNNSA

Amino acid pairing	Separation factor				
	pH = 1	pH = 1.5	pH = 2.5	pH = 3	pH = 3.5
arginine-henylalanine	9.4	11.0			
phenylalanine-alanine		16.7	11.5	6.3	3.6
alanine-glycine		4.8	5.4	7.8	11.9
glycine-aspartic acid		2.7	3.0	3.0	

Selective separation of some amino acids was the aim of the study performed by Caşcaval et al. (2001). The authors studied the separation by reactive extraction with D2EHPA in butyl acetate of amino acids with acidic character (aspartic acid, glutamic acid), basic character (histidine, lysine, arginine) and neutral character (glycine, tryptophan, cysteine, alanine). Due to the rather low extraction degrees of each group of amino acids at the required pH-values for selective separation, for total recovery of the considered amino acids a multistage extraction has to be used (tab. III).

TABLE III

The experimental conditions for the reactive extraction and separation of amino acids from a mixture

Extracted amino acids	pH domain	One stage extraction degree (% w/w)	Number of extraction stages
Neutral amino acids	5–5.5	20-25	9
Acidic amino acids	2–2.5	60-63	3
Basic amino acids	4–4.5	18-25	10

By means of the pH influence on reactive extraction of the considered amino acids, an operation flow for selective separation has been elaborated and demonstrated experimentally.

Using multistage extraction, the total separation of the following amino acids groups has been performed: neutral amino acids (l-glycine, l-alanine, l-tryptophan) at pH 5–5.5, basic amino acids (l-lysine, l-

arginine) and l-cysteine at pH 4–4.5, l-histidine at pH 3–3.5, and acidic amino acids (l-aspartic acid, l-glutamic acid) at pH 2–2.5 (17).

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NEWS

FATIGUE LIFE OF RECIPROC AND MTWO INSTRUMENTS SUBJECTED TO STATIC AND DYNAMIC TESTS

This study evaluated the influence of flexibility and reciprocating movement on the fatigue life of endodontic instruments subjected to static and dynamic tests. The rotary nickel-titanium instruments used in this study were Reciproc and Mtwo. The instruments were initially subjected to a cantilever-bending test and then to static and dynamic fatigue tests. Reciproc instruments were operated in reciprocating movement, whereas Mtwo instruments were worked in continuous rotation. The means of bending resistance (maximum load in grams) of the instruments were 274.9 for Reciproc and 429 for Mtwo. The mean times (in seconds) to fracture of the instruments subjected to static and dynamic tests were 214.5 (static) and 286.3 (dynamic) for Reciproc and 38.9 (static) and 99 (dynamic) for Mtwo. The Student's t test revealed significant differences in all tests ($P < .05$). The results of the present study showed longer fatigue life for instruments with higher flexibility, driven by reciprocating movement, and in the dynamic testing model. These findings reinforce the assumption that use of reciprocating movement is a means to prolong the fatigue life of rotary nickel-titanium endodontic instruments during instrumentation of curved canals (Lopes HP, Elias CN, Vieira MV, Siqueira JF Jr, Mangelli M, Lopes WS, Vieira VT, Alves FR, Oliveira JC,Soares TG. Fatigue Life of Reciproc and Mtwo instruments subjected to static and dynamic tests. *J Endod*. 2013; 39(5): 693 – 696).

Gianina Iovan

CHITOSAN/HYALURONIC ACID POLYELECTROLYTE COMPLEX HYDROGELS IN THE MANAGEMENT OF BURN WOUNDS

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CHITOSAN/HYALURONIC ACID POLYELECTROLYTE COMPLEX HYDROGELS IN THE MANAGEMENT OF BURN WOUNDS (Abstract): **Aim:** This paper is a short review on the state-of-the-art of the use of polyelectrolyte complexes containing polysaccharides for wound and burn healing and treatment using the chitosan/hyaluronic acid polyelectrolyte complex (PEC) hydrogel. **Results and conclusions**: PEC is suitable for wound healing because a wet treatment can be realized and both components of PEC contribute by their properties to the enhanced antimicrobial activity, promote wound healing and prevent wound damage during treatment. **Keywords**: WOUND HEALING, DRESSING, CHITOSAN, HYALURONIC ACID.

Contamination and infection remain serious complications of wound burns and sepsis is a leading cause of mortality and morbidity in patients with major burns. Several factors contribute to the colonization of wounds, including the presence of: serous exudates that contain several proteins providing nutrients for bacteria, necrosed tissue that enhances the proliferation of micro-organisms and their invasion of the underlying viable tissues, and a compromised immune response that prevents the full expression of natural defenses (1). Initially, a burn is sterile, but rapidly becomes colonized, predominantly by Gram positive bacteria in the first instance (2). Once bacterial density reached up to 10^5/g of tissue, wound infection and sepsis are likely to occur. Early application of topical antimicrobial agents is the most effective way of preventing invasive burn infection when combined with early eschar excision (3).

Wound healing is an intricate process in which the skin (or another organ-tissue) repairs itself after injury. It is a particular biological process related to general phenomenon of growth and tissue regeneration. Wound healing progresses by a series of independent overlapping steps such as hemostasis, inflammation, migration/granulation/proliferation and maturation phases of tissue formation, matrix remodeling/reshaping/ and reepithelialization, in which a variety of cellular and matrix components act together to re-establish affected tissue integrity and to replace the

lost tissue (4).

Dressings are applied on open wounds for centuries to protect them from injuries and bacterial invasion. Recently, dressings made from novel polymers are used for the delivery of drugs to acute, chronic, and other types of wounds. Many types of dressings, of occlusive or semi-occlusive nature have been developed. These include collagen/glicosaminoglican sponges, alginate gels, hydrocolloids, hydrogels, polyurethane, collagen, chitosan (CS), pectin and hyaluronic acid-based biomaterials. CS has a beneficial influence in all stages of wound healing (5). Wet dressings have a superior quality because the controlled wet medium at the interface wound- bandage favors wound healing (6).

CHITOSANS (CSs) are polycationic copolymers of β -(1 - 4)-2-acetamido-2-deoxy-D-glucopyranose (deacetylated units) and 2-amino-2-deoxy-D-glucopyranose *N*-acetyl-D-glucosa-mine (acetylated units) as chitin derivatives with different deacetylation degrees (from 70 to 95%) and average molecular weight ranges from 10 to 1.000 kDa (7, 8) (fig. 1).

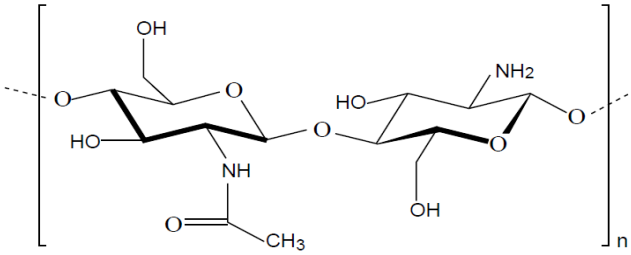

Fig. 1. The CS structure

Chitosan exhibits a good biocompatibility and biodegradability (9) which allows its wide medical applications as topical formulation in ophthalmology, as implants (10) or injection (11), and facilitates the penetration of hydrophilic drugs across mucosal epithelia. Because of the positive charges at physiological pH, CS is a good bioadhesive, increasing the retention to the application sites, efficient for wound healing (12) also because of its bacteriostatic effect (13).

Many studies have reported on the use of chitin, CS, and their derivatives as wound healing accelerators (>75 %) (14), acting in each stage of the healing process by enhancing the functions of the inflammatory cells polymorphonuclear leukocytes, macrophages, fibroblasts or osteoblasts, and by increasing the resistance to wound extension (15).

To further improve the CS properties, chemical modification and copolymerization are applied. These techniques could lead to the formation of entities with unknown toxicological profiles, therefore they should be avoided and the strategies based on non-covalent bonding preferred, this being achieved by electrostatic forces, hydrophobic or hydrogen bonding. Amino groups C_2 position in glucopyranosic units of CS can electrostatically interact with anionic groups (e.g. carboxyl) of polyanions of natural origin (such as pectine, alginate, carrageenan, xanthan gum, carboxymethyl cellulose, chondroitin sulphate, dextran sulphate, hyaluronic acid) or syn-

thetic origin (e.g. polyacrylic acid, polyphosphoric acid, poly(L-lactide) forming polyelectrolyte complexes (PEC) (16).

Hyaluronic acid (HA) was discovered by *Meyer and Palmer* in 1934 in the vitreous humor of bovine eyes. Hyaluronic acid (also called hyaluronan or hyaluronate) is the only nonsulphated glycosaminoglycan found in the extracellular matrix throughout connective, epithelial and neural tissues. Hyaluronic acid is a naturally occurring physiological constituent of the connective tissue, especially in the gingival mucosa (17). It is a glycosaminoglycan, a linear anionic polysaccharide, composed of repeating disaccharides, α-(1,4)-D-glucuronic acid, and β-(1,3)-N-acetyl-D-glucosamine linked by β-(1 - 3) bonds (fig. 2).

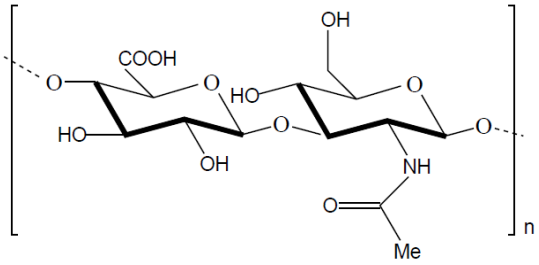

Fig. 2. The hyaluronic acid structure

HA is produced by bacterial fermentation of a *Streptococcus* sp. or is extracted for commercial purpose from rooster combs, umbilical cords, synovial fluids, or vitreous humour. It has been used in ophthalmic surgery, arthritis treatment, in tissue engineering, component of scaffolds for wound healing and implants devices (18). The implants covered with HA and its derivatives reduce adsorption, adhesion and cellular proliferation of *Staphlococcus aureus* at least 100 times (19).

HYDROGELS are macromolecular networks swollen in water or biological fluids. Hydrogels are often divided into three classes depending on the nature of their network, namely entangled networks, covalently cross linked networks, and networks formed by physical interactions, but a clear differentiation between them does not exist. Chemical hydrogels are formed by irreversible covalent bonds, while physical hydrogels are formed by various reversible links. These can be ionic interactions, as in ionically cross linked hydrogels and polyelectrolyte complexes (PEC), or secondary interactions, as in CS/poly (vinyl alcohol) (PVA) complexed hydrogels, grafted CS hydrogels and entangled hydrogels. The latter are formed by solubilization of CS in an acidic aqueous medium and their use is limited by their lack of mechanical strength and tendency to dissolve (20).

Due to the potential toxicity of free unreacted covalent cross linkers, which require an additional purification step during hydrogel manufacturing, the development of alternative types of hydrogels was necessary and desirable. Hydrogels formed by direct interaction between polymeric chains without the addition of cross linkers were prepared by complexation with another polymer or by aggregation after grafting.

POLYELECTROLYTE COMPLEX HYDROGELS (PECs)

Polyelectrolyte complex hydrogels are formed by ionic crosslinking (of ions or ionic molecules with high molecular weight, and in the case of polyelectrolytes with a certain distribution of molecular weight) and are generally biocompatible networks exhibiting interesting swelling characteristic, being an alternative to covalently cross linked hydrogels. Electrostatic attraction between amino cationic groups of CS and anionic carboxyl group of HA is the main and the strongest interaction in this PEC (fig. 3).

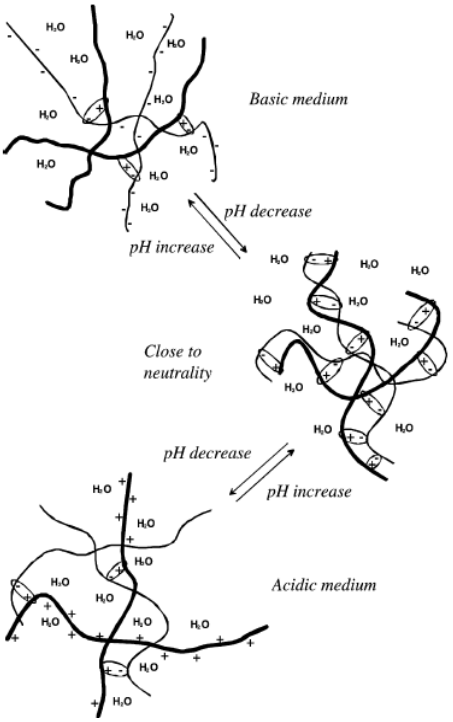

Fig. 3 pH-dependent structure and swelling of PEC containing a polysaccharide (as CS): - negative charges (HA), + positive charges (CS), ⬭ ionic interactions, —— CS; —— HA (adapted from 25)

BIO-SMART HYDROGELS rapidly respond to the action of external stimuli (or local environmental variations) by changes in the internal structure, size, volume, refraction index, and mechanical properties of the network. They are considered superabsorbent materials because they can absorb a high amount of water (solvent), hundred times their weight. Compared to covalently cross linked hydrogels, PEC has a higher swelling sensitivity, especially to pH changes, which led to a wide variety of applications. Bio-smart polysaccharidic hydrogels show good biocompatibility and bacteriostatic properties necessary for wound healing (20).

PEC PREPARATION

CS or its positively charged derivatives, such as glycol CS (21) or N-dodecylated CS (22), and HA are used. The reaction occurs in aqueous solution, this being the main advantage over covalent crosslinking, because biocompatibility is assured and purification before delivery avoided. Both polymers should be in ionized state and bear opposite charges, therefore the reaction can only occur at a pH value close to the pK interval of both polymers; pK_a of CS is ~ 6.5 (23) while that of HA is about 3.0 (24). During complexation, polyelectrolyte may coacervate or form a less compact hydrogel; if interactions are very strong precipitation occurs. Precipitation is very frequent and hinders hydrogel formation. It can be avoided by weakening the electrostatic interaction, adding salts such as NaCl. The presence of salts reduces the interaction between electrolytes by the contribution of the ions in the reaction environment. Phase separation usually does not occur, and a viscous, macroscopically homogeneous mixture, which may gel

when temperature is lowered, is obtained (25). Other factors affecting PEC properties are: pH, temperature, ionic strength, order of mixing, as well as polymer flexibility, molecular weight, degree of deacetylation of CS, and substitution degree of both polymers when derivatives are used. Injectable hydrogels for *in vitro* delivery of an adipogenic factor of insulin and adipose tissue engineering have been prepared by Schiff-base reaction between *N*-succinyl-CS and aldehyde of hyaluronic acid (26) and HA/CS polyelectrolyte complexes were obtained also by electro spinning (27).

PEC PROPERTIES

Stoichiometric PECs contain equal amounts of oppositely charged polyions, so the resulted PEC will have zero overall net charge, are usually insoluble and precipitate from solution (28). CS properties are maintained in PEC and hydrogels, therefore these are very well tolerated by living organisms and can be used in various applications: controlled release systems, cell cultures, enzymes immobilization, tissue regeneration and wound healing. CS-HA PEC protects HA against enzymatic hydrolysis, but only at pH values different of the optimum for enzymatic activity. Cells proliferation and activity in wound healing is reduced comparatively with that of CS (29). Both swelling and release profiles are pH-responsive. In acidic medium polyacid is neutralized, and because of free amino groups of CS positive charges will be present within gels. Mutual repulsion and water penetration together with contraions to neutralize these charges will determine the swelling. In case of a prolonged immersion in water a shrinkage is observed, because of segmental mobility of polyelectrolyte chains in swollen state, which allow the completion of interpolyelectrolyte reaction (30). In basic medium the mechanism is the same, but swelling is induced by negative charges of the polyacid (31). This is the logical reason for sensibility of the swelling to the ions presence and swelling rate is controlled by diffusion of mobile ions and changes in ionization degree. Osmotic pressure and electrostatic repulsion are responsible for swelling, which control the network forces depending on elasticity. If swelling becomes too important, dissolution happens at certain pHs. To prevent dissolution, covalent crosslinking is applied, but this will create biocompatibility problems.

TISSUE REGENERATION AND WOUND HEALING USING CS/HA CONTAINING FORMULATIONS

CS, with a structure similar with that of glycosaminoglycans (GAG) is indicated for preparing PEC hydrogels with GAG polyanions, such as chondroitin sulphate or HA, useful in cartilage reconstruction and wound healing, which mimic the GAG-rich extracellular matrices of articular chondrocytes (32).

In vitro tests showed that these PECs could be used as carriers for chondrocyte transplant and/or scaffold for cartilage-like tissue engineering. PEC CS/ HA as sponges and films allow the culture of various specific cells, such as keratocytes, which produces skin matrix accelerating wound healing after skin ablation without inflammatory reactions and toxicity for animals (29). When PEC is obtained from chitosan and other biocompatible polymers, it can be applied as bandages or powders, which protect the wound, accelerate healing and prevent bacterial contamination (33).

Composite CS/HA films with high transparency can be prepared using glass or

polymethylmethacrylate substrates, but not with PTFE. With increasing HA amount, the films become rougher, increasing the water contact angle and water uptake and decreasing water vapor permeability, BSA adsorption and fibroblasts adhesion, all these being desirable characteristics for wound bandages. Comparatively with Vaseline, CS/HA films are more efficient in wound healing because they do not cause wound damage when the bandage is removed. (34). Rossi *et al*. (35) prepared blends of CS hydrochloride, 5-methyl-pyrrolidinone CS and HA by freeze-drying. Chlorhexidinediacetate was used as antimicrobial agent. HA decreased the hydration capacity but modulated the antimicrobial agent release. It was suggested that the mechanism of the antimicrobial activity of CS consists in the interaction of positively charged CS molecules with negatively charged microbial cell membranes, which leads to disruption of bacterial membrane and loss of protein and intracellular constituents (36).

CONCLUSIONS

PECs of chitosan with hyaluronic acid are suitable for wound healing because a wet treatment can be realized and both components of PEC contribute through their properties to the enhanced antimicrobial activity, promote wound healing and prevent wound damage during treatment. Polyelectrolyte complexation occurs under mild reaction conditions and biocompatible PEC systems are obtained. PEC hydrogels exhibit a highly pH-sensitive swelling, therefore they can be used for pH-controlled drug delivery in different conditions.

ACKNOWLEDGMENTS

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RESEARCH ON THE DEVELOPMENT OF BIOADHESIVE VAGINAL TABLETS CONTAINING 5-FLUOROURACIL

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RESEARCH ON THE DEVELOPMENT OF BIOADHESIVE VAGINAL TABLETS CONTAINING 5-FLUOROURACIL (Abstract): Biomucoadhesive vaginal tablets are modern formulations used in current therapy to achieve controlled release of the active substance at the application site by maintaining the pharmaceutical preparation at that level. This can be achieved by using mucoadhesive substances with different mechanical and physical-chemical properties. Two cellulose derivatives of different viscosity, Metolose 90 SH 4000 and Metolose 90 SH 100000, and two types of polyacrylates with different cross linking degrees, Carbopol 71, low degree of cross linking, and Carbopol 974, high degree of cross linking were used. In a previous study twelve original formulations of bioadhesive vaginal tablets containing 100 mg 5-fluorouracil (5-FU) /tablet (F1-F12) were formulated, prepared and analyzed. The pharmacotechnical characterization of the bioadhesive vaginal tablets containing 5-FU was performed by determining their specific quality characteristics. For the optimization of formulations, the influence of formulation factors on some quality characteristics (mechanical strength, friability, disintegration time) which may be influenced by the nature and amount of auxiliary substances used was studied by SPSS statistical software and statistical analysis ANOVA tests. The results are in favor of formulations F1, F2 containing 20-30% Carbopol 71 and of 37- 47% Microcelac. **Keywords**: 5-FLUOROURACIL, VAGINAL TABLETS, BIOADHESIVE VAGINAL TABLETS

In a previous study (1) twelve original formulations of bioadhesive vaginal tablets containing 100 mg 5-FU/tablet were prepared (2). The concentrations of matrix-forming polymers (3.4), two cellulose derivatives of different viscosity, Metolose 90 SH 4000, Metolose 90 SH 100000, and two types of polyacrylates with different degree of cross linking, Carbopol 71 and Carbopol 974, varied for each series of formulations in the 20 to 50% range. The pharmacotechnical characterization of the bioadhesive vaginal tablets containing 5-FU was performed by determining their specific quality characteristics (5,6). The results reported by the authors show that they fall within the limits stipulated by the regulations in force (5,6).

For the optimization of the formulation we aimed at analyzing the influence of formulation factors, independent variables (Xi), on some quality parameters of hydro-

philic matrix tablets containing 5-FU, namely resistance (Y11), friability (Y22) and disintegration time (Y44).

The use of experimental design in the formulation of matrix tablets and formulation analysis based on these quality characteristics required by bioadhesive vaginal tablets allows ranking the importance of independent variables (Xi) in relation with their degree of influence on the dependent variables (Yi).

In this respect we used the specialized SPSS software and statistical analysis ANOVA tests that are highly flexible, allowing the study of factors with linear constraints in the setup, irregular experimental spacing, and qualitative factors (7,8).

MATERIAL AND METHODS

Materials

We analyzed twelve original formulations of bioadhesive vaginal tablets containing 100 mg 5-FU/tablet.

Method

For the study an experimental design with 5 variables and four levels was selected (tab.I). The statistic analysis were made using SPSS statistical software, and parametric statistical tests - ANOVA

TABLE I

Independent variables (formulation factors)

Independent factors	Symbol	Levels			
		0	1	2	3
Carbopol 71 G NF	X1	0	60	90	150
Carbopol 974 P NF	X2	0	60	90	150
M 4000	X3	0	60	90	150
M 100000	X4	0	60	90	150
Microcelac	X5	0	50	110	140

An experimental study on a system is characterized by a response Y (dependent variable) that varies depending on various factors Xi (independent variables).

RESULTS AND DISCUSSION

The original biomucoadhesive tablets containing 5-FU have the shape of a uniform disk, with entire edges, are flat, white, spotless, and odorless. Mass uniformity is bellow the ± 7.5% limit specified in the Romanian Pharmacopoeia for 300 mg tablets. The results of pharmacotechnical characterization (tab. II) show that the analyzed tablets are within the limits stipulated by the regulations in force for friability, disintegration time and mechanical strength.

The results obtained by the statistical processing of dependent variables show a normal distribution, Gauss curve, which allows the use of parametric tests for statistical processing (fig. 1).

a Dependent Variable: Y11

Independent variables X1-X4 had an insignificant ($p>0.05$) and negative influence (coefficient B) by diminishing mechanical strength, which technologically means that for obtaining tablets with adequate strength 20-30% of bioadhesive excipients should be used (tab. III, IV).

TABLE II

Matrix of experimental design responses

Parameters Formula	Y22 Friability%	Y11 Mechanical Strength(N)	Y44 Disintegration Time (min.)
F1	0.09	94.96	99
F2	0.16	92.52	165
F3	0.38	85.00	196
F4	0.61	60.56	76
F5	0.68	56.42	79
F6	0.72	52.16	67
F7	0.33	94.20	133
F8	0.20	100.92	158
F9	0.22	102.24	188
F10	0.28	100.15	141
F11	0.26	108.64	232
F12	0.25	111.38	282

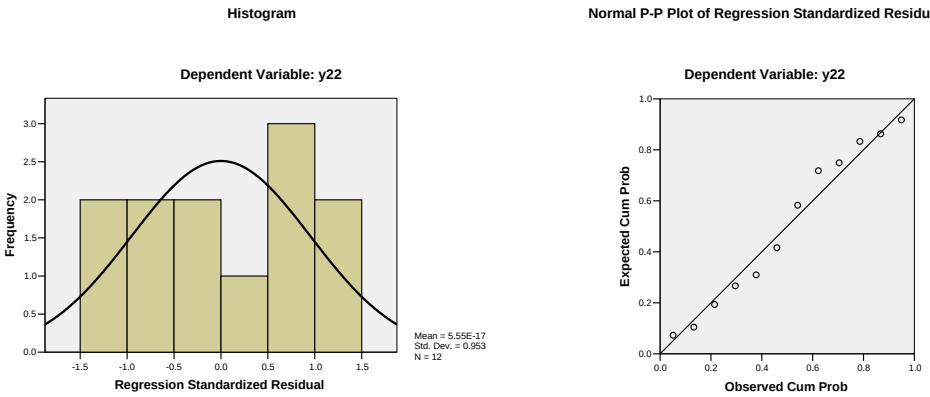

Fig. 1. Histogram and P-P Plot of standardized regression residual for dependent variable Y22

TABLE III

Influence of formulation factors on mechanical strength.

Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	636.523	362.629		1.755	.130
	X1	-2.514	1.791	-5.896	-1.404	.210
	X2	-2.903	2.033	-6.810	-1.428	.203
	X3	-2.484	1.791	-5.826	-1.387	.215
	X4	-2.447	1.791	-5.739	-1.366	.221
	X5	-3.004	1.847	-5.673	-1.627	.155

TABLE III
Influence of formulation factors on friability
Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.095	.107		-.890	.394
	X5	.004	.001	.808	4.339	.001

a Dependent Variable: Y22In the case of friability, dependent variable Y22, the regression model excluded from calculation the independed variables X1-X4, as they were insignificant, significance values p ≅ □, keeping only variable X5, Microcelac, that had a significant (p = 0.001) and positive influence (B=0.004), meaning that an increase in the amount of Microcelac also causes increased tablet friability

TABLE IV
Influence of formulation factors on disintegration time
Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4046.764	634.386		6.379	.001
	X1	-18.749	3.133	-11.243	-5.985	.001
	X2	-21.543	3.557	-12.918	-6.057	.001
	X3	-18.559	3.133	-11.129	-5.924	.001
	X4	-17.931	3.133	-10.752	-5.723	.001
	X5	-20.460	3.231	-9.878	-6.333	.001

a Dependent Variable: Y44

The dependent variable Y44, disintegration time, is significantly influenced (p = 0.001) by independent variables X1-X5. The higher the percentage of mucoadhesive substance in the formulation the longer the duration of time the drug stays in contact with vaginal mucosa, long enough to produce its therapeutic effects.

CONCLUSIONS

Using the specialized *SPSS* statistical software and ANOVA we can say that the analyzed quality parameters, strength, friability and disintegration time are influenced by the nature of excipients and drug-excipient ratio, the best formulations being those containing 20-30% C71 and 37-47% Microcelac, formulations F1 and F2, respectively.

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NEWS

FERULIC ACID DERIVATIVES WITH INSECTICIDAL ACTIVITY

A recent study by Huang GY et al. showed that plant viruses cause many serious plant diseases and are currently suppressed with the simultaneous use of virucides and insecticides. The use of such materials, however, increases the amounts of pollutants in the environment. To reduce the environmental contaminants, virucides with insecticidal activity is an attractive option. A series of substituted ferulic acid amide derivatives and the corresponding hydrogenated ferulic acid amide derivatives were synthesized and evaluated for their antiviral and insecticidal activities. The majority of the synthesized compounds exhibited good levels of antiviral activity against the tobacco mosaic virus (TMV). Furthermore, the compounds displayed good insecticidal activities against insects with piercing-sucking mouthparts, which can spread plant viruses between and within crops. The bioassay showed that the compounds not only inhibited the plant viral infection, but also prevented the spread of plant virus by insect vectors. These findings therefore demonstrate that the ferulic acid amides represents a new template for future antiviral studies (Huang GY, Cui C, Wang ZP et al. Synthesis and characteristics of (Hydrogenated) ferulic acid derivatives as potential antiviral agents with insecticidal activity. Chemistry Central Journal 2013; 7:33 doi:10,1186/1752-153x-7-33).

Maria Drăgan

SEVERE ACUTE RESPIRATORY INFECTIONS SURVEILLANCE IN ROMANIA - IS IT ANY GAIN TO PUBLIC HEALTH?

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SEVERE ACUTE RESPIRATORY INFECTIONS SURVEILLANCE IN ROMANIA - IS IT ANY GAIN TO PUBLIC HEALTH? (Abstract): This article **aims** to present the operation and usefulness of severe acute respiratory infection (SARI) surveillance in Romania. **Material and methods** : The information obtained from surveillance data analysis using *EpiInfo* program revealed a greater severity of influenza in the second post-pandemic season (2011/2012) compared to the previous one (2010/2011). Detected risk factors were evidence-based arguments for recommending preventive measures in certain population groups. **Conclusions**: This surveillance system has proven useful, being able to provide information on the severity of influenza and other etiologies involved in severe acute respiratory infections.
Keywords: SURVEILLANCE, SEVERITY, RISK FACTORS

Hospital based sentinel surveillance for influenza severity was implemented during pandemy with the support of WHO-EURO. There has been no measure of severity until then. Severe Acute Respiratory Infections (SARI) Surveillance continued in the next two post-pandemic seasons, having as objectives to estimate SARI incidence in a catchment area, to describe the etiology of SARI cases and to evaluate underlying risk conditions, in order to recommend evidence based public health measures.

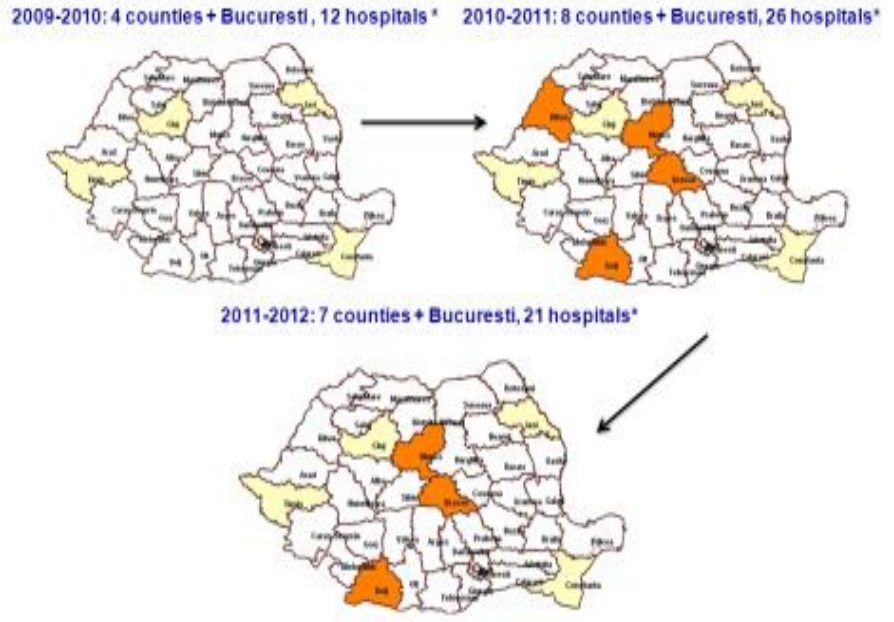
MATERIAL AND METHODS

System description. We used the WHO-EURO case definition for SARI (1, 2). We calculated a denominator based on the number of hospitalizations in the previous three years in each sentinel hospital after county of residence of the patient and the extent of each county in average admissions for three years was applied to county population. The sum of these populations was the population of the area of accessibility of the system ("catchment area"). For season 2010/2011 its value was 6,413,821, and for 2011/2012, were 5,813,728. Number of participating sentinel hospitals was changed over time (fig.1). The work began in four counties (Constanta, Cluj, Iasi, Timis) and Bucharest Municipality, out of 42 Romanian counties. In the first post-pandemic season (2010/2011) four counties were added (Bihor, Brasov, Dolj, Mures) and in the second (2011/2012) one of these (Bihor) was excluded due to lack of data.

Data were collected on a sheet of SARI surveillance case. Three levels of validation were used: the first was made by the hospital epidemiologist, the second by the epide-

miologist from County Public Health Authority and the third at national level - National Center for Communicable Disease Surveillance and Control (NCCDSC), where the SARI surveillance coordinator epidemiologist made final data validation and subsequent introduction the data in the national database. Weekly analysis of data was performed using *Epi Info* program and weekly information was posted on the NCCDSC site. Data for SARI cases were weekly loaded in the electronic platform TESSy (The European Surveillance System).

Because the case definition for SARI was changed in season 2011/2012, as recommended by WHO-EURO, updated in May 2011 (2), analytical results presented below will refer only to SARI cases with fever higher than or equal to 38 °C associated with coughing and shortness of breath or difficulty breathing, as signs and symptoms common to both case definitions.



*infectious diseases hospitals, pediatrics, pulmonology, emergency hospitals

Fig. 1. SARI sentinel, Romania, during pandemic and the first two post-pandemic seasons

A sampling algorithm was established and included in surveillance methodology. Before the onset of influenza season (week 10% positive samples for the same subtype / variant of the total number of those taken in the same week), samples from all SARI cases reported were identified and investigated for the etiology of influenza, and 25% of the negative (according to WHO recommendations), would be evaluated for other etiologies (RSV, hMPV, parainfluenza viruses, coronaviruses, *Str. pneumoniae*). After the onset of flu season, only one sample per week, taken from each sentinel hospital, the first case of SARI detected and reported, the etiology was investigated

only for influenza.

All deaths registered in possible (case that meets the clinical criteria), probable (case that meets the clinical criteria and has an epidemiological link to a laboratory confirmed case) or confirmed (case that meets the clinical criteria and is laboratory confirmed with influenza) SARI cases were to be reported immediately by telephone on CNSCBT and within 24 hours to be sent to the same destination the Sheet flu/ SARI death, by fax or e-mail

RESULTS

Epidemiological findings. In the second post-pandemic season (2011/2012) 346 SARI cases were validated (out of 349 reported), compared to 438 (out of 449) in the previous season.

SARI incidence decreased from 6.06‰ in 2010/2011, to 5.41‰ in 2011/2012. Graph representing the weekly incidence rates (fig. 2) shows two peaks in weeks 5 and 9 of season 2010/2011. In 2011/2012, two peaks, but having lower amplitude, were recorded in weeks 4 and 6.

Male / female ratio (M / F) was almost equal in the two seasons (1.1 in 2010/2011, 1.2 in 2011/2012). The median age was lower in 2011/2012, for SARI cases in general, and also for those influenza virus type A confirmed (tab. I, II).

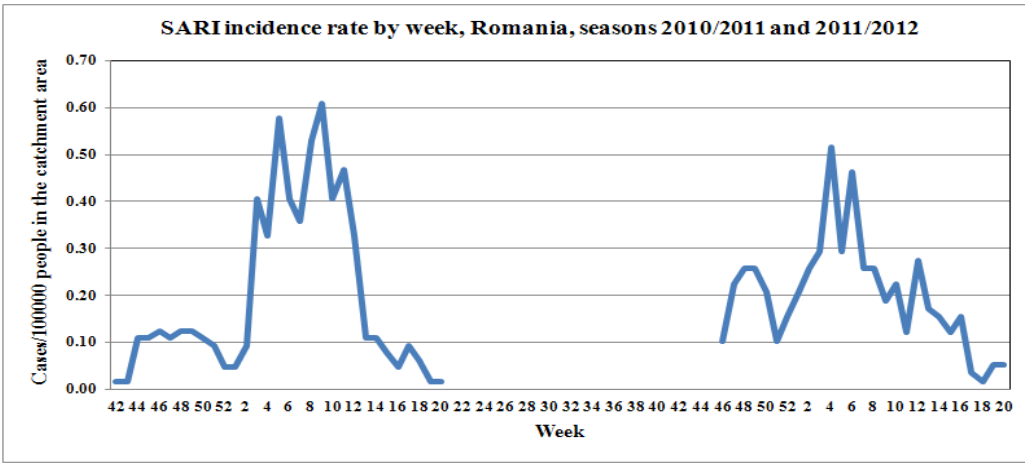


Fig. 2. SARI incidence rate by week, Romania, season 2010/2011 and 2011/2012
Source: National Institute of Public Health, Romania, National Centre for Communicable Diseases Surveillance and Control

TABLE I

Median age in SARI cases and SARI cases confirmed with influenza, in the first post-pandemic season (2010/2011), Romania

Median age (years)	SARI overall (N=389)	SARI confirmed with A/H1N1 (N=75)	SARI confirmed with B (N=74)
Cases	**21** (0-86)	**33** (0-82)	11 (0-82)
Deaths	37 (15-79)	31 (15-61)	73 (37-77)

TABLE II

Median age in SARI cases and SARI cases confirmed with influenza, in the second post- pandemic season (2011/2012), Romania

Median age (years)	SARI overall (N=315)	SARI confirmed with A/H3N2 (N=62)	SARI confirmed with B (N=1)
Cases	**3** (0-88)	**26** (0-88)	49 (N=1)
Deaths	11 (1-83)	1 (N=1)	NA (N=0)

Analysis by age distribution of SARI cases and confirmed SARI cases showed that while in season 2010/2011 most SARI cases and also A/H1N1 confirmed SARI cases have been registered to the same group age, 15-49 years, in 2011/2012 most cases registered were children, but the highest A/H3 positivity rate was recorded in the age group ≥ 65 years.

Laboratory findings. In season 2010/2011, laboratory investigations were performed for 378 cases of SARI (97.2%), with an overall positivity rate of 51.3% and 39.7% for influenza. In 2011/2012, 263 cases with SARI were investigated in laboratory (83.5%). The overall positivity rate was higher than the previous season (65.8%), but lower for influenza (24%). In 2010/2011, the first week SARI surveillance detections were dominated by Respiratory Syncytial Virus (RSV). A/H1N1 influenza virus was detected and became dominant between weeks 4 and 7 (late January-early weeks of February 2011). Influenza B was more frequently detected between weeks 8 and 12 (end of February to end of March 2011). In 2011/2012, the first weeks of monitoring were dominated by parainfluenza virus detections (PIV). Then RSV was detected (November 2010), co-existing with A/H3 influenza virus from week 52 (late December). A/H3 influenza virus detections have become dominant since week 5 (early February) (fig. 3). Starting with week 6, laboratory investigations were detected only for influenza etiology, as mentioned in surveillance methodology. Testing for other etiologies have been claimed and paid by the County Public Health Authorities.

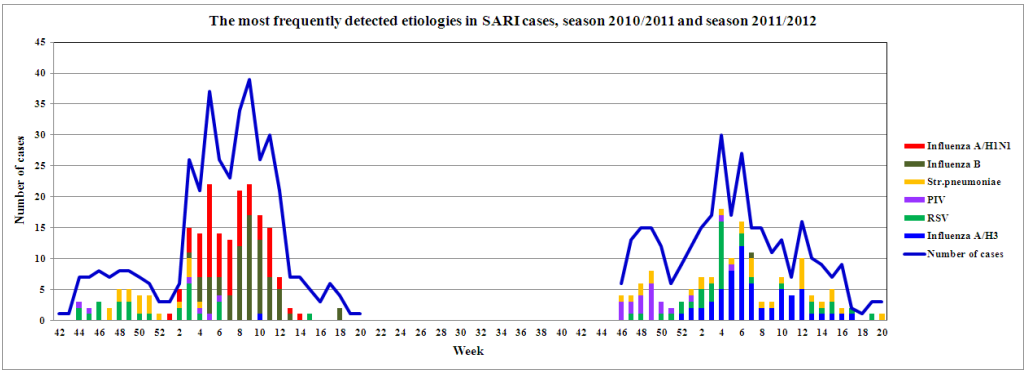


Fig. 3. The most frequently detected etiologies in SARI cases, season 2010/2011 and season 2011/2012

Comparing the distribution of various etiologies detected in SARI cases in the first two post-pandemic seasons (fig. 4), we observed equal proportions of influenza A/H1N1 and influenza B (37%) in 2010/2011, while the A/H3N2 represented only 1%. In 2011/2012, the highest proportion (42%) was recorded for influenza A/H3N2. Influenza B attended only 1%. Unlike other European countries (3) A/H1N1 pandemic virus was not detected in any SARI case. Other etiologies were represented by RSV, parainfluenza viruses and *Str. pneumoniae*. The National Reference Laboratory for Influenza in National Institute for Research and Development in Microbiology and Immunology (NIRDMI) "Cantacuzino" also detected hMPV, Bocavirus, Adenovirus, *H. influenzae*, parainfluenzae, but in smaller proportions.

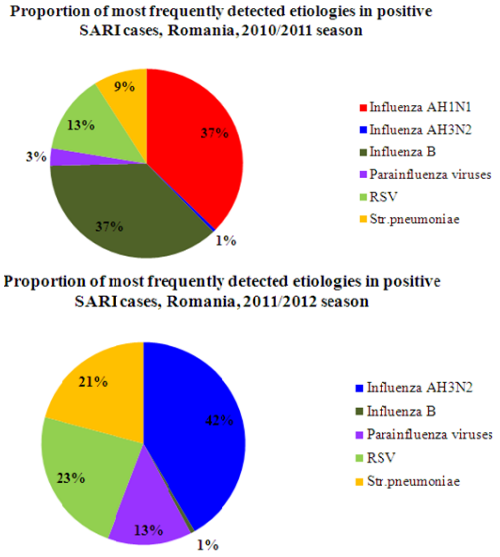


Fig. 4 Etiological distribution in positive SARI cases in the first two post pandemic seasons, Romania

For 2010/2011 season, the National Reference Laboratory for Influenza communicated to NCCDSC 9 isolations and sequencing in SARI cases: 5 A/H1N1/ California/7/09-like, 1 A/ H3N2/Victoria/ 210/09- like and 3 B/Brisbane/60/08-like. Eight antiviral sensitivity tests have been performed, 7 strains being sensitive for Oseltamivir and one with uncertain result. For 2011/2012 season, 14 isolations in SARI cases have been communicated for A/H3N2-A/Perth/16/2009 like and 8 sequencing in confirmed A/H3N2 SARI cases (13%): 4 Clade A/Victoria/208, group 6; 3 - Clade A/Victoria/208, group 3C and 1 - Clade A/Victoria/208, group 3A. Fourteen antiviral sensitivity tests have been performed, all the strains being sensitive for Oseltamivir and Zanamivir.

Comparison for complications. The proportion of complications in the second post pandemic season (2011/2012) was lower compared to 2010/2011, both in SARI cases overall and in SARI confirmed with influenza type A. We also found lower proportion for ARDS and the same discrepancy between ARDS and ventilation. This suggests that the severity of ARDS was not as high, but, on the other hand, it is possible that the data were not very well updated. A very low number of deaths in SARI cases (6) was registered in 2011/2012, compared to the previous season (27), and just one out of 6 was confirmed with influenza A/H3N2 (a 21 month old, immune-compromised girl), compared to 19 in 2010/2011, most of them caused by A/H1N1.

A lower proportion of SARI cases were admitted in ICUs in 2011/2012, compared to the previous season, both in SARI overall (14.9% *vs.* 36.2%) and in SARI confirmed with influenza (9.5% *vs.* 34%).

The proportion of SARI cases with secondary bacterial pneumonia increased from 26.5% in 2010/2011 (103 cases), to 29.8% in 2011/2012 (94 cases). Only 2 cases out of

103 were positive for *Str. pneumoniae* in 2010/2011 and none of the 94 cases in 2011/2012. We also observed an increase in the proportion of SARI cases positive for *Str. pneumoniae* in 2011/2012 (9.8%), compared to the previous season (4.6%). For 30.7% A/H1N1 confirmed SARI cases (23) and for 24.3% B confirmed SARI cases (18) a secondary bacterial pneumonia was mentioned in 2010/2011, while in the next season the value for A/H3 confirmed SARI cases with secondary bacterial pneumonia (22) was higher (35.5%). None of the A/H1N1 and B confirmed SARI cases in 2010/2011 and none of the A/H3 confirmed SARI cases in 2011/2012 was positive for *Str. pneumoniae*.

Comparison for seasonal vaccination. The proportion of vaccinated SARI cases was very low in both seasons: 2.3% in 2010/2011 and 1% in 2011/2012. This fact illustrates the low vaccination coverage in general population: 6% in the first post-pandemic season and 3% in the second one. Just one A/H1N1 and one B confirmed SARI case had been vaccinated against influenza. None of SARI cases confirmed with influenza A/H3 or B in 2011/2012 had been vaccinated.

Comparison for risk factors. For 2010/2011, we found as significant risk factors for A/H1N1 confirmed SARI cases (95% CI), obesity for total cases, chronic kidney disease for female and obesity and compromised immunity for male (tab. III).

For 2011/2012, we found chronic heart diseases and compromised immunity as significant risk factors for total A/H3 confirmed SARI cases (95% CI), the same for female and, additionally, pregnancy for childbearing age female (15-45 years old). No risk factor was found for male (tab. IV).

TABLE III

Significant risk factors in A/H1N1 confirmed SARI cases, Romania, 2010/2011 season

RISK FACTOR	TOTAL		
	OR	Z-Statistic	P-Value
Obesity	3.14 [1.61 - 6.14]	3.3603	0.0008
	FEMALE		
Chronic kidney disease	13.14 [1.38 - 124.73]	2.2473	0.0248
	MALE		
Obesity	4.12 [1.71 - 9.89]	3.1693	0.0015
Compromised immunity	4.16 [1.31 - 13.21]	2.4267	0.0152

TABLE IV

Significant risk factors in A/H3 confirmed SARI cases, Romania, 2011/2012 season

RISK FACTOR	TOTAL		
	OR	Z-Statistic	P-Value
Chronic heart diseases	2.82 [1.35-5.88]	2.7631	0.0057
Compromised immunity	3.35 [1.06-10.56]	2.0642	0.039
	FEMALE		
Chronic heart diseases	4.18 [1.49-11.73]	2.7193	0.0065
Compromised immunity	9.01 [1.06-76.40]	2.0158	0.0438
Pregnancy (childbearing age female = 15-45 years old)	8.12 [1.11-59.19]	2.0676	0.0387
	MALE		
None			

DISCUSSION

Before 2009 pandemy, many discussions and debates were held on influenza severity and on a possible dedicated surveillance system. From the proposed models, we chose and implemented the classic SARI Surveillance. Like any other case based surveillance system, SARI Surveillance is very time consuming and also resource consuming, in terms of staff and finance for laboratory investigations.

All the above-mentioned analysis and comparison between seasons refer only to SARI cases having fever of ≥ 38°C, associated with cough, and shortness of breath or difficulty breathing. This approach changed the logistic regression results for 2010/2011, when another case definition was in place. At that time, we found some additional significant risk factors for A/H1N1 confirmed SARI cases (95% CI) in female: obesity, chronic liver disease, and pregnancy (the last, in childbearing age female).

To improve surveillance, the discussions continue in our team about the involvement of *Str. pneumoniae* in SARI cases etiology. Many cases detected positive could be, in fact, just carriers, given that for most of them the sample was represented by a nasal and pharyngeal swab and not by tracheo-bronchial aspirate.

CONCLUSIONS

We find this surveillance system useful because it is capable to provide information about influenza severity and about the involvement of other etiologies in severe acute respiratory infections. We found a lower severity of influenza in the second post-pandemic season compared to the previous one, suggesting that influenza A/H3N2 was less aggressive than A/H1N1. The underlying risk conditions are evidence based arguments to issue recommendations for influenza prevention in certain risk groups.

We consider that the case based sentinel SARI Surveillance must to continue, and to explore influenza and, depending on resources, other etiologies in SARI cases: at least RSV in children and elder people and *Str. pneumoniae* in cases with secondary bacterial pneumonia. We chose these two etiologies because specific preventive measures (specific immunoglobulin and a vaccine, respectively) could be targeted to certain risk groups.

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We present our acknowledgements to all the clinicians, epidemiologists, microbiologists, nurses and statisticians involved in SARI Surveillance in Romania.

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PROFESSIONAL AND MANAGEMENT ISSUES IN THE SURVEY AND CONTROL OF NOSOCOMIAL INFECTIONS IN GENERAL SURGERY HOSPITALS

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PROFESSIONAL AND MANAGEMENT ISSUES IN THE SURVEY AND CONTROL OF NOSOCOMIAL INFECTIONS IN GENERAL SURGERY HOSPITALS (Abstract): Nosocomial infections (NI) are evidenced in health care facilities around the world as representing the main health and mortality problem in the assisted patients. This prompts for the constant monitoring of NI and implementing preventive and control measures. We designed and administered a 10-item questionnaire referring to NI to the health care personnel of two general surgery units from municipal two hospitals (A and B). The results proved to be poor, especially for hospital B. We investigated the measures against the sources of infection, pathogen carriers and ways of transmission, as well as the management of patients with increased susceptibility to NI. The use of antibiotics and disinfectants was also studied. **Key words:** NOSOCOMIAL INFECTIONS, QUESTIONNAIRE, SURGERY HOSPITAL.

Nosocomial infections have a special place in human pathology, being considered a major population health issue for all the health care units (1, 2 , 3, 4). NI are characterized by a high incidence and severe clinical and functional consequences that lead directly or indirectly to the abrupt increase of treatment costs and cause economic, moral and social damage.

Postoperative infectious complications with septic-purulent clinical pictures do not only lead to avoidable unnecessary suffering, but also prolong the hospital stay from 5.6 to 35.4 days, depending on the unit profile, a mean increase of 14.07 days (5,4,6,7), plus also great economic loss.

MATERIAL AND METHODS

a. A questionnaire containing 10 questions (Annex I) was designed and administered to the health care personnel of two general surgery units – from hospital A and B respectively – in order to assess the level of professional information required for the awareness, prevention and control of NI.

b. We studied the measures taken against the infection sources identified by clinical and laboratory check-up of the personnel (in years 2007 and 2010), as well as the attitude towards the pathogens carriers.

c. The measures taken against the ways of transmission were studied by assessing the control of septic and aseptic routes,

current and final disinfection, aseptic techniques and sterilization of equipment.

d. We surveyed the measures taken in the case of patients with high risk of NI.

e. We surveyed the use of antibiotics and disinfectants

RESULTS

a. Forty (40) questionnaires were administered in hospital A and 25 in hospital B, to the nurses and patient caretakers of the two general surgery units (tab. 1)

TABLE I

Hospital	0	Nurse / Caretaker	1	Nurse / Caretaker	4	Nurse / Caretaker	5	Nurse / Caretaker	6	Nurse / Caretaker
A	80 %	62,5 % / 37,5 %	20 %	75 % / 25 %	0 %		0 %		0 %	
B	0 %		0 %		16 %	100 %	80 %	45 % / 55 %	4 %	100 %

Legend: A – hospital A, B- hospital B; 0-correctly filled in questionnaire; 1,4,5,6 – Wrong answers

b. In hospital B clinical and laboratory examinations were performed in 40 health care personnel members from the surgical unit and 40 from the operating block in the years 2007 and 2010.

In the surgical unit there were two documented cases of healthy HBsAg carriers, one nurse diagnosed in 2010 and a stretch carrier diagnosed when hired, in 2007; both individuals were under hepatoprotective therapy.

There were 3 cases of healthy HBsAg carriers in the operating block (one nurse and two caretakers), diagnosed in 2007, under hepatoprotective therapy, as well as two cases of HCV (caretakers) diagnosed in 2010, under Interferon therapy. In none of the above 7 cases could the infection source be identified.

In hospital A 34 personnel members of the surgical unit and 16 from the operating block underwent clinical and laboratory examinations in 2010, 100% were clinically healthy, with negative tests for HBsAg, HCV-Ab, anti-HIV Ab, VDRL.

In 2007 clinical and laboratory examinations were performed in only 12% of personnel, all being healthy and negative for HBsAg, HCV-Ab, anti-HIV Ab, VDRL.

c. Surgery units from hospital A and B are on the last floor of the building, have a good connection with the hospital patient reception, the operating block, the ICU, sterilization services, personnel rooms, dining rooms, bathrooms, storage rooms.

Patient wards in both hospitals were divided into septic and a septic. The rooms are large enough for patient stretchers and wheelchairs. Beds allow access to the patient from three sides and are equipped with signaling systems.

Wound care wards included a gynaecological table, dressing cart, cupboards for instruments, sterile and non-sterile material, antiseptic solutions.

The operating blocks in the two hospitals included several operating rooms and annexes, directly linked to the ICU wards. They are isolated from circulation, access being through a buffer room and with strict observance of asepsis and anti-sepsis rules.

Current and final disinfection in the two sections use disinfectants approved by the Ministry of Health, in the recommended concentrations.

The following are used:

BICLOSOL – chlorine disinfectatnt for surfaces, drinking water, dishes, linen and clothing.

CIDEX OPA – high level disinfectant used especially for endoscopic equipment

THOR – used for surfaces, thermo-sensitive equipment, metallic instruments, pre-disinfection of instruments before sterilization, textiles

TERRALIN protect – used for the disinfection of medical devices, surfaces, sensitive materials

SUPRASEPT – high level disinfectant, used for thermo-sensitive materials, metallic instruments, medical devices, surfaces

MIKROYD AF Liquid – rapid spray disinfectant

MEDICARINE – chlorine tablets – used for washable surfaces, various objects, equipment, toilets and bathrooms

The health care personnel performing the disinfection took into account the resistance to disinfectants, the factors influencing disinfection, observed the general disinfection practice rules and the methods of application of chemical disinfectants according to the object treated.

Instruments of the two surgical units were sterilized using devices approved by the national health authority (Ministry of Health). Instructions for used were displayed visibly for every sterilizer.

Health care personnel affirmatively observed aseptic techniques, correct use of protection equipment and accessories (gloves, masks, eyeglasses, gowns), the operative preparation of the skin, the mucosa and/or clothing following contamination with biological fluids, the prevention of infection transmission through contaminated instruments, prevention of open wounds and mucosal membranes exposure, prevention of skin injury by needles or sharp instruments.

d. The measures taken in the case of patients more susceptible to NI were focused on isolation, increase of general body resistance, chemoprophylaxis and artificial immunization in certain cases; overall the measures were modest. The main emphasis in the daily activity was on the diagnosis and performance of the surgical act.

e. In both hospitals the main measure taken against NI prevention was antibiotic therapy. The frequency of active NI cases evidenced retrospectively by studying the records over a period of 8 months was 9.34% (A) and 9.60% (B) respectively, as compared to a reported frequency of 0.88% in hospital A and 0% in hospital B.

Antibiotic therapy in hospital A was prescribed in 62.83% of the cases as monotherapy, 28.3% as antibiotic associations, while in 8.84% no antibiotics were prescribed in the hospital. We could not document whether antibiotics were prescribed and bought by the patients themselves.

In hospital B 35.21% of NI cases were treated with monotherapy, 64.78% with drug associations and in 4.22% no antibiotics were prescribed in the hospital. Nor in this case could we document whether antibiotics were prescribed and bought by the patients themselves.

DISCUSSION

Results obtained after distributing the questionnaire to members of the health care personnel, especially those in the surgery unit of hospital B, revealed considerable problems regarding the professional knowledge necessary for NI prevention and control.

It is more than imperative to develop continuous training programmes for the health care personnel, with full responsibility and adoption by the medical staff of the adequate prophylactic practices, for applying the NI prevention measures and evaluating their efficiency (8, 9, 10, 11).

Nosocomial transmission can be reduced by application of standard prevention measures and patient confinement, taking into account the transmission channel of the infectious agents in a hospital environment (12, 13).

Hygiene of the hands remains the essential element of standard prevention measures and the most important method in the practical activity for reducing nosocomial transmission of infectious agents (13, 14, 15). Scientific motives, indications, methods and products used for hand hygiene are extremely well known (16-24) and observing this professional behavioural act is above all discussion.

All health care, clinical and laboratory personnel must be submitted to more rigorous and thorough controls, taking into account that hepatitis B and C viruses as well as HIV transmission are of utmost importance both for the personnel and patient health, because of the severity of late consequences of persistent infections and the limitations of the current treatment methods (25). Exposure to these germs must be considered – according to law - a medical emergency, for which immediate measures are required in order to prevent the spreading of the infection (26, 25, 27).

Antibiotics should not replace real and effective prevention and control measures of NI. Misuse of antibiotics explains but does not justify why infectious agents become more and more resistant to antibiotics. More and more frequently we witness the decision of clinicians to use reserve antibiotics in the treatment of infections, composite drugs which are considerably more expensive and which increase costs involved in patient treatment (28,29,30,31, 32,33,34), without addressing the functional deficiencies of the hospital and the professional staff deficiencies.

CONCLUSIONS

Knowledge of the fundamental principles of NI monitoring, prevention and control is undoubtedly deficient and explains both the underreporting of NI, as well as its real frequency, which is significantly higher than the one found in official accounts.

Learning and correctly applying the NI prevention measures by the health care personnel during medical assistance is axiomatic and can improve the quality of NI control.

Empirical “protective” antibiotic treatment, clearly expensive and unnecessary, must be avoided and its indications should be updated.

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DEZVOLTAREA ŞI VALIDAREA UNEI METODE GC – MS PENTRU DETERMINAREA NICOTINEI DIN TUTUN

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THE DEVELOPMENT AND VALIDATION OF A GC – MS METHOD FOR NICOTINE DETERMINATION FROM TOBACCO (Abstract): A gas chromatographic - mass spectrometric method (GC/MS) for determination of nicotine from tobacco products was developed and validated. **Material and method:** It was used an Agilent Technologies 7890A Chromatograph equipped with a DB 5 MS column (30 m x 0.25 mm, 0.25 µm) and Agilent Technologies 5975C inert MSD detector. The temperature program began from 100˚C and increase with 10˚C/min to 190˚C than with 20˚C/min to 280˚C, constant for 5 min, with He as carrier gas (1 ml/min) and MS detection. The temperature of the source was 230˚C and the temperature of the quadrupole was 150˚C. The parameters of validation were studied according to the international requirements. **Results:** Finally, the method established has following parameters: is linear in the 1.01-201.8 µg/ml range, has the detection limit of 3.6 µg/ml, the quantification limit of 10.8 µg/ml, is precise (RSD < 5%, n=9) and accurate (mean recovery is 100.28% in 99.08 – 100.96 µg/ml range). **Conclusion:** The method can be used to determine nicotine from tobacco products, with good results. **Key words**: NICOTINE, GC-MS, VALIDATION

Nicotina este principalul alcaloid extras din frunzele de tutun, *Nicotiana tabacum*, familia *Solanaceae*. Tutunul uscat din care se obțin țigaretele, țigările de foi şi tutunul de pipă, are o compoziție chimică complexă, conținând nicotină, alături de alți alcaloizi secundari, acizi organici, hidrați de carbon, rezine, uleiuri eterice, enzime, alți compuşi. Țigareta se poate asemăna cu o retortă chimică, fumul de tutun rezultat din arderea unei țigarete conținând 4.000 de compuşi, dintre care peste 60 de substanțe au potențial cancerigen.

Consumul cronic de tutun, respectiv tabagismul sau nicotinismul, considerat prima cauză de mortalitate prematură şi morbiditate evitabilă, a atins astăzi amploarea unei "epidemii mondiale", deoarece, conform statisticilor OMS, numărul fumătorilor la scară planetară se cifrează la aproximativ 1,1 miliarde. În ultimele decenii, tutunul nu mai este considerat un produs care dăunează sănătății, ci ca un produs care curmă viața. Statistic, după OMS, tabagismul produce la nivel mondial, în fiecare oră, 560 de decese (134.000 pe zi; 4,9 milioane pe an) (1). Tabagismul poate fi definit ca un comportament susținut de o dependență farmacolo-

gică faţă de nicotină, intoxicaţia tabagică fiind cea mai răspândită dintre toate toxicomaniile cunoscute în prezent. Fumatul reprezintă una din cele mai grave şi complexe probleme de sănătate publică, prin efectele provocate asupra organismului.

Nicotina, responsabilă de dependenţa tabagică, acţionează asemănător acidului cianhidric, absorbţia sa fiind foarte rapidă la nivelul tuturor mucoaselor. Doza letală la om este de 60 mg. Produce dependenţă de 6-8 ori mai mare decât alcoolul, dar la fel de mare ca şi cocaina, 95-100% dintre fumători fiind dependenţi. Acţiunea toxică a nicotinei se manifestă la nivelul sistemului nervos central, la nivelul aparatului cardiovascular, respirator, tubului digestiv; afectează metabolismul glucidic, lipidic, proteic (2).

În lucrarea de faţă ne-am propus elaborarea şi validarea unei metode simple şi rapide de determinare, prin cromatografie de gaze cuplată cu spectrometria de masă (GC – MS), a nicotinei din tutun.

MATERIAL ŞI METODĂ

Reactivi şi aparatură. S-a utilizat un cromatograf de gaze Agilent Technologies 7890 A, injector Agilent Technologies 7683 B Series şi detector spectrometru de masă Agilent Technologies 5975C inert MSD.

Reactivii utilizaţi (metanol şi diclormetan) sunt de puritate cromatografică, iar etalonul de nicotină a fost procurat de la firma Merck (densitate 1,009 g/ml). Soluţia stoc de nicotină, de concentraţie de 200 μg/ml, a fost preparată în amestec metanol / diclormetan (1/1, v/v).

Condiţii cromatografice. Analiza a fost efectuată pe o coloană capilară DB 5 MS (30 m x 0,25 mm; 0,25 μm) cu o fază mobilă formată din heliu (debit 1 ml/min); temperatura injectorului = 250°C, temperatura sursei MSD = 230°C, temperatura cvadrupolului = 150°C, gradient de temperatură în compartimentul coloanei (10°C/min între 100 - 190°C, 20°C/min între 190 - 280°C, constant 5 min); volumul injectat = 0,3 μl în modul SPLIT (1 / 10). Achiziţia s-a efectuat în modul SCAN (10 – 450 UAM). Soft utilizat: Chemstation, Agilent Techologies.

Soluţii standard. Soluţiile standard de nicotină au fost preparate în amestec metanol / diclormetan (1/1, v/v), în domeniul de concentraţie 1,01 – 201,8 μg/ml.

REZULTATE ŞI DISCUŢII

Identificarea nicotinei din probele analizate s-a realizat pe baza comparării timpului de retenţie şi al spectrului de masă înregistrat în vârful acestuia, din cromatograma probelor, cu timpul de retenţie al picului corespunzător nicotinei din cromatograma unei soluţii standard conţinând nicotină, şi cu spectrele de masă din biblioteca spectrală Willey (3).

Validarea metodei. Pentru validarea metodei de determinare a nicotinei prin cromatografie de gaze cuplată cu spectrometrie de masă, au fost urmăriţi următorii parametri: liniaritatea, limita de detecţie, limita de cuantificare, precizia (precizia sistemului, precizia metodei, precizia intermediară) şi exactitatea. Toate testele de validare au fost efectuate în conformitate cu cerinţele internaţionale în vigoare (4-9).

Liniaritatea. Pentru studiul liniarităţii, pornind de la soluţia stoc de nicotină (201,8 μg/ml) au fost preparate un număr de 3 seturi de soluţii de lucru, în domeniul de concentraţie 1,01 – 201,80 μg/ml. Pentru fiecare soluţie în parte s-au efectuat analizele în conformitate cu modul descris, s-au înregistrat cromatogramele şi s-au calculat ariile picurilor corespunzătoare nicotinei. Valoarea medie calculată (tab. I) s-a reprezentat grafic în funcţie de concentraţie (fig. 1).

TABELUL I

Ariile picurilor obținute în cazul studiului liniarității metodei de determinare a nicotinei prin GC-MS

Nr. det.	Nicotină µg/ml	Arie pic			
		I	II	III	Medie
1	1,01	8222266	8222243	8310784	8251764
2	2,02	8532264	8515113	8467305	8504894
3	5,05	9250301	9260584	9256597	9255827
4	10,09	10635143	10680832	10535786	10617254
5	15,14	11735280	11876283	11901127	11837563
6	20,18	13045078	13173306	13114807	13111064
7	30,27	15723175	15805883	15638440	15722499
8	40,36	18302348	18295130	18113730	18237069
9	50,45	20715903	20773050	20760442	20749798
10	60,54	23529690	23284476	23285925	23366697
11	70,63	25925125	25749226	25718447	25797599
12	80,72	28669167	28444229	28703648	28605681
13	90,81	31154353	31182582	30995956	31110964
14	100,9	33635114	33459437	33430843	33508465
15	110,99	36018603	36200992	36032968	36084188
16	121,08	38542738	38734483	38581342	38619521
17	131,17	41375240	41382384	41353258	41370294
18	141,26	43827881	43854923	43975547	43886117
19	161,44	49308802	49420389	49437142	49388778
20	181,62	53061887	53404152	53520378	53328806
21	201,8	58426340	58034957	58094618	58185305

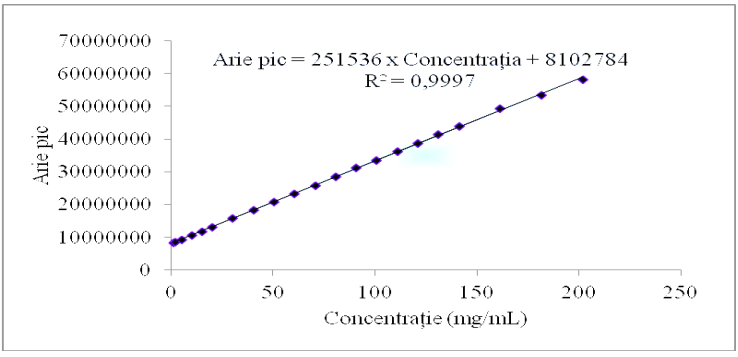

Fig. 1. Dreapta de calibrare pentru determinarea nicotinei prin GC-MS

S-a calculat ecuația şi eroarea standard a dreptei de regresie, precum şi coeficientul de regresie (R^2). Astfel, ecuația dreptei de calibrare este:

$$Arie\ pic = 251535{,}6 \times Concentrația\ (\mu g/ml) + 8102784$$

Valorile calculate pentru eroarea standard a dreptei de regresie (SE) este 271159,5, iar pentru coeficientul de regresie (r^2) este 0,9997.

Din datele prezentate rezultă că metoda de determinare a nicotinei prin GC-MS este liniară pe domeniul de concentraţie studiat.

Folosind parametrii statistici calculaţi (eroarea standard şi panta dreptei de regresie), s-au calculat limita de detecţie (LD) şi limita de cuantificare (LQ), utilizând relaţiile:

$$LD = \frac{3,3 \cdot SE}{\text{Panta}} = \frac{3,3 \cdot 271159,5}{251535,6} = 3,6 \mu g / mL \quad ; \quad LQ = \frac{10 \cdot SE}{\text{Panta}} = \frac{10 \cdot 271159,5}{251535,6} = 10,8 \mu g / mL$$

Precizia. Pentru determinarea preciziei, s-au studiat precizia sistemului, precizia metodei şi precizia intermediară.

Pentru determinarea preciziei sistemului sau a repetabilităţii injecţiei, aceeaşi soluţie conţinând nicotină, în concentraţie de 100,9 μg/ml, a fost analizată de 10 ori, în aceleaşi condiţii de operare. Rezultatele experimentale şi calculul statistic s-a realizat pe baza deviaţiei standard – SD şi a deviaţiei standard relativă – RSD % (tab. II).

Pentru determinarea preciziei metodei, s-au efectuat câte trei determinări pe soluţii diferite, aflate la trei nivele de concentraţie (100 μg/ml ± 30%). Pentru toate cele nouă cromatograme obţinute, s-a calculat aria picului corespunzător nicotinei şi (pe baza ecuaţiei dreptei de regresie) concentraţia reală din probă (tab. III).

TABELUL II

Precizia sistemului (repetabilitatea injecţiei)

Nr. determinării	Arie pic
1	33776882
2	33596284
3	33635053
4	33475707
5	33476349
6	33775885
7	33609431
8	33421825
9	33538452
10	33404652
Media	**33571052**
SD	**126336,1**
RSD	**0,3763%**

TABELUL III

Precizia metodei pentru determinarea nicotinei prin GC-MS

Nr. det.	Concentraţie teoretică (μg/ml)	Arie pic	Concentraţie calculată (μg/ml)	%
1	70,63	25765760	70,22	99,42
2		25846522	70,54	99,87
3		25787033	70,31	99,54
4	100,90	33735374	101,90	101,00
5		33565101	101,23	100,32
6		33740976	101,93	101,02
7	131,17	41317675	132,05	100,67
8		41426286	132,48	101,00
9		41413721	132,43	100,96
Date statistice			**Media (%)**	**100,42**
			SD	**0,6590**
			RSD (%)	**0,6562**

Pentru determinarea preciziei intermediare, s-au efectuat câte trei determinări pe soluții diferite aflate la trei nivele de concentrație (100 μg/ml ± 30%). Pentru toate cele nouă cromatograme obținute, s-a calculat aria picului corespunzător nicotinei şi (pe baza ecuației dreptei de regresie) concentrația reală din probă (tab. IV).

TABELUL IV

Precizia intermediară pentru determinarea nicotinei prin GC-MS

Nr. det.	**Concentrație teoretică (μg/ml)**	**Arie pic**	**Concentrație calculată (μg/ml)**	**%**
1	70,63	25764109	70,21	99,41
2		25732428	70,09	99,23
3		25939261	70,91	100,40
4	100,90	33456945	100,80	99,90
5		33752212	101,97	101,06
6		33574085	101,26	100,36
7	131,17	41414853	132,43	100,96
8		41432299	132,50	101,02
9		41426742	132,48	101,00
Date statistice			**Media (%)**	**100,37**
			SD	**0,7147**
			RSD (%)	**0,7121**

În urma prelucrării statistice a datelor experimentale obținute (tabelele II, III, IV), se constată că valorile RSD sunt mult mai mici decât limita maximă admisă de 5%, de unde rezultă că metoda de determinare a nicotinei prin GC – MS este precisă.

Exactitatea. *Pentru determinarea exactității metodei*, s-au efectuat câte trei determinări prin metoda adausului de soluții diferite aflate la trei nivele de concentrație (100 μg/ml ± 30%). Pentru toate cele nouă cromatograme obținute, s-a calculat aria picului corespunzător nicotinei şi (pe baza ecuației dreptei de regresie) concentrația reală din probă. S-a calculat regăsirea medie, minimă şi maximă (tab. V).

TABELUL V

Exactitatea metodei de determinare a nicotinei prin GC-MS

Nr. det.	Concentrație teoretică (μg/ml)	Arie pic	Concentrație calculată (μg/ml)	Regăsire (%)
1	70,63	25705694	69,98	99,08
2		25814063	70,41	99,69
3		25777616	70,27	99,49
4	100,90	33715665	101,83	100,92
5		33663327	101,62	100,71
6		33497876	100,96	100,06
7	131,17	41403480	132,39	100,93
8		41413616	132,43	100,96
9		41329236	132,09	100,70
Date statistice			**Regăsire medie**	**100,28**
			Minim	**99,08**
			Maxim	**100,96**

Din datele experimentale obținute, s-a constatat că valorile medii (100,28%) se încadrează în limitele admise (± 5÷10%), ceea ce confirmă că metoda de determinare a nicotinei prin GC – MS este exactă.

Pentru aplicarea în practică a metodei dezvoltate şi validate, s-au preparat probe de tutun provenind din țigări aflate în rețeaua comercială a municipiului Iaşi (Marlboro şi Kent). Astfel, nicotina a fost extrasă din tutun, cu amestec metanol / diclormetan (1/1, v/v), şi determinată prin GC – MS, în condițiile metodei. Din datele experimentale obținute, s-a constatat faptul că, în probele de tutun analizate, cantitatea de nicotină este în limitele specificate.

CONCLUZII

Constatările noastre au permis elaborarea şi validarea unei metode de determinare cantitativă a nicotinei prin GC-MS, stabilindu-se condițiile de lucru: coloană cromatografică, regimuri termice, cantitatea de probă analizată, modul de identificare a nicotinei, liniaritatea dreptei de calibrare, limita de detecție, limita de cuantificare, precizia (precizia sistemului, precizia metodei, precizia intermediară) şi exactitatea. Rezultatele experimentale obținute arată că metoda este liniară (r^2 = 0,9997) pe domeniul de concentrații studiat (1 – 200 μg/ml), este sensibilă (LD = 3,6 μg/ml, LQ = 10,8 μg/ml), precisă (RSD < 5%, n = 9) şi exactă (regăsire medie = 100,28%). Metoda a fost aplicată cu rezultate bune pentru determinarea nicotinei din tutunul utilizat la prepararea țigărilor comerciale.

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RELAȚIA ÎNTRE NIVELUL EDUCAȚIONAL MATERN ŞI ALIMENTAȚIE LA UN LOT DE PREŞCOLARI DIN ORAŞUL BACĂU

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RELASHIONSHIP BETWEEN MATERNAL EDUCATIONAL LEVEL AND CHILD FEEDING IN A SERIES OF PRESCHOOL CHILDREN IN BACĂU TOWN. (Abstract): Child growth and development are decisively influensed by feeding. This depends on parent's financial resources and food habits, most often tradition-related. **Aim:** to determine how important mother's educational level is in this respect. **Material and methods:** the study was carried out on a series of 153 preschool children from two kindergartens in Bacău town. Their parents filled out a questionnaire on the frequency of weekly food consumption. **Results:** 64.5 %of the mothers of the children included in this study had a higher education. Most children had milk 4-7 times a week, cheese 2-3 times a week, eggs and chicken 2-3 times a week, and fruits 4-7 times a week. The diffrences in feeding provided by mothers with higher education and those less educated were statistically insignificant, food habits being quite similar. **Conclusions:** A good knowledge of food habits in a poplation is essential for developing programs aimed at changing the unhealthy ones. **Key words:** FOOD INTAKE, MATERNAL EDUCATIONAL LEVEL, FOOD HABITS

Creşterea şi dezvoltarea sunt două procese ce caracterizează copilăria şi adolescența şi care sunt influențate de ereditate şi particularitățile mediului fizic extern. În cel din urmă, alimentația joacă un rol hotărâtor în asigurarea normalității creşterii/dezvoltării copiilor cu dependență puternică de nivelul socio-economic al familiei şi cel de instruire al părinților. Nivelul superior de instruire al părinților poate asigura o alimentație adaptată necesităților particulare fiecărei etape de dezvoltare a copilului (1,2). Deşi comportamentele nutriționale se transmit de la o generație la alta este importantă intervenția, prin mijloace educaționale, pentru a asigura o alimentație optimă, cantitativ şi calitativ, îndeosebi, în perioada de preşcolar, etapă în care obiceiurile alimentare se formează şi pot fi modelate cu uşurință, prin intermediul colegilor şi educatorilor, care, în mod frecvent, constituie un model pe care copilul îl respectă şi îl urmează cu multă încredere. În acest context, colaborarea între familie, medici şi educatori este împortantă, în direcția implementării unor programe naționale de îmbunătățire cantitativă şi calitativă a alimentației (3, 4, 5).

MATERIAL ŞI METODE

Studiul s-a realizat pe un lot de 155 preşcolari, proveniți de la două grădinițe din

oraşul Bacău. Informaţiile au fost acumulate pe baza unei fişe de anchetă epidemiologică cu referire la studiile mamei, modul de alimentaţie zilnică a copiilor, cu aprecierea obiceiurilor alimentare în relaţie cu zona geografică respectivă, cu accent pe consumul săptămânal de produse lactate, carne şi preparate din carne, peşte, ouă, grăsimi, derivate de cereale, legume, fructe şi produse zaharoase (6, 7).

Prelucrarea şi evaluarea datelor s-a realizat prin metode epidemiologice clasice şi statistico-matematice, îndeosebi testul Pearson.

REZULTATE ŞI DISCUŢII

1. Nivelul educaţional al părinţilor şi comportamentele alimentare

Cercetările noastre au evidenţiat faptul că mamele cu studii superioare deţin multe informaţii legate de aspectele de sănătate şi cele educaţionale, insistând asupra aspectelor formative importante, la vârsta preşcolară. Chiar dacă, în unele cazuri, nivelul economic al familiei era modest, mamele reuşesc să asigure o alimentaţie adecvată copilului, adesea învingând anumite comportamente nutriţionale legate de tradiţii. În zona Moldovei tradiţiile, mai ales cele alimentare, sunt considerate importante şi respectate la nivel populaţional. Pornind de la aceste considerente, am încercat să apreciem modul în care se asigură alimentaţia preşcolarilor, în relaţie cu nivelul educaţional matern.

În cadrul lotului examinat, 64,5% din mame aveau studii superioare, 22,6% liceale sau şcoală profesională şi 12,9% postliceale (fig. 1).

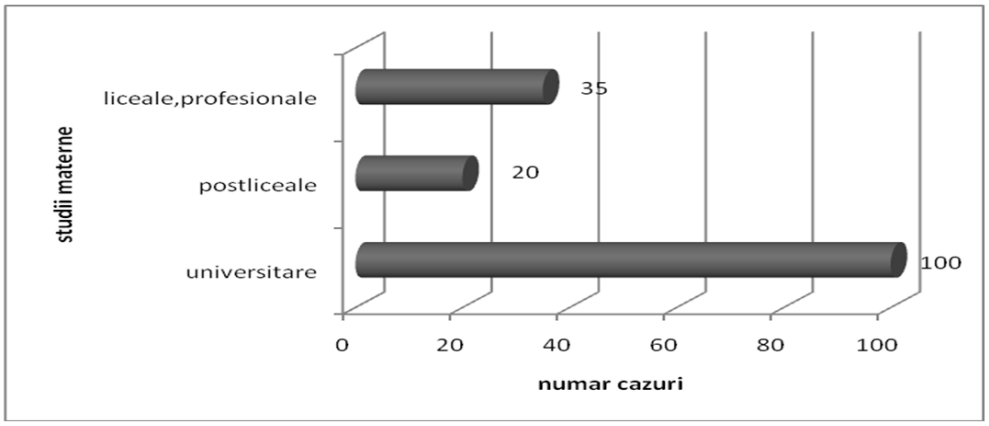

Fig. 1. Repartiţia lotului de mame în relaţie cu nivelul studiilor

În numai 6 (3,9 % cazuri) mamele aveau studii profesionale, ceea ce a impus asocierea acestora cu cele cu pregătire liceală pentru a evita dispersia prea mare a lotului.

2. Frecvenţa consumului săptămânal al unor anumite produse alimentare.

Laptele a fost consumat în majoritatea cazurilor (70, 3%), de 4-7 ori pe săptămână şi doar o dată pe săptămână (7, 1 %).

Diferenţele în ce priveşte ritmul consumului săptămânal de lapte au fost nesemnificative statistic ($p>o,o5$, $\chi^2 = 2,24$), ceea ce se explică prin obiceiuri alimentare asemănătoare, chiar şi în condiţiile în care informaţiile deţinute de mamă erau diferite (tab. I).

TABELUL I

Consumul săptămânal de lapte de către copiii incluşi în studiu în relaţie cu nivelul educaţional al mamelor

Nivelul studiilor materne	Frecvenţa consumului săptămânal de lapte (zile)		
	1	2-3	4-7 *
Studii universitare	9	18	73
Postliceale	2	6	12
Liceale, profesionale	0	11	24
Total Nr.	11	35	109
%	7, 1	22, 6	70, 3

***pentru valorile mai mici de 10 proporţiile sunt orientative**

3. Consumul brânzeturilor s-a dovedit a fi mai ales de 2-3 ori pe săptămână (47,7%), comparativ cu familiile în care aportul de brânzeturi era zilnic (21,9%), sau cele în care acesta era absent (7,7%). Diferenţele s-au dovedit a fi puternic semnificative statistic ($p<0,005$). Insuficienţa sau lipsa acestor produse din alimentaţia preşcolarilor este o eroare gravă perturbând, astfel aportul de proteine de calitate superioară, grăsimi, vitamine şi elemente minerale (fig. 2).

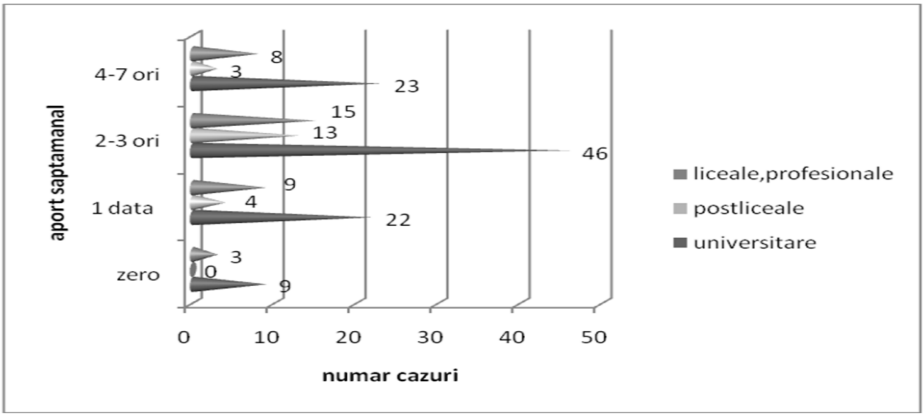

Fig. 2. Consumul săptămânal de brânzeturi în relaţie cu nivelul studiilor materne

Diferenţele nivelului de consum au avut o relaţie nesemnificativă statistic cu studiile materne ($p >0,05$, $\chi^2 = 4,0$), situaţie ce poate fi explicată, cel puţin în parte, prin faptul că obiceurile alimentare ale preşcolarilor sunt asemănătoare, indiferent de pregătirea mamei şi confirmă faptul că tradiţia este mai puternică comparativ cu informaţiile cotidiene actualizate normelor moderne.

Programele naţionale de orientare în domeniul alimentaţiei, vor avea impact favorabil dacă debutează în perioada de preşcolar, şi în cooperare cu familia.

La această categorie de vârstă consumul optim de brânzeturi va fi de 3-4 ori pe săp-

tămână. Studiul nostru a evidenţiat un aport dominant de 2-3 ori pe săptămână (49,0%), dar şi existenţa a 3,9% de situaţii cu un consum absent şi similar 3,9% cu unul excesiv (4-7 ori/săptămână).

Rezultatele cercetărilor au dovedit existenţa unor diferenţe nesemnificative statistic ($p > 0,05$, $\chi^2 = 9,01$).

4. Consumul de carne pasăre este recomandat prioritar pentru preşcolari. În lotul inclus în studiu aportul dominant a fost de numai 2-3 ori pe săptămână (48,4%). 36,8% de 4-7 ori, dar şi 2,6% de consum absent. Deficienţele legate de consumul de carne de pasăre apar la toate grupurile de preşcolari, indiferent de studiile materne, diferenţele constatate fiind nesemnificative statistic ($p>0,05$, $\chi^2 = 1,96$) (tab. II).

TABELUL II

Distribuţia cazurilor în relaţie ce aportul săptămânal de carne de pasăre

Nivelul studiilor materne	Frecvenţa consumului de carne de pasăre/săptămână*			
	Zero	1	2-3	4-7
Studii universitare	2	14	47	37
Postliceale	1	1	10	8
Liceale, profesionale	1	4	18	12
Total nr.	4	19	75	57
%	2,6	12,2	48,4	36,8

*** Pentru valorile mai mici de 10 proporţiile sunt orientative**

5. Produsele de origine vegetală. În alimentaţia preşcolarilor alături de produsele de origine sunt necesare şi cele vegetale, adecvate cantitativ şi calitativ, pentru a evita dezechilibre nutriţionale.

Produsele cerealiere studiate au fost pastele făinoase şi orezul, produse bogate în proteine vegetale, amidon şi oferă un aport caloric crescut. În majoritatea cazurilor (55, 5%) acestea au fost incluse în consum de 2-3 ori pe săptămână, prin contrast cu 5, 2% din cazuri în care consumul a fost absent sau în 12,9%, în exces, de 4-7 ori pe săptămână. Diferenţele pe grupuri de preşcolari şi categoriile de studii materne au fost nesemnificative statistic ($p> 0,05, \chi^2 - 9,35$).

Cartofii sunt legumele cele mai recomandate pentru preşcolari datorită digestibilităţii lor uşoare. În studiul nostru au fost incluşi în meniul săptămânal de 2-3 ori (54, 8%), dar în 3, 9% răspunsurile au fost negative şi 22, 6% consumul de doar o dată pe săptămână. Repartiţia situaţiilor de consum pe grupuri de preşcolari au evidenţiat diferenţe nesemnificative statistic ($p > 0,05$ $\chi^2 = 2,2$), care se pot explica prin faptul că mamele sunt ancorate în tradiţii, astfel că greşelile constatate nu ţin de nivelul lor educaţional.

6. Consumul de fructe a fost asigurat cel mai frecvent de 4-7 ori pe săptămînă (81,9%), iar în 14,2 %, de 2-3 ori şi cu 3,9% de consumuri insuficiente. Programele de suplimentare a aportului de fructe, iniţiat de guvern, au puţine şanse de reuşită deoarece se adresează doar la 3,9% din copii. În restul familiilor consumul este prezent, iar un consum în exces al fructelor ar aduce o cantitate mai mare de vitamine, minerale şi glucide (fig. 3).

În majoritatea cazurilor, în populaţia din zona inclusă în studiu, consumul de fructe era optim şi nu impunea o suplimentare prin program.

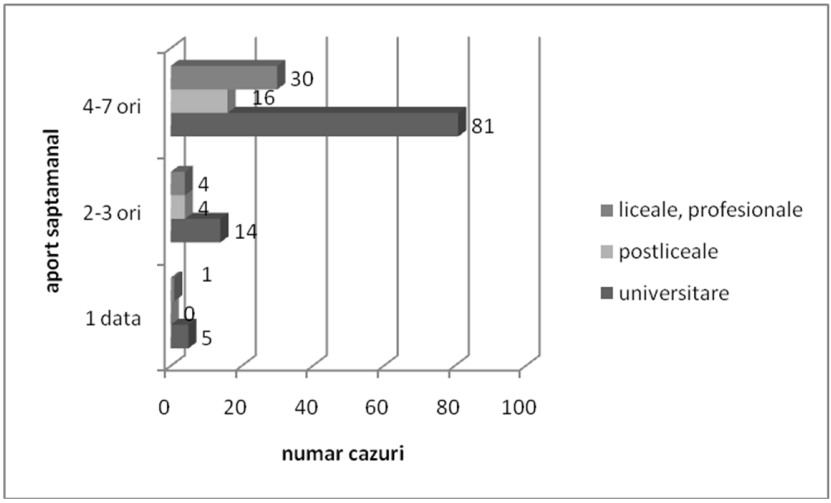

Fig. 3. Frecvența consmului săptămânal de fructe la lotul de preşcolari inclus în studiu, în relație cu nivelul educațional al mamelor

Şi în acest caz pregătirea educațională a mamei nu a avut influență statistică semnificativă ($p>0,05$ $\chi^2 = 2,34$).

7. Consumul de dulciuri considerat "modelul permanent al copilăriei", are caracter tradițional când părinții recurg la dulciuri drept recompensă (8,9). În cazul lotului de copii studiat a fost dominant aportul de 4-7 ori pe săptămână (56,8%), 2-3 ori (30,9%) şi absența acestuia afirmată de 1,2% dintre părinți, situație nefavorabilă deoarece celula nervoasă foloseşte glucidele drept unică sursă de energie (tab. III).

TABELUL III

Frecvența consumului de dulciuri la preşcolarii incluşi în studiu

Nivelul studiilor materne	Frecvența consumului de dulciuri/săptămână			
	Zero	1	2-3	4-7
Studii universitare	2	10	35	53
Postliceale	0	4	4	12
Liceale, profesionale	0	3	9	23
Total nr.	2	17	48	88
%	1,2	10,9	30,9	56,8

Consumul de dulciuri nu a avut relații semnificative statistic ($p>0,05$, $\chi^2 = 5,17$) cu nivelul studiilor materne, deci obiceiurile alimentare ale preşcolarilor, nu au dovedit a avea relație directă cu nivelul educațional al mamei.

CONCLUZII

În studiul realizat au fost dominante mamele cu studii universitate, deci care deținеau multe informații legate de modul de îngrijire şi de alimentația corectă a preşcolarilor.

Consumul de lapte a avut în majoritatea cazurilor un ritm de 4-7 ori pe săptămână; cel de brânzeturi, ouă şi carne de pasăre de 2-3 ori, iar comparația între grupurile de studii materne au marcat diferențe nesemnificative statistic ceea ce explică existența unor obiceiuri alimentare asemănătoare, indiferent de gradul educațional al mamei.

Aportul de paste făinoase şi cartofi a fost practicat îndeosebi de 2-3 ori/săptămână, iar fructele, în majoritatea cazurilor de 4-7 ori.

Se poate considera că realizarea unor programe naționale de orientare nutrițională sanogenă se impun a fi stabilite în raport de cunoaşterea obiceiurilor alimentare ale populației, iar în cazul preşcolarilor studiați nu s-a dovedit a fi necesară suplimentarea cantității de fructe, deoarece acestea sunt incluse în mod adecvate în rația zilnică.

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NOUTĂȚI

HAFNIA SPP.- DIAGNOSTIC ŞI TRATAMENT

În acest studiu au fost incluse 68 de tulpini de Hafnia identificate pâna le nivel de specie pe baza secvențierii au fost învestigate în scopul stabilirii caracterelor fenotipice utile şi accesibile în toate laboratoarele. Au fost investigare patru teste; utilizarea malonatului, fermentarea salicilinei şi a arabinozei şi expresia beta glucozidazei care au incadrat corect tulpinile investigate fie ca *H.alvei* sau *H.paralvei*. Testarea sensibilității la antibiotice a fost făcută pentru 35 tupini utilizând E test . Toate tulpinile au fost sensibile la aminoglicozide, chinolone, carbapeneme şi monobactam. Majoritatea tulpinilor au avut CMI pentru colistin mai mare sau egal cu 2 µg/ml. De asemenea, 70% din tulpinile testate produc o toxină citolotică activă pe liniile Vero care ar putea avea un rol în patogenia gastroenteritei. (Abbott S, Moler S, Green N, Tran R et all.Clinical and laboratory Diagnostic characteristics and Cytotoxigenic Potential of Hafnia alvei and Hafnia paralvei Strains. *J Clin Microbiol* 2011:3191-3197).

Ecaterina Enache

ASPECTE CLINICO-EPIDEMIOLOGICE ŞI PARTICULARITĂŢI ALE SENSIBILITĂŢII LA ANTIBIOTICE ÎN INFECŢIA GONOCOCICĂ

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CLINICO-EPIDEMIOLOGICAL FEATURES AND ANTIMICROBIAL RESISTANCE PATTERN IN GONOCOCCAL INFECTION (Abstract): **Aim**: To analyze clinical and epidemiological features in patients with gonococcal infection attended Dermato-Venerology Clinic in Iasi and regional dermato-venerology offices and to evaluate gonococcal antimicrobial resistance pattern. **Methods:** The study was carried out on 129 patients clinically diagnosed and bacteriologically confirmed with gonococcal infection who were subsequently submitted to a questionnaire. We studied their demographic characteristics (sex, age, nationality, marital status), clinical features (site of infection, symptoms, concurrent STI, previous history of gonorrhoea) and behavioral aspects (education, number and type of sexual partners, safe sexual practices). **Results:** We found in our patients a strong association of gonorrhoea with young male individual, poor educational level and with clinical symptoms of urethritis. The level of antimicrobial resistance is higher than in other European countries. **Conclusions:** The poor health-seeking behavior, symptoms not specific enough, resistance pattern, lack of accessible and sensitive diagnostic methods lead to undiagnosed and probably mistreated gonorrhoea. **Key words**: GONOCOCCAL INFECTION, EPIDEMIOLOGY, ANTIMICROBIAL RESISTANCE

Infecţia gonococică reprezintă o problemă majoră de sănătate publică, atât prin incidenţa în creştere la nivel mondial cât şi datorită emergenţei şi răspândirii tulpinilor de gonococ rezistente la antibiotice. Dezvoltarea rezistenţei gonococului la antibioticele utilizate curent compromite atât managementul individual al cazurilor de gonoree cât şi eficienţa programelor de control. Nivelul ridicat al preocupărilor pentru controlul infecţiei gonococice peste tot în lume este justificat şi datoriă implicării gonoreei în cresterea de aproximativ 500 de ori a riscului de transmitere a HIV (1).

Controlul infecţiei gonococice întâmpină, de asemenea, dificultăţi legate de de insuficienţa datelor asupra incidenţei şi prevalenţei cazurilor asimptomatice sau cu simptomatologie necaracteristică, accesibilitate redusă la mijloace şi metode sensibile de diagnostic, reticenţă la raportarea cazurilor confirmate, mai ales în medicina privată (1).

Scopul studiului este de a realiza anali-

za caracteristicilor clinice şi epidemiologice ale infecţiei gonococice la pacienţii asistaţi în Clinica de Dermatologie din Iaşi şi în cabinetelor dermato-venerologice din teritoriu, în perioada septembrie 2004 - decmbrie 2008.

MATERIAL ŞI METODĂ

Lotul de studiu a fost constituit din 129 pacienţi, diagnosticaţi clinic şi confirmaţi bacteriologic cu infecţie gonococică. După recoltarea prelevatelor cu tampoane din dacron acestea au fost introduse imediat în mediul de transport - Amies cu cărbune. Însamânţarea probelor s-a realizat pe agar Thayer Martin selectiv şi neselectiv, cu incubare 24-48 ore la 35°C, în atmosferă umedă, cu 5-10% CO_2. Identificarea izolatele s-a făcut pe baza caracterelor microscopice, de cultură şi biochimice (galerii API NH, Bio Merieux) (2). Pacienţii confirmaţi bacteriologic cu infecţie gonococică au răspuns la un chestionar pentru a analiza particularitaţile demografice (sex, vârstă, nationalitate, stare civilă), clinice (localizare, simptomatologie, antecedente de infecţie gonococică sau alte boli cu transmitere sexuală), educaţionale şi comportamentale (nivel educaţional, număr de parteneri sexuali, caracteristicile sursei de infecţie, utilizarea metodelor de protecţie). Pentru un număr de 113 tulpini s-a făcut testarea sensibilităţii la penicilină 10UI, tetraciclină 30µg, ciprofloxacin 5µg, spectinomicină 100µg, cefixim 5µg, ceftriaxonă 30µg, şi au fostinterpretat rezultatele conform criterilor interpretative ale CLSI, cu tulpina de referinţa *N. gonorrhoeae* ATCC 49226 (3).

REZULTATE

1. *Caracteristici demografice*.

Au fost confirmate bacteriologic 129 cazuri de infecţie gonococică, 116 cazuri (89.92%) la bărbaţi şi 13 cazuri (10.07%) la femei, raportul fiind de 11,2/1.

Vârsta subiecţilor din lotul investigat a fost de 13- 64 ani, cu o medie de 28,3 ani, majoritatea cazurilor (101/78,29%) fiind semnalate la grupurile de vârstă 15-34 ani

Majoritatea pacienţilor au provenit din mediul urban (103/79,85%), cu domiciliul în judetul Iaşi (107/82,94%). Pacienţii investigaţi au fost români (71/86.64%), rhomi (1/1,11%) şi moldoveni (1/1.24%); 52(40,31%) fiind căsătoriţi, 35 (27,13%) sunt căsătoriţi iar 41 (31,78%) trăiesc în concubinaj (tab.I).

2. *Caracteristici clinice*

Toţi pacienţii de sex masculin au prezentat simptome de uretrită acută purulentă, cu debut la o perioda variind între 2 şi 7 zile de la contactul infectant (medie 3,5 zile. La 2 (1,55%) pacienţi secreţia a fost mucopurulentă iar alţi 2 (1,55%) au prezentat simptome de afectare testiculară. Toate pacientele diagnosticate au fost în contact cu bărbaţi suferinzi de uretrită gonococică şi au prezentat clinic inflamaţia mucoasei cervicale şi scurgere vaginală mucopurulentă. Nu s-au izolat tulpini de gonococ cu localizare faringienă sau rectală.

Antecedente de infecţie gonococică au prezentat 15 (11,62%), 20 (15,5%) au fost diagnosticaţi cu sifilis iar 12 (9,3%) au avut vegetaţii veneriene.

În 32 (24,8%) din cazurile investigate a fost evidenţiată practicarea tratamentului înainte de prezentarea la medic (ciprofloxacin, ofloxacin, peniciline, doxiciclina) iar 10 (7,75%) nu au precizat medicaţia administrată.

S-a evidenţiat prezenţa îmbolnăvirilor predominant la elevi şi la cei cu un nivel de şcolarizare redus.

Toţi pacienţii diagnosticaţi cu infecţie gonococică s-au declarat eterosexuali, doar 2 indivizi au semnalat un contact sexual

extragenital; 114 dintre ei (88,37%) au avut mai mult de 5 parteneri sexuali iar 19 (14,72%) au avut mai mult de 4 parteneri în ultimul an.

TABELUL I

Unele caracteristicile epidemiologice ale pacienţilor cu gonoree incluşi în studiu

Caracteristicile pacientului		**Nr.**	**%**
Sex	Masculin	116	89,92
	Feminin	13	10,07
Mediu de proveniență	Urban	103	79,85
	Rural	26	20,15
Vârstă	<15	3	2,32
	15-24	61	47,28
	25-34	40	31,0
	35-44	15	11,62
	45-54	6	4,51
	>55	4	3,1
Nivel de pregătire	gimnaziu	70	54,26
	studii medii	51	39,53
	studii superioare	8	6,2
Status marital	căsătoriți	17	40,47
	necăsătoriți	10	23,8
	concubinaj	14	33,33
	văduvi	1	2,38
Orientare sexuală	heterosexuali	127	97,61
	homosexuali	2	2,38
Număr de parteneri sexuali	1	1	2,38
	2 - 4	5	11,9
	>5	13	30,95
	>10	23	54,76
Antecedente de ITS	Da	13	30,95
	Nu	29	69,04

3. *Caracteristici educaţionale şi comportamentale*

Nici unul dintre cei intervievaţi nu utilizează, în mod constant, prezervativul şi doar 10(7,75%) îl folosesc ocazional.

Sursa de infecţie a fost cunoscută la toate femeile din lotul de studiu iar la bărbaţi în 44 cazuri (34,1%); 39 (30,23%) au afirmat că nu cunosc identitatea sursei iar 28 (21,7%) au declarat că au contactat infecţia de la o prostituată. 2 dintre bărbaţi (1,55%) s-au infectat in străinătate.

4. *Sensibilitatea la antibiotice*

Dintre tulpinile de gonococ izolate de la pacienţii luaţi în studiu pentru113 tulpini s-a testat sensibilitatea la antibiotice.

TABELUL II

Nivelul rezistenţei la antibiotice a tulpinilor de gonococ

	2004		2005		2005		2006		2008	
	S	R	S	R	S	R	S	R	S	R
P 10UI	0	100 (35)	0	100 (28)	0	100 (23)	10,52 (2)	89,47 (17)	7,69 (1)	92,30 (12)
TE 30µg	14,28 (5)	85.71 (30)	14.28 (4)	85.71 (24)	17,39 (4)	82,60 (19)	15,78 (3)	84,21 (16)	15,38 (2)	84,61 (11)
CIP 5µg	25,71 (9)	74,28 (26)	21,42 (6)	78,57 (22)	26,08 (6)	73,91 (17)	21,05 (4)	78,94 (15)	23,07 (3)	76,92 (10)
CFM 5µg	100 (35)	0	100 (28)	0	95,65 (22)	4,34 (1)	94,73 (18)	5,26 (1)	100 (13)	0
CRO 30µg	100 (35)	0	100 (28)	0	100 (23)	0	100 (19)	0	100 (13)	0
SPT 100µg	100 (35)	0	100 (28)	0	100 (23)	0	100 (19)	0	100 (13)	0

P=penicilină; TE=tetraciclină; CIP=ciprofloxacin; CFM=cefixim; CRO=ceftriaxonă; SPT= spectinomicină; S= sensibil; R= rezistent.

În toţi cei 5 ani luaţi în studiu s-a remarcat un nivel foarte ridicat al rezistenţei la penicilină (84,21-100%) şi la tetraciclina (82,6-85,71) iar rezistenţa la ciprofloxacin a depăşit nivelurile semnificative clinic; la cefixim avem două tulpini cu sensibilitate redusă; nu s-au izolat tulpini rezistente la ceftriaxonă sau spectinomicină (tab.II).

DISCUŢII

Numărul de cazuri de gonoree raportate este în scădere la nivel mondial deşi incidenţa altor infecţii cu transmitere sexuală este în creştere (1). În zona Moldovei se înregistrează aceeaşi scădere a numărului de cazuri raportate de gonoree (4,5), motiv pentru care studiul sensibilitatăţii la antibiotice este realizat pe tulpini izolate până in 2008, pentru a avea relevanţă statistică. Lotul nostru de pacienti a fost format în majoritate din bărbaţi, simptomatologia acută caracteristică determinând prezentarea

acestora la medicul de familie sau dermatovenerolog. Femeile cu infecţie gonococică, frecvent asimptomatice sau cu simptomatologie nespecifică, fie nu se adresează medicului, fie se adresează medicului de familie sau ginecolog. În condiţiile în care metode sensibile şi specifice de depistare şi de identificare a *N. gonorrhoeae* nu sunt disponibile de, rutină aceste cazuri nu sunt diagnosticate corect şi cu mare probabilitate nu sunt tratate eficient. Toate cazurile de cervico-vaginita diagnosticate de noi au fost contacte ale unor pacienţi cu uretrita gonococică.

În lotul investigat s-a demonstrat o incidenţă semnificativă a infecţiei la grupurile de vârstă 15-35 ani, majoritatea cazurilor fiind din mediu urban, celibatari şi de etnie română. Nivelul educaţional al pacienţilor este scăzut, lucru susţinut atât de nivelul redus de şcolarizare cât şi de datele legate de cunoaşterea şi utilizarea metodelor de protecţie, numărul mare de parteneri sexuali, frecvent necunoscuţi, sau de incapacitatea indivizilor de a preciza medicaţia administrată. Grupul de vârstă 15-19 ani, cu incidenţă mare a infecţiei şi cu comportament cu risc ca urmare a vârstei mici de debut a vieţii sexuale, numărul mare de parteneri sexuali şi necunoaşterea metodelor de protecţie, impune includerea prioritară în programelor educaţionale de prevenţie a BTS.

Toţi pacienţii incluşi în studiu s-au declarat eterosexuali, deşi datele din literatură subliniază incidenţa crescută la bărbaţii homosexuali (6), motiv pentru care nu am izolat tulpini faringiene sau rectale de gonococ.

La insuficienţa reţelei de laboratoare care să poată depista şi identifica *N. gonorrhoeae*, se adaugă lipsa de adresabilitate a personalului medical. Examenul microscopic, utilizat de rutina pentru identificarea etiologiei gonococice, este insuficient de sensibil şi de specific, mai ales în infecţii cronice, pluritratate sau pentru diagnosticul infecţiei gonococice la femei. Metodele de diagnostic molecular (foarte sensibile, ce permit diagnosticul comcomitent a mai multor BTS) sunt inaccesibile în acest moment în România (1,2) .

Eficienţa redusă a programelor de control este dependentă şi de accesul larg la antibiotice al pacienţilor, ce favorizează automedicaţia şi indicatiile terapeutice ale personalului medical fără expetiză în terapia acestei infecţii. Astfel s-a ajuns la apariţia tulpinilor multiplu rezistente la antibioticele utilizate curent în terapia sindromică.

Cercetările efectuate pe lotul de pacienţi luaţi în studiu a evidenţiat un nivel foarte ridicat al rezistenţei la penicilină, tetraciclină şi ciprofloxacin, concordant cu datele raportate intr-un alt studiu realizat în judeţul Suceava (5), dar nu s-a constatat rezistenţă la ceftriaxonă şi spectinomicină. În două cazuri s-a semnalat o scădere a sensibilităţii la cefixime. În zona Moldova antibioticul de elecţie pentru tratamentul infecţiilor gonococice ramâne ceftriaxona. Nivelurile de rezistenţă au fost mai mari decât cele raportate în alte tări europene (7,8) dar comparabile cu cele din tările asiatice (6).

Raportarea cazurilor de gonoree întâmpină dificultăţi, boala fiind privită cu reticenţă de către pacient, mai ales în serviciile medicale private. Rapoartele sistemelor de supraveghere europene semnalează subraportarea din ţările Europei de Est (7), motiv pentru care alocarea fondurilor pentru pro-

gramul național de prevenție şi combatere a BTS este suboptimal

CONCLUZII

Slaba adresabilitate la medic şi inaccesibilitatea unor metode sensibile şi specifice de diagnostic duc la o subdiagnosticare a infecției gonococice şi, în consecință, la aplicarea unui tratament incorect. Controlul infecției gonococice în Moldova implică tratamentul corect al cazurilor diagnosticate, cu antibiotice active asupra tulpinilor circulante, depistarea activă a cazurilor, mai ales la femei cu infecții asimptomatice, supravegherea sensibilității la antibiotice a tulpinilor circulante dar şi instituirea unor programe eficiente de prevenție, educaționale pentru înlăturarea unor comportamente prezente în special la grupurile populaționale cu risc crescut.

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CERCETĂRI EXPERIMENTALE PRIVIND EFECTELE UNUI EXTRACT DIN *CETRARIA ISLANDICA* ASUPRA STRESULUI OXIDATIV LA ANIMALE DE LABORATOR

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EXPERIMENTAL RESEARCHES ON THE EFFECTS OF A *CETRARIA ISLANDICA* EXTRACT ON OXIDATIVE STRESS IN LABORATORY ANIMALS (Abstract): **Aim:** Experimental researches on the effects of a *Cetraria islandica* extract, with or without magnesium association, on oxidative stress in rats. **Material and methods:** The experiments were carried out on Wistar rats, treated intraperitoneally for 14 days at single daily dose as follows: Group 1**:** Saline solution (SS) (0.5 ml/100g bw/day); Group 2**:** Subcutaneous pellets, SS; Group 3: Levamisole (LEV) (10mg/kbw/day); Group 4: Subcutaneous pellets, LEV; Group 5: Prednison (PDN) (5mg/kbw/day); Group 6: Subcutaneous pellets, PDN; Group 7: $MgSO_4$ (5mg/kbw/day); Group 8: Subcutaneous pellets, $MgSO_4$; Group 9: *C. islandica* (Ci) extract (21.56mg/kbw/day); Group 10: Subcutaneous pellets, Ci; Group 11: Ci+$MgSO_4$; Group 12: Subcutaneous pellets, Ci+$MgSO_4$. At the end of the experiment blood samples were collected for assessing the following oxidative stress parameters: malonaldehyde, catalase, glutathione peroxidase, glutathione levels. **Results**: In the rats with or without experimentally-induced granuloma, repeated administration of Ci for 14 days resulted in a decrease of malonaldehyde, catalase, glutathione peroxidase activity, and an increase in glutathione levels. The association of magnesium augmented the antioxidant effect of Ci in this experimental model. **Conclusions**: In our study experimental conditions *C. islandica* extract determined adaptogenic-antistress effects, confirmed by its actions on oxidative stress parameters. **Key words**: *CETRARIA ISLANDICA,* IMMUNOMODULATION, OXIDATIVE STRESS.

Există o varietate largă de imunomodulatori de origine vegetală cu importante efecte antioxidante şi/sau adaptogene (1,2). În grupul adaptogenilor se încadrează şi principiile active din *Cetraria islandica.* Studiile din literatura de specialitate au evidenţiat că principiile active conţinute în *C. islandica* prezintă acţiune antimicobacteriană, antifungică, antivirală, antitumorală şi imunomodulatoare (3,4,5).

Unele studii clinice au arătat că magneziul nu este implicat direct în menţinerea balanţei oxidanţi/antioxidanţi la pacienţii supuşi unui stres cronic, dar suplimentarea cu magneziu la aceşti pacienţi a fost urmată de creşterea depozitelor de magneziu, creşterea nivelului seric al vitaminei E şi, consecutiv, ameliorarea proceselor de pe-

roxidare lipidică (6).

Scopul studiului a constat în cercetarea experimentală a efectelor de tip antioxidant ale extractului din *C. islandica* în monoterapie sau în asociere cu Mg.

MATERIAL ŞI METODĂ

Pentru experiment au fost utilizate 12 loturi de câte 6 şobolani Wistar, cu greutate cuprinsă între 180-200g, cu repartiţie uniformă pe sexe, proveniţi de la Institutul de Sănătate Publică din Iaşi, trataţi conform schemei: **L1** = SF= Ser fiziologic (0,5ml/100g c/zi); **L2** = SFp = Pelete subcutanate, ser fiziologic (0,5ml/100g c/zi); **L3** = LEV = Levamisol (10mg/kg c/zi); **L4** = LEVp = Pelete subcutanate, Levamisol (10mg/kg c/zi); **L5** = PDN = Prednison (5mg/kg c/zi); **L6** = PDNp = Pelete subcutanate, prednison (5mg/100g c/zi); **L7** = $MgSO_4$ = $MgSO_4$ (5mg/kg c/zi); **L8** = $MgSO_4$p = Pelete subcutanate, $MgSO_4$ (5mg/kg c/zi); **L9** = Ci = Extract din *C. islandica* (21,56mg/kg c/zi); **L10** = Cip = Pelete subcutanate, Ci (21,56mg/kg c/zi); **L11** = Ci + $MgSO_4$= Ci (21,56mg/kg c/zi) + $MgSO_4$ (5mg/kg c/zi); **L12** = Ci + $MgSO_4$p = Pelete subcutanate, Ci (21,56mg/kg c/zi) + $MgSO_4$ (5mg/kg c/zi).

Substanţele au fost administrate în doză unică zilnică, la ora 8 dimineaţa, timp de 14 zile, pe cale intraperitoneală. Testarea Ci s-a realizat comparativ cu LEV, imunomodulator de sinteză, cu frecvente implicaţii practice, şi respectiv, PDN, un analog glucocorticosteroidian, derivat cu puternice efecte antiinflamatoare şi imunosupresoare. Efectele Ci au fost analizate în monoterapie şi în asociere cu Mg, sub forma $MgSO_4$.

În a opta zi a experimentului, la animalele din loturile notate cu „p", s-a practicat testul granulomului, apoi s-a continuat administrarea substanţelor conform schemei de mai sus. Doza din Ci reprezintă 1/80 din DL_{50}. După 14 zile de tratament s-a recoltat sânge din plexul retroorbitar şi au fost determinaţi următorii parametri ai stresului oxidativ: activitatea malondialdehidei (MAD), catalazei, glutation peroxidazei şi a glutationului redus. Activitatea enzimelor a fost determinată printr-o reacţie de culoare citită la un spectrofotometru tip UV-VIS (HP 8453). Rezultatele au fost apoi prelucrate statistic folosind testul „t-Student". Valoarea $p<0,05$ a fost considerată semnificativă statistic.

Cercetările au fost realizate în conformitate cu recomandările Comisiei de Etică a Universităţii de Medicină şi Farmacie „Gr.T. Popa" din Iaşi, în strictă concordanţă cu reglementările etice internaţionale, privind lucrul pe animale de laborator (7).

REZULTATE ŞI DISCUŢII

La loturile martor cu granulom indus experimental şi tratate cu SF s-a constatat o creştere semnificativă statistic a nivelurilor MAD (fig. 1) comparativ cu valorile acestei enzime la animalele tratate cu SF, fără granulom, acest aspect certificând existenţa unor procese peroxidative intense la animalele cu granulom.

Peroxidarea lipidică este dăunătoare reacţiilor ulterioare de neutralizare a radicalilor liberi produşi. Aldehida malonică produsă prin peroxidarea lipidelor pare să fie atât factorul determinant al polimerizării prin legătură încrucişată, cât şi promotorul cromoforului fluorescent, fenomene ce determină alterări structurale proteice şi chiar ale materialului genetic (ADN) (8,9).

LEV a demonstrat efecte antioxidante prin reducerea semnificativă statistic a valorilor serice ale MDA comparativ cu lotul SFp. La animalele din lotul LEVp valorile serice ale MDA au fost semnifica-

tiv statistic mai crescute comparativ cu nivelul de la animalele tratate cu LEV fără granulom, dar semnificativ statistic mai reduse comparativ cu cele de la lotul SFp. PDN a determinat scăderea semnificativă statistic a valorilor serice ale MDA indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom şi comparativ cu cel indus de LEV.

Ci a determinat scăderea semnificativă statistic a valorilor serice ale MDA indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom.

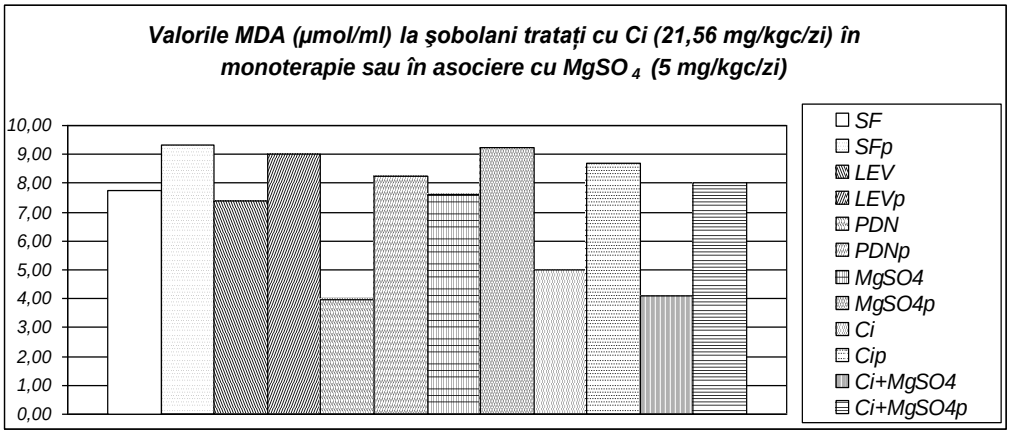

Fig. 1. Activitatea MDA la 14 zile de la debutul studiului

Magneziul nu a influenţat semnificativ statistic nivelurile serice ale MDA, dar a potenţat efectul antioxidant al extractului din *C. islandica*.

S-a constatat că Ci a determinat scăderea peroxidării lipidice semnificativ statistic mai puternică comparativ cu cea indusă de LEV, dar semnificativ statistic mai redusă comparativ cu cea indusă de PDN.

La loturile SFp nivelurile crescute de catalază (fig. 2) au demonstrat falimentul mecanismelor endogene de luptă antioxidantă.

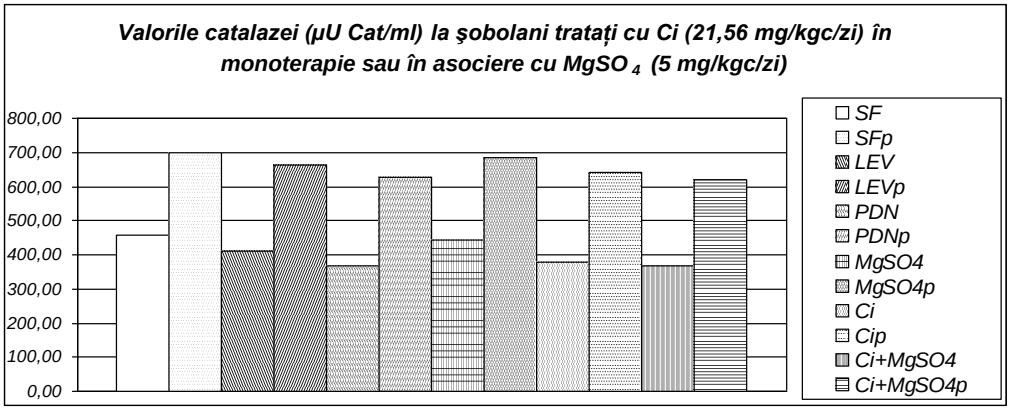

Fig. 2. Activitatea catalazei la 14 zile de la debutul studiului

LEV a determinat scăderea semnificativă statistic a valorilor catalazei comparativ cu lotul SFp. La animalele din lotul LEVp valorile catalazei au fost semnificativ statistic mai crescute comparativ cu nivelul de la animalele din lotul LEV, dar semnificativ statistic mai reduse comparativ cu cele de la lotul SFp. PDN a determinat scăderea semnificativă statistic a valorilor catalazei indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom şi comparativ cu cel indus de LEV.

Ci a determinat scăderea semnificativă statistic a valorilor catalazei indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom. Mg nu a influenţat semnificativ statistic nivelurile catalazei, dar a potenţat efectul antioxidant al Ci.

La lotul SFp nivelurile crescute de glutation peroxidază (fig. 3) a demonstrat condiţii experimentale de stres oxidativ.

LEV a determinat scăderea semnificativă statistic a valorilor glutation peroxidazei comparativ cu SFp. La animalele din lotul LEVp valorile glutation peroxidazei au fost semnificativ statistic mai crescute comparativ cu nivelul de la animalele din lotul LEV, dar semnificativ statistic mai reduse comparativ cu cele de la SFp. PDN a determinat scăderea semnificativă statistic a valorilor glutation peroxidazei, indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom şi comparativ cu cel indus de LEV.

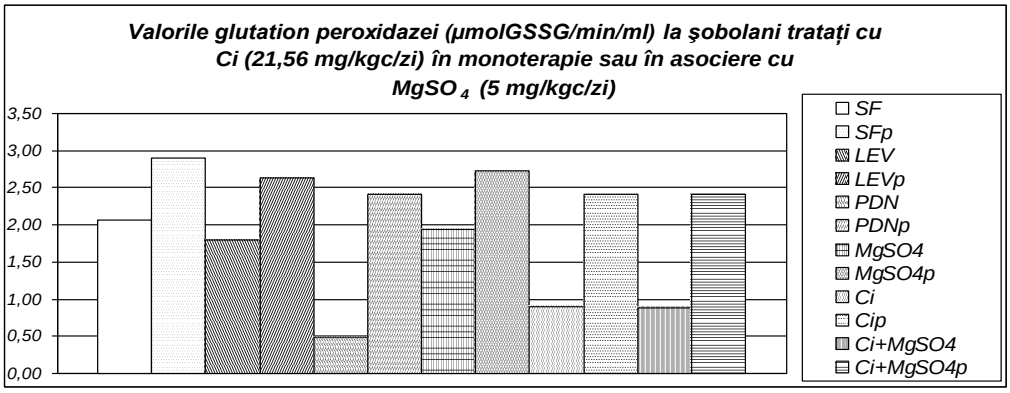

Fig. 3. Activitatea glutation peroxidazei la 14 zile de la debutul studiului

Ci a determinat scăderea semnificativă statistic a valorilor glutation peroxidazei, indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom. Mg nu a influenţat nivelurile glutation peroxidazei, atât în monoterapie, cât şi în cazul asocierii cu extractul din *C. islandica*.

S-a constatat că efectele Ci la animalele cu granulom indus au fost similare cu cele determinate de PDN în aceleaşi condiţii experimentale.

La lotul SFp nivelurile scăzute de glutation redus (fig. 4) au demonstrat consumul său ca substrat al glutation peroxidazei.

LEV a determinat creşterea semnificativă statistic a valorilor glutationului redus comparativ cu lotul SFp. La animalele cu granulom indus experimental şi tratate cu LEV valorile glutationului redus au fost

semnificativ statistic mai scăzute comparativ cu nivelul de la animalele tratate cu LEV fără granulom, dar semnificativ statistic mai crescute comparativ cu cele de la lotul SFp. PDN a determinat creşterea semnificativă statistic a valorilor glutationului redus, indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom şi comparativ cu cel indus de LEV.

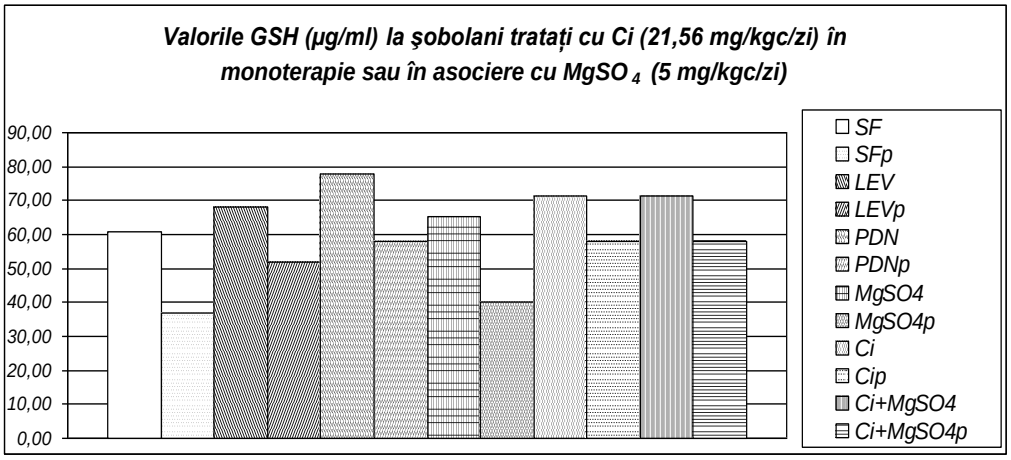

Fig. 4. Valorile glutationului redus la 14 zile de la debutul studiului

Ci a determinat creşterea semnificativă statistic a valorilor glutationului redus indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom. Magneziul nu a influenţat nivelurile glutationului redus, atât în monoterapie, cât şi în cazul asocierii cu extractul din *C. islandica.*

Datele obţinute au demonstrat că extractul vegetal din *C. islandica* a determinat efecte antistres-adaptogene evidenţiate de acţiunea sa asupra enzimelor ce caracterizează balanţa oxidanţi/antioxidanţi (10).

CONCLUZII

Studiile experimentale imunofarmacologice, privind extractul obţinut din produsul vegetal *C. islandica* (codificat Ci), în doză reprezentând 1/80 din DL_{50} (21,56 mg/kgc/zi), administrat în monoterapie sau în asociere cu Mg, sub formă de $MgSO_4$, au condus la următoarele concluzii:

Ci a determinat scăderea semnificativă statistic a valorilor serice ale MDA, catalazei şi glutation peroxidazei, indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom. Magneziul nu a influenţat semnificativ statistic nivelurile serice ale MDA, catalazei şi glutation peroxidazei, dar a potenţat efectul antioxidant al extractului din *C. islandica*. Efectele extractului din *C. islandica* la animalele cu granulom indus au fost similare cu cele determinate de PDN în aceleaşi condiţii experimentale.

Extractul din *C. islandica* a determinat creşterea semnificativă statistic a valorilor glutationului redus, indiferent de condiţiile experimentale, efectul fiind mai puternic la animalele fără granulom. Magneziul nu a influenţat nivelurile glutationului redus, atât în monoterapie, cât şi în cazul asocierii cu extractul din *C. islandica*.

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NOUTĂȚI

VALOAREA PREDICTIVĂ A INDICILLOR ANTROPOMETRICI PENTRU MORTALITATEA ŞI MORBIDITATEA PACIENŢILOR DIALIZAŢI

Evaluarea *status*-ului nutriţional al pacienţilor dializaţi este importantă deoarece malnutriţia protein calorică este un factor care influenţează morbiditatea şi mortalitatea acestora. Scopul acestui studiu a fost să argumenteze valoarea predictivă a parametrilor antrpopometrici şi modalitatea în care aceste două caracteristici se corelează. Studiul a inclus 242 pacienţi. Măsurătorile antropometrice au fost făcute în timpul iernii şi s-au referit la date privind pliul cutanat, circumferinţa braţului, înălţime şi greutate. Procentul de grăsime corporală a fost calculat în funcţie de pliul cutanat prezent în regiunea tricepsului, bicepsului, suprascapulară şi suprailiacă. Indicele de masă corporală (BMI) rata catabolizării proteinelor şi factorii de risc cardio-vasculari au fost de asemenea valori luate în discuţie. În urma analizei datelor au fost găsite corelaţii semnificative între parametrii antropometrici (analiza statistică folosită fiind regresia Cox extinsă). De asemenea, fiecare parametru investigat s-a corelat independent cu mortalitatea şi morbiditatea pacienţilor investigaţi. Parametrul cu valoarea predictivă cea mai mare a fost circumferinţa braţului, indicele de masă corporală fiind considerat doar un factor de risc. (Stosovic M, Stanojevic M, Ogrizovic S, Jovanovic, The predictive value of anthropometric parameters on mortality in haemodialysis patients. *NephrolDial Transplant.* 2011; 26: 1367-1374).

Ecaterina Enache

PREVENIREA TRANSMITERII INFECȚIEI ÎN LABORATOARELE DE TEHNICĂ DENTARĂ DIN IAŞI

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PREVENTION OF INFECTION TRANSMISSION IN DENTAL LABORATORIES (Abstract). The professional activity in a dental laboratory is associated with a high risk for infection transmission through the manipulation of impressions and prostheses contaminated with patients' saliva and blood. **Aim:** To assess the compliance of dental technicians in Iasi town dental laboratories with the methods used for preventing infection transmission in their practices. **Material and methods:** This questionnaire-based survey included 61 dental technicians aged 22 to 58 years from 29 dental laboratories. The 17 questions were related to the protection equipment, air ventilation, and disinfection of surfaces, instruments, impressions, and prostheses. SPSS 14 system, Chi-Square test were used for data analysis, and the levels of statistical significance were set at $p<0.05$. **Results:** 90.2% of the subjects claim the need of prosthetic items disinfection upon arrival at the laboratory from the dental office ($p<0.05$). The protection equipment is used by 49.1% of the dental technicians included in this survey (full equipment 18.0%, safety glasses 45.9%, mask 37.7%, rubber gloves 19.6%). 59.1% of the laboratories are provided with air ventilation, while surface disinfection is common practice in 85.2%. Of the study subjects 62.1% disinfect the impressions, 26.1% the final prosthesis, and 25.0% disinfect the interocclusal registrations ($p<0.05$). **Conclusions:** Educational programs aimed at increasing dental technicians' awareness of infection control by adopting the most effective prevention measures are essential for reducing the incidence of technicians, dentists, and patients; exposure. **Key words:** PREVENTION, INFECTION CONTROL, EXPOSURE, DENTAL LABORATORY

Controlul infecției reprezintă un domeniu esențial al medicinei dentare, care este asociată unui risc crescut de transmitere a infecției prin contact direct cu fluidele contaminate, indirect prin intermediul unor obiecte contaminate sau prin transmitere încrucişată („cross infection") (1). Potențialul de transmitere a infecțiilor în laboratorul de tehnică dentară este datorat manipulării şi transportului între cabinetul dentar şi laborator a amprentelor, a fazelor intermediare de tratament protetic şi a protezelor contaminate cu bacterii, virusuri sau fungi provenind din saliva şi sângele pacientului (2).

Laboratoarele de tehnică dentară pot dispune de o zonă de recepție pentru evaluarea şi decontaminarea pieselor protetice provenind din cabinetele dentare sau pot funcționa ca o zonă izolată care impune dezinfecția amprentelor, fazelor intermediare şi a protezelor în cabinetul dentar, înainte de intrare în laborator. Ambele

metode sunt eficiente alegerea efectuându-se în funcție de locație, calificarea personalului şi sistemul de organizare (3,4).

Comunicarea eficientă şi coordonarea dintre cabinet şi laborator trebuie să asigure aplicarea procedurilor de curățare, dezinfecție şi sterilizare în vederea eliminării riscului de expunere ocupațională a personalului medical dar şi a pacientului, evitând totodată deteriorarea materialelor protetice prin supraexpunere la acțiunea dezinfectantelor şi dublarea inutilă a protocoalelor specifice de control al infecției (5).

Aplicarea Precauțiilor Universale este esențială în laboratorul de tehnică dentară, ca şi în cabinetul dentar, impunând utilizarea echipamentului de protecție format din halat, mască facială, mănuşi şi ochelari şi aplicarea protocoalelor specifice de igienizare a mâinilor, dezinfecție a dispozitivelor, echipamentelor şi suprafețelor şi sterilizare a instrumentelor reutilizabile (6).

MATERIAL ŞI METODĂ

În scopul evaluării cunoştințelor şi atitudinilor tehnicienilor dentari față de procedurile de control al infecției am instituit un studiu pe bază de chestionar la care au participat 61 de tehnicieni din 29 de laboratoare de tehnică dentară din Iaşi. Lotul a fost format din 31 de bărbați şi 30 de femei cu vârsta cuprinsă între 22 şi 56 de ani. Chestionarul a cuprins 17 întrebări referitoare la necesitatea dezinfecției pieselor protetice în cursul transferului dintre cabinetul dentar şi laboratorul de tehnică dentară, utilitatea măsurilor de prevenire a transmiterii infecției, utilizarea echipamentului de protecție, dezinfecția suprafețelor din laboratorul de tehnică dentară şi soluțiile dezinfectante utilizate. Rezultatele au fost prelucrate utilizând sistemul SPSS 14, testul Chi-Square ($p<0,05$).

REZULTATE

Prelucrarea datelor chestionarului a demonstrat că majoritatea subiecților (93,4%) declară că sunt preocupați şi au cunoştințe privind controlul infecție. 90,2% consideră necesară decontaminarea pieselor protetice provenind din cabinetul dentar la intrarea în laboratorul de tehnică dentară, fără diferențe semnificative în funcție de sex (86,6% dintre femei, 93,5% dintre bărbați). ($p<0,05$) Deasemenea 81,9% dintre tehnicieni (80,0% dintre femei şi 83,8% dintre bărbați) apreciază ca necesară decontaminarea probelor şi a lucrărilor protetice care urmează a fi transportate din laborator către cabinetul dentar (fig.1).

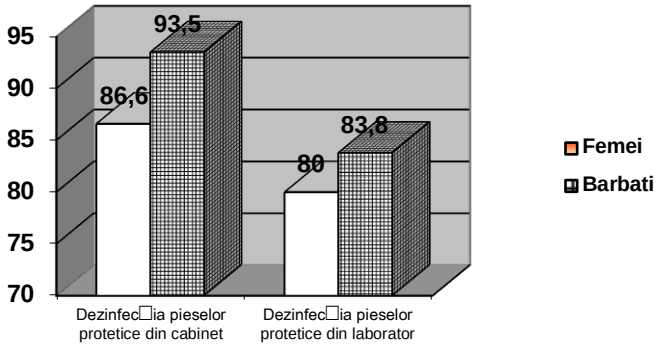

Fig. 1. Distribuția procentuală a tehnicienilor care consideră necesară dezinfecția pieselor protetice în laboratorul şi cabinetul dentar

Măsurile de dezinfecţie şi sterilizare sunt considerate importante pentru tehnicianul dentar de către 86,9% dintre subiecţi. (83,3% dintre femei şi 93,3% dintre bărbaţi). Utilitatea acestor protocoale pentru medic şi pacient este apreciată de 36,4% şi, respectiv,15,5% dintre tehnicieni (fig. 2).

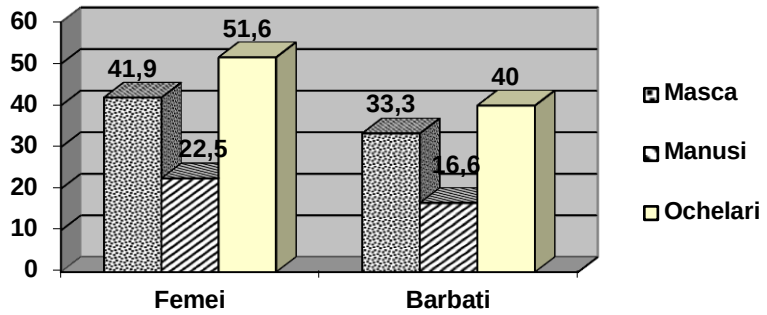

Fig. 2. Distribuţia procentuală a tehnicienilor care consideră utile măsurile de dezinfecţie aplicate în laboratorul de tehnică dentară.

Echipamentul de protecţie, componenta esenţială a Precauţilor Universale, este utilizat de către 49,1% dintre tehnicieni. Subiecţii de sex feminin apelează la echipamentul de protecţie în proporţie mai ridicată (59,0% dintre femei) comparativ cu cei de sex masculin (44,2% dintre bărbaţi) (fig.3).

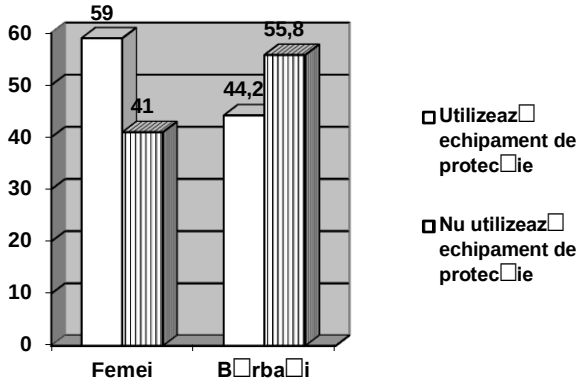

Fig. 3. Distribuţia procentuală a utilizării echipamentului de protecţie în funcţie de sex

Ochelarii de protecţie sunt utilizaţi de către 45,9% dintre tehnicieni (51,6% dintre femei şi 40,0% dintre bărbaţi) în comparaţie cu masca - 37,7% (41,9% dintre femei şi 33,3% dintre bărbaţi) şi mănuşile de cauciuc -19,6% (22,5% dintre femei şi 16,6% dintre bărbaţi). 18,0% dintre tehnicieni declară că utilizează echipamentul complet de protecţie (masca, mănuşi şi ochelari) (fig. 4).

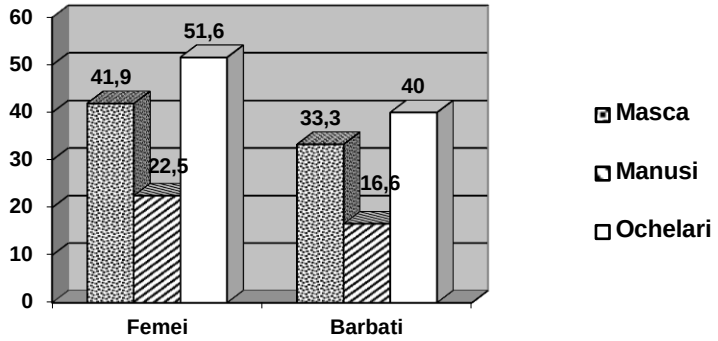

Fig. 4. Utilizarea componentelor echipamentului de protecție în funcție de sex

Evaluarea răspunsurilor referitoare la măsurile de control al contaminării aerului a relevat faptul că 59,1% dintre laboratoare dispun de sisteme de ventilație ($p<0,05$).

Dezinfecția suprafețelor de lucru se practică în 85,2% dintre laboratoarele de tehnică dentară. In majoritatea laboratoarelor dezinfecția suprafețelor se efectuează la începutul şi la sfârşitul zilei de lucru (52,4%) sau numai la începutul zilei (40,9%) ($p<0,05$). In ceea ce priveşte piesele protetice subiecții incluşi în studiu declară că efectuează dezinfecția amprentelor venite din cabinetul dentar (26,1%), a fazelor intermediare de tratament protetic (25.0%) şi a protezelor (26,1%) ($p<0,05$) (fig.5).

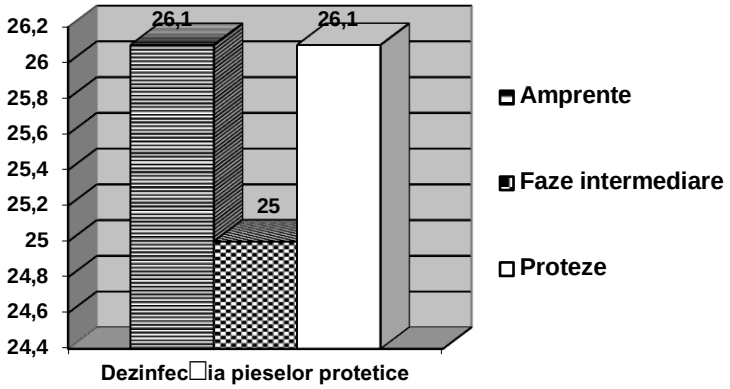

Fig. 5. Dezinfecția pieselor protetice în laboratoarele de tehnică dentară.

Decontaminarea pieselor de mână şi a instrumentarului rotativ se practică de către 36,0% dintre tehnicienii dentari. Majoritatea subiecților utilizează în acest scop proceduri de dezinfecție (31,1%) iar sterilizarea pieselor de mână este menționată de către 4,9% dintre aceştia.

Rata de răspuns la întrebarea privind antisepticele utilizate pentru dezinfecție a fost de 31,2%. Produsele menționate pentru dezinfecția suprafețelor sunt: Perform, alcool alb, Surfanios, Domestos, Zeta 3. Pentru piesele de mână şi instrumentarul rotativ sunt utilizate produse ca: Hexanios, Zeta 1,

Perform iar pentru dezinfecţia pieselor protetice : Hexanios, Zeta 7, Perform, Cloramina, alcool .

DISCUŢII

Personalul medical din laboratoarele de tehnică dentară trebuie să adere la standardele profesionale, legale şi etice în privinţa controlului infecţiei (7,8). Rezultatele studiului relevă faptul că majoritatea tehnicienilor (93,4%) declară că sunt preocupaţi şi posedă cunoştinţe privind mijloacele de prevenire a transmiterii infecţiei în laboratorul dentar. Această atitudine se reflectă în susţinerea necesităţii dezinfecţiei pieselor protetice atât la intrarea cât şi la ieşirea din laborator de către 90,2% şi respectiv 81,9% dintre subiecţi, fără diferenţe semnificative în funcţie de sex. Percepţia subiecţilor participanţi la studiu asupra utilităţii acestor măsuri relevă preocuparea privind protecţia tehnicienilor (86,9%), ignorând importanţa acestora pentru medic şi pacient.

Răspunsurile la întrebările referitoare la echipamentul de protecţie demonstrează că acesta este utilizat doar de jumătate dintre tehnicieni, femeile apelând la aceste bariere fizice de protecţie în proporţie mai ridicată decât bărbaţii. Preocuparea pentru protecţia ochilor este demonstrată de proporţia ridicată a tehnicienilor care utilizează ochelarii de protecţie (45,9%). Această proporţie este mai mare comparativ cu rezultatele raportate de AlDwairi (35,2%)(9) dar mult mai mică decât cea prezentată de Jagger şi colaboratorii (74,2%) (10). Mănuşile de cauciuc sunt folosite de către tehnicieni în proporţie foarte redusă (19,6%) comparativ cu datele raportate de în literatură: AlDwairi şi colaboratorii (24%)(9), Jagger şi colaboratorii (44%) (10) şi Akeredolu şi colaboratorii (60,5%) (11). Aceste rezultate demonstrează ignorarea riscului de expunere la infecţie prin leziuni provocate de obiecte ascuţite în cursul manevrării şi prelucrării pieselor protetice.

Procedurile de control al calităţii aerului în laboratorul de tehnică dentară trebuie să urmărească reducerea riscului de expunere la pulberi, gaze şi agenţi patogeni cu transmitere pe cale aeriană (12). Răspunsurile subiecţilor incluşi în studiu susţin că 59,1% dintre laboratoare dispun de sisteme de ventilaţie ($p<0,05$).

Evaluarea procedurilor de control al transmiterii infecţiei pe cale indirectă prin intermediul suprafeţelor contaminate a relevat faptul că în 85,2% dintre laboratoare se practică dezinfecţia suprafeţelor de lucru. În jumătate dintre laboratoare dezinfecţia suprafeţelor se efectuează la începutul şi la sfârşitul zilei de lucru (52,4%).

Sursa principală de contaminare în laboratorul de tehnică dentară este reprezentată de piesele protetice contaminate cu sângele şi saliva pacienţilor care trebuie să fie dezinfectate, în condiţiile unei comunicări eficiente, în cabinet sau laborator. Rezultatele studiului demonstrează că o proporţie redusă dintre tehnicieni efectuează dezinfecţia amprentelor (26,1%), a fazelor intermediare de tratament protetic (25.0%) şi a protezelor (26,1%) comparativ cu datele prezentate de alţi autori ca Akeredolu şi colaboratorii şi Hatzikyriakos şi colaboratorii care raportează că în 74,4% , respectiv 74,8% dintre laboratoarele dentare amprentele şi piesele protetice sunt dezinfectate la intrarea în laborator (11,13).

Piesele de mână şi instrumentarul rotativ (freze) utilizate în laboratorul dentar sunt dezinfectate doar de o treime dintre subiecţi iar sterilizarea pieselor de mână este menţionată de către 4,9% dintre aceştia. La întrebarea referitoare la antisepticele utilizate pentru dezinfecţie, rata de răspuns

a fost foarte redusă (31,2%), demonstrând lipsa de preocupare şi cunoştințe în acest domeniu .

CONCLUZII

Evaluarea condițiilor de desfășurare a activității în laboratoarele de tehnică dentară privind aplicarea protocoalelor de control al infecției demonstrează necesitatea implementării unor măsuri energice de creştere a nivelului de cunoştințe în acest domeniu. Organizarea acțiunilor de educație medicală şi inițierea de programe specifice trebuie să urmărească creşterea complianței tehnicienilor dentari față de măsurile de control al infecției.

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FOOD–DRUG INTERACTIONS: GRAPEFRUIT JUICE

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FOOD–DRUG INTERACTIONS: GRAPEFRUIT JUICE (Abstract): Food-drug interactions are increasingly recognized as important clinical events which may change significantly the bioavailability of oral administrated drugs. Grapefruit juice (GFJ) demonstrated multiple interactions with drugs leading to loss of the therapeutic effects or increased side-effects. GFJ decreases pre-systemic metabolism through a) competitive or mechanism-based inhibition of gut wall CYP3A4 isoenzymes and b) P-glycoprotein (P-gp), c) multidrug resistance protein-2 (MRP2) or d) organic anion-transporting polypeptide (OATP) inhibition. Although, GFJ presents high amounts of flavonoids (e.g. naringin, naringenin), furanocoumarins (e.g. 6',7'--dihydroxybergamottin, bergamottin) are the main chemicals involved in the pharmacokinetic interactions. As compounds of GFJ show additive or synergistic effects, all the major furanocoumarins are necessary for the maximal inhibitory effect. Also, related citrus fruits (sweeties, pummelo and sour orange) or various plants containing furanocoumarins may present pharmacological interactions, yet to be discovered. **Key words:** GRAPEFRUIT JUICE, PHARMACOKINETIC INTERACTION, CYP

Food-drug interactions are increasingly recognized as noteworthy clinical events as they may shift the effect of the drug outside of its therapeutic window (leading either to loss of effect or undesired side effects) (1).

The discovery that grapefruit juice (GFJ) can increase the oral availability of some medications was an accidental discovery made in 1989 when GFJ was used to mask the taste of ethanol in a study involving the calcium channel blocker felodipine (2, 3). Subjects consuming GFJ concomitantly with felodipine presented a lower dehydrofelodipine/felodipine area under the curve (AUC) ratio, decreased diastolic blood pressure and an increased heart rate (4).

As population has become more aware of the health benefits of antioxidants found in fruits and vegetables, grapefruit and other fruit juices are frequently consumed worldwide. Especially in the elderly, GFJ is often administrated with medication as it is thought that linking drug administration to a regular event like a meal can improve the patient's adherence to the treatment (5).

GFJ significantly decreases pre-systemic metabolism due to inhibition of: a) gut wall CYP3A4 isoenzymes, b) P-glycoprotein (P-gp), c) multidrug resistance protein-2 (MRP2), d) organic anion-transporting polypeptide (OATP) and e) esterase activity (4-11).

The **most important compounds** of GFJ considered to be involved in the pharmacokinetic interaction are flavonoid glycosides

(narirutin, naringin, naringenin, quercetin, kaempferol, hesperidin, neohesperidin, didymin, and poncirin), furanocoumarins (6',7'-dihydroxybergamottin (DHB), GF-I-1, GF-I-4, bergamottin (GF-I-2), GF-I-5 (bergamottin--6',7'-epoxide), GFI-6, bergapten, epoxybergamottin] and sesquiterpens (nootkatone) (12-15).

Naringin, which gives the pungent taste, is the main flavonoid in GFJ, found in concentrations of 450 mcg/mL (10% of the dry weight of juice) (16). Although naringenin, the metabolite of naringin, demonstrated inhibitory effects on CYP3A4, recent evidence clearly indicate that furanocoumarins are the chemicals responsible for in *vivo* significant GFJ interactions (17, 18).

Among different strains of GFJ, origin, packages and ways of processing, the amount of active ingredients may vary among batches and lots (5).

GF-I-2 and GF-I-4 appear mainly in pulp in comparison to sac, peel or seed of grapefruits. The composition of GF-I-1, GF-I-2 and GF-I-4 in grapefruit pulp are similar to those in juice product, excluding the suspect that furanocoumarins might be the byproducts during juice processing.

Furanocoumarin derivatives have been found in various plants belonging to families like *Rutaceae*, *Apiaceae, Moraceae* and *Fabaceae*. 6',7'-dihydroxybergamottin and bergamottin are the furanocoumarins found in the highest concentration in fresh grapefruit (in the low micromolar range) (16).

The precipitate obtained after the centrifugation of GFJ (which contains GF-I-1, GF-I-2, GF-I-4, GF-I-5 and GF-I-6) presents as strong inhibition of CYP3A4 activity as that of the original juice sample. The supernatant (rich in DHB) presents much weaker CYP3A4 inhibition (17, 18).

a) The cytochrome P450 (**CYP**) enzyme family plays a major role in the metabolism a wide array of xenobiotics including drugs, chemical carcinogens, insecticides and other environmental pollutants (4).

CYP3A4 isoenzyme is found in the intestine and liver and accounts for about 40% to 60% of all CYP450 isoenzymes (19). Intestinal CYP3A4 is found in apical enterocytes and varies 11-fold in protein content among individuals. Intestinal and hepatic CYP3A4 content appear to be regulated independently of each other (5). The susceptibility of CYP3A4 to modulation by food constituents may be related to its high level of expression in the intestine, as well as its broad substrate specificity (13).

Inhibition of the CYP system occurs mainly through two **mechanisms**.

The first and most common mechanism is known as competitive inhibition and results from the competition between the inhibitor and substrate for the same CYP isoenzyme required for substrate metabolism and elimination. The effects of competitive inhibition can be observed after administration of the first dose of the inhibitor.

The second mechanism is known as mechanism-based inhibition. This effect is thought to be caused by furanocoumarins that was initially metabolized by CYP3A4 to a reactive intermediate and then bonded covalently to the enzyme, a process termed "suicide" or "mechanism-based" inhibition.

The structurally modified and inactivated CYP3A4 undergoes rapid proteolysis within the cell and consequently, the return of CYP3A4 activity would require *de novo* enzyme synthesis. The fact that reduced intestinal CYP3A4 protein by GFJ is not associated with increased CYP3A4 mRNA, indicates that there is not an effective feedback mechanism within the enterocyte to up-regulate CYP3A4 synthesis (5, 16). Furanocoumarins inactivate CYP3A4 by the binding of the furanoepoxide to the apoprotein, presumably at or near their respective substrate domains (4). Separating the administration of GFJ and substrate drug by a few hours does not minimize grapefruit-drug interactions (20).

The onset of the interaction can occur within 30 minutes. Complete recovery of the CYP3A4 may take 48 to 72 hours after the last exposure to GFJ. The half-life of disappearance of inhibitory effect of GFJ on CYP3A4-mediated drug metabolism was estimated at 12 hours.

In the case of nisoldipine, increased drug AUC is observed up to 72 h after a 7-day pretreatment period of GFJ (200 ml) three times daily ; repeated administration of juice might be expected to cause a cumulative increase in magnitude of pharmacokinetic interaction (5). GFJ does not affect the pharmacokinetics of these drugs when they are administered intravenously (13).

In a study conducted with human intestinal microsomes by Paine and coworkers, DHB induced a substrate-independent reversible (within 30 minutes) and mechanism--based inhibition on CYP3A4 (21). In contrast, BG (bergamottin), being more lipophilic, was a substrate dependent reversible inhibitor and a substrate-independent mechanism based inhibitor (three hours) (4).

GF-I-1, GF-I-2 and GF-I-4 are not detected in juice samples of apple, grape, orange and tangerine, only trace amounts of GF-I-2 and GF-I-4 appeared in lemon juice, suggesting that furanocoumarins are fairly selective in GFJ.

Depending on KI values (the inhibitor concentration required for half-maximal rate of inactivation) on nifedipine oxidation in human liver microsomes, recent data indicated that dimers and trimers induce a stronger inhibition of CYP3A4 activities than monomers. The inhibitory potencies of furanocoumarins are similar to those shown by a typical inhibitor, metyrapone or strong CYP3A4 inhibitor, ketoconazole (for dimer and trimer structures). Between the two monomers, DHB is more potent than GF-I-2 for CYP3A4 inactivation. A plain core structure, tricyclic ring containing a furan moiety in linear position, is more important than the side chains for furanocoumarins in CYP3A4 inhibition.

In contrast to the results from the extract of GFJ which show similar inhibitory properties on CYP3A4, CYPlA2, CYP2C9, and CYP2D6, isolated GF-I-2 presents clear inhibition of CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, with stronger potencies on CYP2C9 and CYP2D6.

All the major furanocoumarins are necessary for the maximal inhibition of CYP3A4 activity observed in GFJ. Each furanocoumarin contributes to the GFJ-drug interaction due to either stronger CYP3A4 inhibitory potency (like dimers GF-I-1 and GF--I-4) or higher natural abundance (like DHB and GF-I-2) (17, 18).

In conclusion, GFJ - drug interactions are caused by additive or synergistic effects of several flavonoids and furanocoumarins in the juice rather then a single potent component (22)

It is interesting to note that related citrus fruits (sweeties, pummelo and sour orange) which contain furanocoumarins are also susceptible to drug interactions. For example, sweetie is a stronger inhibitor of CYP3A4 than GFJ. Kim et al., 2006 (23) reports that commercial black mulberry fruit juice inhibits CYP3A-catalyzed midazolam 1'-hydroxylation suggesting that a mechanism-based inhibitory component is present in these fruit juices as in the case of GFJ. Seville orange juice with DHB and GF-I-2 at half level than GFJ showed similar effect on human CYP (17, 18).

More, residues prepared from star fruit, pomegranate and common papaw significantly inhibited CYP3A4 activity in comparison with white grapefruit juice (control). No significant inhibition was noted with valencia orange, mango, kiwi fruit, dragon fruit or passion fruit (24).http : //www.power netdesign.com/grapefruit/references/index.htm - ref131

b) **P-gp** is an adenosine 5'-triphosphate--dependent efflux pump encoded by the mul-

tidrug resistance (MDR) 1 gene located on the luminal surface of epithelial cells of the small intestine, the bile canalicular membrane of the liver and the proximal tubule of the kidney (5). In addition to GFJ, orange juice and pomelo juice also have been shown to inhibit the activity of P-gp *in vitro* which results in increase blood levels of the drug substrate (4). Okura et al., 2008 (1) reports that GFJ potentiates the antinociceptive effect of morphine through inhibition of intestinal P-glycoprotein.

c) **MRP2** is a efflux protein closely related to Pgp in terms of its expression and function (13).

d) **OATP-B** is a transmembrane sodium- and ATP - independent transporter present in the intestinal mucosa which promotes uptake of drugs into the enterocytes. GFJ inhibition of OATP results in reduced blood levels of the drug substrate (25). There are authors who consider that orange juice (and apple juice) decrease the bioavailability of fexofenadine through an inhibition of the influx transporter OATP (6, 26, 27). Among GFJ components, naringenin and naringin inhibit the OATP-B–mediated uptake of estrone-3-sulfate into human embryonic kidney cells and interact with simvastatin and saquinavir in *in vitro* experiments (11).

e) Recent data indicate that GFJ also decreases the **esterase** activity leading to enhancement of exposure to the active drugs in rats (28). In rat and human hepatic or gut fractions and rat gut lumen, GFJ inhibited the hydrolysis of enalapril and lovastatin, which are known to be metabolized principally by esterases, lovastatin being metabolized also by CYP3A4. The oral bioavailability of lovastatin in humans was increased 15-fold by GFJ (11).

Unfortunately, there are no routine clinical tests available to estimate the extent of pharmacokinetic interaction before exposure (5). The human intervention trials examining the pharmacokinetic interaction of GFJ revealed a great inter-individual variability, where subjects with the highest content of CYP3A4 showed the largest reduction of this enzyme after the consumption of grapefruit (4).

There are several factors associated with greater risk of developing side-effects such as :

- pre-existing medical conditions,
- elderly patient (multiple medication and reduced autonomic responsiveness) (5),
- consumption of high doses of a susceptible drug together with GFJ for the first time (sudden decrease of intestinal CYP3A4 activity),
- severe liver disease (higher importance of intestinal metabolism),
- consumption of drugs with narrow therapeutic index and metabolized by CYP enzymes (16)
- administration of drugs with low bioavailability with other inhibitors of cytochrome P450.

Physicians and pharmacists should be aware of the possibility that some drugs (anti-arrhythmics - amiodarone ; hypnotics - diazepam ; calcium channel blockers - felodipine, nicardipine, nifedipine ; anti-convulsant - carbamazepine ; immune modulators - cyclosporine, hormons - ethinyl estradiol, HMG-CoA reductase inhibitors - atorvastatin, lovastatin, simvastatin ; corticosteroids - methylprednisolone ; macrolide antibiotics - erythromycin etc.) can interact with GFJ and inform the patients about it. Also, in order to facilitate knowledge sharing, regulatory agencies (such as FDA) require some drugs such as cyclosporine, sirolimus, simvastatin, lovastatin, and felodipine to carry a warning label regarding the possibility of an interaction) (4, 29, 30).

In future it is possible to develop grapefruit furanocoumarins as additives to certain drugs in order to improve their oral bioavailability and reduce the variability. On the other hand, as the pharmacogenetic-based

approach is not such a near reality (31), citrus juice manufacturers can develop GFJs without furanocoumarins, which would reduce the potential for a drug interaction (4, 5).

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POLIMERI RETICULAŢI ACRILICI UTILIZAŢI PENTRU OBŢINEREA DE NOI MATERIALE DENTARE

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RETICULATE POLYMERS USED IN OBTAINING NEW DENTAL MATERIALS (Abstract) : This paper seeks the characterization of new polymers acrylic cross-linked structure, prepared in the presence of a template that creates the polymer synthesized the property of saving template form. **Material and method** : For carrying out synthesis of polymers reticulate-based on dimethacrylate monomer was left to the mono-, di-and trietilenglicol dimethacrylate. Cross-linked polymerization reaction was initiated with benzoyl peroxide. **Results** : New structures are obtained witch respond to the need to diversify and improve the biomaterials used in dentistry. The materials are chemically thermally and mechanically inert. Reticulate polymers are non-biodegradable and therefore do not produce intoxication and not degrade biological fluids. **Key words** : RETICULATE POLYMERS, TEMPLATE POLYMERIZATION, COMPLETE DENTURES

Asimilarea compuşilor macromoleculari sintetici în medicină şi terapeutică, domenii interdisciplinare, trebuie să răspundă complexităţii problemelor de utilizare ce rezultă din contactul temporar sau de lungă durată al materialelor polimerice cu ţesuturile şi substanţele biologice (1). În acest context, sunt definite ca biomateriale polimerice acei polimeri sau compozite polimerice atestate ca biocompatibile în contact cu biostructurile. Prin crearea de noi polimeri şi noi structuri, ştiinţa materialelor pregăteşte tehnologii noi, o dată cu descoperirea de noi fenomene fizice şi proprietăţi specifice la scară atomică (2). Polimerii reticulaţi, pot fi reţele tridimensionale ale catenelor legate chimic între ele sau pot rezulta în urma legării covalente a catenelor liniare (3).

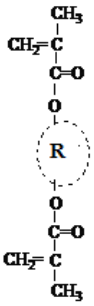
Cea mai folosită răşină utilizată în stomatologie are ca bază de plecare un monomer (metacrilatul de metil) al cărui polimer are în momentul actual o multitudine de denumiri comerciale (4).

Lucrarea de faţă are ca scop obţinerea şi caracterizarea unor noi polimeri acrilici cu structură reticulată, preparaţi în prezenţa unui templat care crează polimerului sintetizat proprietatea de a memora forma templatului. Aceşti polimeri, denumiţi "inteligenţi" datorită memorării formei templatului îşi găsesc în medicina dentară aplicatibilitate practică (5). O traiectorie importantă este înglobarea vitaminei B_{12} în structura polimerului acrilic în scopul tratamentului aftelor bucale, precum şi în scop profilactic prin eliberarea acesteia în saliva pacientului şi reînglobarea ei prin imersia materialelor în B_{12}.

MATERIAL ŞI METODĂ

Metoda de polimerizare utilizată în acest studiu este o metodă nouă numită polimerizare templată în care am utilizat monomeri prezentaţi în tabelul I.

TABELUL I

Denumire	Structură	Provenienta
Etilenglicol dimetacrilat		Reactiv organic, 98%, Merck.
Monoetilenglicol dimetacrilat (MEGD) unde **R** este $-CH_2-CH_2-$	Dietilen glicol dimetacrilat (DEGD) unde **R** este $-(CH_2)_2-O-(CH_2)_2-$	Trietilen glicol dimetacrilat (TEGD) unde **R** este $-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2$

Ca şi moleculă templat am folosit ciancobalamina care este forma de vitamină B_{12}, cu utilizare pe scară largă din punct de vedere clinic, datorită disponibilităţii şi stabilităţii acesteia. Este transformată în factori activi în interiorul organismului. Pentru realizarea sintezelor de polimeri reticulaţi pe bază de monomeri dimetacrilici s-a plecat de la mono-, di- şi trietilenglicol dimetacrilat. Reacţia de polimerizare reticulantă (fig. 1), a fost iniţiată cu peroxid de benzoil.

Reţinerea, eliberarea şi reîncărcarea cu materialul templat au fost testate cantitativ cu ajutorul spectroscopiei în UV cu ajutorul unui Spectrofotometru UV-VIZ Specord 200 Analytic Jena. Analizele de degradare termică au fost făcute pe un aparat Paulik-Erdey- Budapest Derivatograf, iar spectrele IR pe un Spectrofotometru Specord M90 Carl Zeiss Jena

REZULTATE ŞI DISCUŢII

Polimerul format în urma legării covalente a catenelor macromoleculare este un polimer reticulat iar legăturile formate se numesc reticulări. Aceşti polimeri se disting prin stabilitatea lor dimensională, termică şi chimică, precum şi prin abilitatea crescută de a păstra informaţia formei lor (memoria formei). Influenţa condiţiilor de preparare a polimerilor reticulaţi ai monomerilor dimetacrilici asupra caracteristicilor produşilor obţinuţi sub formă de perle a fost studiată pe un număr de 6 probe pentru obţinerea cărora s-a variat natura monomerilor şi cantitatea de templat. În tabelul II sunt date compoziţiile polimerilor obţinuţi precum şi câteva caracteristici specifice polimerilor. Un studiu al influenţei cantităţii de templat şi a naturii monomerilor asupra structurilor obţinute trebuie să ţină seama de complexitatea reacţiilor care au loc în timpul reticulărilor monomerilor bisnesaturaţi care participă la reacţie.

Cu cât densitatea polimerului este mai

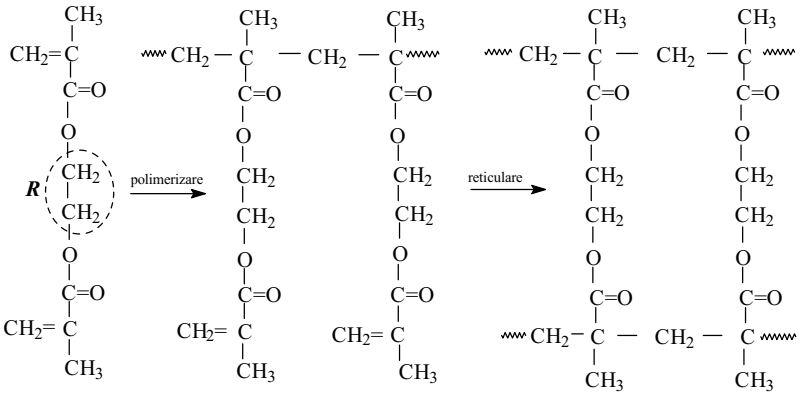


Fig. 1. Polimerizarea-reticularea monoetileglicol dimatacrilatului

TABELUL II
Compoziţia şi proprietăţile polimerilor preparaţi în prezenţa vitaminei B_{12}

	COMPOZIŢIE		PROPRIETĂŢI	
Cod proba	Monomer	Templat %, g	Den-sitate	Stabilit termică T_{max}, ^{0}C
M1	MEGD	25	0,32	425
M2	MEGD	50	0,42	415
D1	DEGD	25	0,41	525
D2	DEGD	50	0,82	530
T1	TEGD	25	0,57	542
T2	TEGD	50	1,00	557

mare, ca atât reticularea este mai puternică. Se observă că materialele polimere cel mai puternic reticulate sunt cele obţinute pe baza de TEGD. Având în vedere faptul că produşii puternic reticulaţi sunt cei mai stabili termic şi chimic, am ales pentru testele de reţinere şi eliberare a templatului proba T2, preparată din monomerul cu catena cea mai lungă şi flexibilă, care în timpul polimerizării permite templatului să rămână în reţeau tridimensională care este heterogenă. Considerând cantitatea iniţială de vitamină B_{12} ca fiind 100%, o cinetică de elibarare a vitaminei pe parcursul a 8 zile, a fost urmărită prin introducerea polimerului într-un pahar cu apă, la temp de 37^{0}C, iar apa a fost schimbată în fiecare zi (fig. 2). Se constată că cea mai mare cantitate de vitamina B_{12} se elibereaza în primele 5 zile, apoi rămâne o cantitate constantă, foarte mică de vitamină, care în condiţiile statice date rămâne constantă.

Structurile preparate prin copolimerizarea reticulantă radicalică a monomerilor mono- şi polinesaturaţi în prezenţa unui templat formează reticulările în jurul tem-

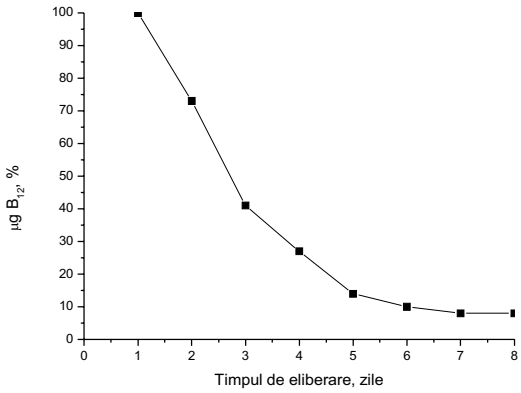


Fig. 2. Eliberarea vitaminei B_{12} în apă, la 37^{0}C

platului, iar eliberarea acestuia lasă goluri în materialul polimeric, goluri care păstrează memoria formei templatului.

Caracterizarea spectrală a polimerilor reticulaţi sintetizaţi a evidenţiat structura complexă a acestor polimeri. Un spectru tipic pentru poli(MEGD) este dat în fig. 4. Se observă existenţa grupărilor vinilice nereacţionate la 1640 cm^{-1} datorate vibraţiilor C=C şi la 950 cm^{-1} datorate =CH_2.

Analiza IR a poli(DEGD) arată structura complexă a polimerului ce conţine benzi de adsorbţie la 1734, 1265, 1160 şi 1060 cm^{-1} atribuite grupării C=O şi grupării -COOR ; la 2890, 2855 cm^{-1} şi la 2960, 2930 cm^{-1} sunt atribuite vibraţiilor simetrice şi asimetrice -CH_3 (fig. 5).

În seria monomerilor M, D, T, creşterea lungimii lanţului dintre cele două grupări nesaturate determină schimbări evidente asupra mecanismului de formare a structurilor macroreticulate. Cu cât distanţa dintre cele două unităţi de ester metacrilic este mai mică, cu atât efectul de împiedicare sterică

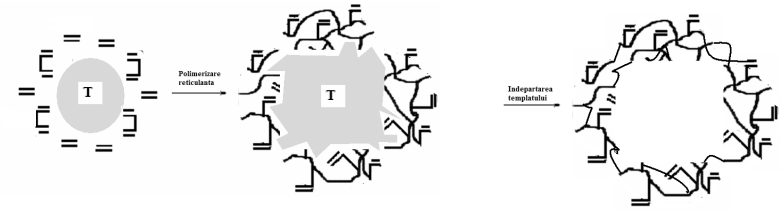


Fig. 3. Modul de formare a polimerului reticulat, în prezenţa templatului

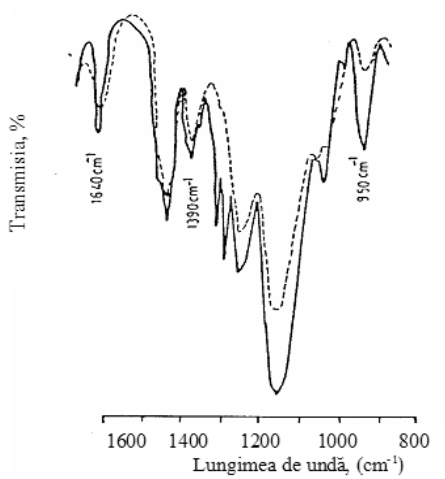


Fig. 4. Spectrul IR al polimerului pe baza de MEGD (—— proba M2 şi _____ proba M1)

creşte şi în consecinţă numărul de reticulări este mai mic. Scăderea masei moleculare medii dintre două reticulări este o consecinţă a creşterii densităţii de reticulare. Cel mai înalt grad de reticulare se observă la poli(TEGD) deoarece monomerul de plecare are lanţul dintre grupările nesaturate cel mai mare. Reacţiile de reticulare sunt favorizate în seria TEGD > DEGD > MEGD în comparaţie cu reacţiile de propagare liniară din cauza efectului excluderii de volum.

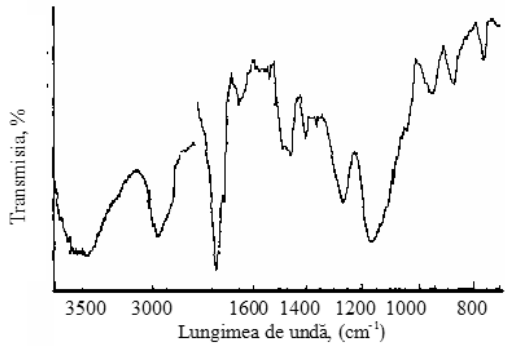


Fig. 5. Spectrul IR al poli(DEGD), proba D1

CONCLUZII

1. Materialele dentare sintetizate fac parte din clasa polimerilor reticulaţi, sinteza lor presupunând unirea macromoleculelor liniare de PMM prin legături covalente cu monomeri monofuncţionali, în cazul nostru MM, sau cu monomeri polifuncţionali, aşa cum este EGDMA
2. Prin metoda polimerizării template se obţin polimeri sintetici cu selectivitate predeterminată care permit difuzia moleculei imprint înăuntrul şi în afara matricei polimere. Locul creat după îndepărtarea templatului se constituie ca "o memorie moleculară indusă", denumită "intellectual cavity", capabilă de recunoaştere selectivă. Polimerii obţinuti în prezenţa templaţilor au o memorie selectivă, reţinând în mod preferenţial produşi cu structură asemănătoare templatului.
3. Întrebuinţarea unor monomeri dimetacrilici, în scopul obţinerii de structuri reticulate polare şi hidrofile răspunde necesităţii diversificării şi îmbunătăţirii biomaterialelor utilizate în stomatologie, cunoscut fiind faptul că polimerii reticulaţi sunt materiale inerte din punct de vedere chimic, termic şi mecanic.

   Polimerii reticulaţi sunt nebiodegradabili şi prin urmare nu produc intoxicări şi nu degradează fluidele biologice.

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UNELE CORELAŢII ÎNTRE DEZVOLTAREA FIZICĂ ŞI ALIMENTAŢIE. OBSERVAŢII PE UN LOT DE ADOLESCENŢI DIN MUNICIPIUL CÂMPULUNG MOLDOVENESC

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SOME RELATIONS BETWEEN PHYSICAL GROWTH AND NOURISHMENT. OBSERVATION ON A GROUP OF TEENAGERS FROM CAMPULUNG MOLDOVENESC (Abstract) : The raise and growth of children and youth are processes influenced by the action of internat (inheritance) and external factors (environmental). Among the exterlan factors, nourishment plays an essential role. **Material and method** : The study was made on 369 high school students. There has been made an appreciation of height and weight of these students. There has also been made a nutritional survey on groups of food. **Results** : Height is prevalent high (40.4%) and very high (29.8%) Weight is especially at average (53.8%) and high values (23.1%). The nutritional survey on groups of food shows a raising input of milk, meat (over 100%) at males and females of 11-14 years old, and also of eggs. On the other hand, fish scores a constant shortcoming (-50%). Corny and dried vegetables products are in high quantities whereas the input of fruit is insufficient (over -20%). **Conclusion** : These studies let us know the peculiarities of youth's nourishment, which allow a correct orientation of prevention programs. **Key words** : HEIGHT, WEIGHT, NOURISHMENT

Perioada copilăriei şi adolescenţei se particularizează printr-o creştere şi o dezvoltare intensă (1,2,3). Creşterea este un process cantitativ (de acumulare a substanţei organice), în timp ce dezvoltarea este un proces calitativ (de accentuare a complexităţii structurilor şi funcţiilor). Aceste două procese sunt influenţate de acţiunea unor factori interni (ereditari) şi externi (de mediu). Multă vreme s-a insistat asupra acţiunii unuia din factori, celălalt fiind minimalizat. În prezent, se ştie că aceşti doi factori acţionează concomitent, ajustându-şi reciproc efectele. Un copil ajunge la valorile taliei înscrise în codul genetic doar în condiţiile adecvate de mediu (4,5,6).

Deoarece în cadrul factorilor de mediu, alimentaţia joacă un rol esenţial, ne-am propus realizarea unor aprecieri asupra corelaţiei dintre dezvoltarea fizică şi alimentaţie.

MATERIAL ŞI METODE

Studiul s-a realizat în oraşul Câmpulung Moldovenesc, pe un lot de 369 elevi, în vârstă de 13-17 ani, 292 (79,2%) de sex masculin şi 77 (20,8%) feminin, provenind dintr-o colectivitate în care admiterea se face pe baza selecţiei după anumiţi indicatori de dezvoltare fizică, iar în timpul şcolarizării, exerciţiile fizice sunt incluse în program. Caracteristicile lotului de studiu se referă la aprecierea dezvoltării staturo-

-ponderale (talie, greutate), urmată de încadrarea în clase sigmatice (foarte mică, mică, medie, mare şi foarte mare).

Ancheta alimentară s-a realizat prin metoda statistică, pe o perioadă de 3 ani, în lunile februarie (A1), mai (A2) şi octombrie (A3). Aprecierea s-a făcut cantitativ, pe grupuri de alimente, iar valorile cuprinse între ±10% din normă au fost considerate normale (7, 8). Prelucrarea şi interpretarea datelor s-a realizat folosind testul Pearson.

REZULTATE ŞI DISCUŢII

1. Unele caracteristici ale lotului studiat

Talia a fost de nivel mare (40,4%) şi foarte mare (29,8%), lipsind valorile foarte mici sau patologice, situaţie explicată prin selecţie iniţială.

S-au evidenţiat unele diferenţe ale *valorilor dominante*, astfel, la clasa a IX-a au fost prezente cele foarte mari, la a X-a şi a XI-a, cele mari, iar la a XII-a, cele medii. Aceste date permit a se aprecia că şi în condiţii foarte bune de viaţă, copilul ajunge doar la nivelul înscris în codul său genetic.

Comparând *valorile taliei* elevilor din clasa a IX-a şi a X-a, s-au observat diferenţe semnificative statistic ($p < 0,001$), similară fiind şi scăderea observată în cazul comparaţiei dintre clasa a XI-a şi a XII-a ($p < 0,001$) (tab. I).

Analiza greutăţii corporale pentru lotul inclus în studiu a evidenţiat, în special, valori medii (53,8%) şi mari (23,1%) deoarece selecţia la admitere face ca cele foarte

TABELUL I

Distribuţia cazurilor în relaţie cu nivelul taliei

Clasa		Înălţime (clasa sigmatică)				Total
		mică*)	medie	mare	f. mare	
A IX-a	*nr. elevi*	0	16	30	52	98
	%	0,0	16,3	30,6	53,1	100,0
A X-a	*nr. elevi*	0	20	51	27	98
	%	0,0	20,4	52,0	27,6	100,0
A XI-a	*nr. elevi*	1	29	45	20	95
	%	1,1	30,5	47,4	21,1	100,0
A XII-a	*nr. elevi*	4	40	23	11	78
	%	5,1	51,3	29,5	14,1	100,0
Total	*nr. elevi*	5	105	149	110	369
	%	1,4	28,5	40,4	29,8	100,0

*) pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL II

Distribuţia cazurilor în relaţie cu nivelul taliei

Clasa		Greutate (clasa sigmatică)				Total
		mică*)	medie	mare	f. mare	
A IX-a	*nr. elevi*	0	17	30	51	98
	%	0,0	17,3	30,6	52,0	100,0
A X-a	*nr. elevi*	0	19	32	47	98
	%	0,0	19,4	32,7	48,0	100,0
A XI-a	*nr. elevi*	1	43	29	22	95
	%	1,1	45,3	30,5	23,2	100,0
A XII-a	*nr. elevi*	1	42	18	17	78
	%	1,3	53,8	23,1	21,8	100,0
Total	*nr. elevi*	2	121	109	137	369
	%	0,5	32,9	29,6	37,2	100,0

*) pentru valorile mai mici de 10, proporţiile sunt orientative

mici şi cele patologice să lipsească, iar distribuţia acestora, pe clase şcolare, să aibă o evoluţie particulară (tab. II).

În clasa a IX-a şi a X-a, au fost dominante valorile foarte mari, pe când la a XI-a şi a XII-a, cele medii. Diferenţele între valorile măsurate la clasa a IX-a şi a X-a sunt nesemnificative statistic ($p > 0,05$), iar pentru celelalte clase, nu au fost îndeplinite condiţiile de cazuri minime, astfel că aprecierea nu s-a putut realiza.

2. Aspecte privind componentele nutriţionale

Caracteristicile alimentaţiei au fost apreciate pe grupuri de alimente: lapte, carne, ouă, peşte, grăsimi, produse cerealiere, cartofi, alte legume, leguminoase uscate, fructe, produse zaharoase. În interpretarea rezultatelor, s-au luat în consideraţie recomandările diferite, care apar în relaţie cu grupurile de vârstă şi sex. Consumul de lapte a fost asigurat, în majoritatea cazurilor, în limite normale.

Consumul de lapte. Excepţie face anul 2005 când, în A2 au existat valori de + 22,8% sau +31,5% la sexul masculin şi de +31,5% la feminin, cu valori în uşor exces, care nu ridică probleme deosebite, deoarece laptele bogat în proteine de calitate asigură creşterea normală a tinerilor (tab. III).

Carnea şi preparatele din carne s-au consumat în exces, urmare a particularităţilor activităţilor din această colectivitate, contribuindu-se, astfel, la optimizarea proceselor de creştere şi dezvoltare. La sexul masculin, consumul celor mai mari cantităţi de carne apare la grupul 11-14 ani, iar la cel feminin, aportul a fost dublu şi chiar mai mare la ambele grupuri de vârstă (11-14 şi 15-19 ani) ($p < 0,005$), cu toate că, în mod normal, necesităţile sunt mai mici, comparativ cu sexul masculin (tab. IV).

Ouăle au fost consumate în cantităţi corespunzătoare, în toate cele trei etape anuale şi în cei trei ani luaţi în studiu.

Consumul de peşte a fost deficitar, chiar şi sub 50% din necesar.

TABELUL III

Evaluări pe ani şi grupuri de vârstă a consumului de lapte pe lotul inclus în studiu

Grupul de vârstă (ani)		**11-14**			**15-19**		
An calendaristic		*2005*	*2006*	*2007*	*2005*	*2006*	*2007*
Sex	**Anotimp**						
Masculin	A1	+5,66	-17,59	+2,40	+13,20	-11,70	+9,70
	A2	+22,8	-8,04	-0,20	+31,50	-1,40	+6,80
	A3	-9,53	-5,20	-2,09	-3,06	+1,50	+4,90
Feminin	A1	+13,2	+11,70	+9,70	+13,20	+11,70	+9,70
	A2	+31,5	-1,40	+6,80	+31,50	-1,40	+6,80
	A3	-3,06	+1,50	+4,90	-3,06	+1,50	+4,90

A1 = luna februarie; A2 = luna mai; A3 = luna octombrie

TABELUL IV

Consumul de carne la elevii din lotul studiat

Grupul de vârstă (ani)		**11-14**			**15-19**		
An calendaristic		*2005*	*2006*	*2007*	*2005*	*2006*	*2007*
Sex	**Anotimp**						
Masculin	A1	+107,03	+108,60	+121,20	+71,03	+72,30	+82,80
	A2	+111,10	+66,20	+106,10	+74,30	+37,30	+70,20
	A3	+106,90	+108,50	+102,10	+70,95	+72,20	+66,90
Feminin	A1	+131,30	+133,20	+147,30	+107,03	+108,60	+121,20
	A2	+135,90	+85,80	+130,30	+111,10	+66,20	+106,10
	A3	+131,20	+133,08	+125,80	+106,90	+108,50	+102,10

A1 = luna februarie; A2 = luna mai; A3 = luna octombrie

TABELUL V

Consumul de carne la elevii din lotul studiat

Grupul de vârstă (ani)		11-14			15-19		
An calendaristic		*2005*	*2006*	*2007*	*2005*	*2006*	*2007*
Sex	**Anotimp**	%	%	%	%	%	%
Masculin	A1	+61,50	+61,50	+61,50	+33,04	+33,04	+33,04
	A2	+63,20	+61,50	+61,50	+34,80	+33,04	+33,04
	A3	+59,07	+59,70	+58,40	+31,40	+31,90	+30,80
Feminin	A1	+85,40	+85,40	+85,40	+70,00	+70,00	+70,00
	A2	+87,90	+85,40	+85,40	+70,00	+70,00	+70,00
	A3	+83,10	+83,90	+82,40	+67,90	+68,60	+67,20

A1 = luna februarie; A2 = luna mai; A3 = luna octombrie

TABELUL VI

Consumul de leguminoase uscate

Grupul de vârstă (ani)		11-14			15-19		
An calendaristic		*2005*	*2006*	*2007*	*2005*	*2006*	*2007*
Sex	**Anotimp**	%	%	%	%	%	%
Masculin	A1	+364,7	+429,4	+300,0	+209,8	+252,9	+166,6
	A2	+300,0	+300,0	+300,0	+166,6	+166,6	+166,6
	A3	+364,7	+300,0	+300,0	+209,8	+166,6	+166,6
Feminin	A1	+563,8	+656,2	+471,4	+563,8	+656,2	+471,4
	A2	+471,4	+471,4	+471,4	+471,4	+471,4	+471,4
	A3	+563,8	+471,4	+471,4	+563,8	+471,4	+471,4

A1 = luna februarie; A2 = luna mai; A3 = luna octombrie

Grăsimile s-au consumat în cantităţi care au depăşit normele la ambele sexe şi la cele două grupuri de vârstă. La sexul feminin, aportul de grăsimi totale a ajuns şi la un nivel dublu faţă de norme (+119,20% în 2007, A2) ($p < 0,005$). Raportul dintre grăsimile animale şi cele vegetale a fost echilibrat.

Produsele cerealiere au înregistrat la sexul masculin, grupul 11-14 ani, un consum care a depăşit necesarul cu 60%, iar la grupul 15-19 ani, cu 30%, pentru ca la sexul feminin depăşirea să fie de 85% la 11-14 ani şi de 65% la 15-19 ani ($p < 0,005$). Deşi acest aport crescut de cereale reprezintă un factor de risc pentru obezitate, elevii din această colectivitate au compensat, datorită programului permanent, de exerciţii fizice (tab. V).

Cartofii au fost consumaţi în cantităţi mari, la sexul masculin depăşind necesarul cu +21,90% (15-19 ani) şi cu +40,20% (11-14 ani), iar la sexul feminin s-au înregistrat niveluri maxime de +105,20% (15-19 ani) şi +128,05% (11-14 ani) ($p < 0,005$).

Leguminoasele uscate (fasolea, mazărea) au fost incluse în meniurile din această colectivitate, depăşind necesarul de 3-4 ori la sexul masculin (11-14 ani) sau de 1,5-2 ori la 15-19 ani, iar la sexul feminin, depăşirea a ajuns şi la 4-6 ori faţă de valoarea normală ($p < 0,005$) (tab. VI).

Consumul de fructe a fost deficitar, ajungându-se până la o carenţă de -60% la întregul lot studiat.

Produsele zaharoase au fost consumate în cantităţi echilibrate la sexul masculin, dar mai crescute la feminin.

CONCLUZII

Dezvoltarea fizică a adolescenţilor incluşi în lotul studiat a fost foarte bună, datorită modulul de alimentaţie impus de selecţia practicată la intrarea în colectivitate. Talia şi greutatea corporală au înregistrat valori normale, încadrate în clase sigmatice. Alimentaţia evaluată statistic doar pe grupuri de alimente a evidenţiat un consum echilibrat pentru lapte; carnea a fost asigurată în

cantităţi aproape duble faţă de necesar ; în schimb, consumul de peşte a înregistrat un deficit constant de până la 50% din necesar. Produsele cerealiere au depăşit constant necesarul, iar leguminoasele uscate au fost asigurate în cantităţi mari, comparativ cu fructele care au înregistrat un deficit constant.

Rezultatele studiului pot permite cunoaşterea caracteristicilor generale ale alimentaţiei, într-o anumită colectivitate, creând astfel posibilitatea orientării preventţionale a programelor nutriţionale.

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NOUTĂŢI

MYCOPHENOLATUL MOFETIL LA COPIII CU SINDROM NEFROTIC STEROID/CICLOFOSFAMID REZISTENT

Un grup de autori brazilieni îşi propune în acest studiu să evalueze rezultatele terapiei cu Mycophenolatul mofetil (MMF) la copiii cu sindrom nefrotic idiopatic (ISN) care s-a dovedit a fi atât steroid cât şi ciclofosfamid-rezistent. Tratamentul a fost urmat minimum 6 luni iar datele înregistrate au sumat peste 2 ani de urmărire. Copiii au fost împărţiţi în 2 grupuri : grupul 1 (34 copii) a cuprins copii care au primit ciclosporină A (CsA) înainte de iniţierea terapiei cu MMF ; grupul 2 (18 copii) a cuprins pacienţi care au primit numai MMF. În grupul 1, remisiunea completă sau parţială a fost înregistrată la 7 (20,6%) respectiv 13 (38,6%) pacienţi. Nu s-a obţinut răspuns terapeutic la 14 copii (41,2%). În grupul 2, remisiunea completă sau parţială a fost prezentă la 5 (27,8%), respectiv şase (33,3%) copii. Fără răspuns terapeutic au fost 7 copii (38,9%). Opt pacienţi au dezvoltat boală renală cronică. Cele mai întâlnite efecte secundare au fost tulburări gastrointestinale (11 cazuri, 21%), infecţii recurente severe (1 caz, 1,9%). MMF s-a dovedit eficient terapeutic n 59, 5% din cazuri. Aceste efecte favorabile nu au fost confirmate în studiile pe termen lung după întreruperea tratamentului. Analiza statistică a rezultatelor studiului prezentat de autorii brazilieni cu privire la terapia cu MMF nu relevă o diferenţă semnificativă între rezultate la copiii care au primit anterior CsA sau cei care au primit direct MMF (Raposo de Mello V, Rodrigues MT, Mastrocinque TH et al. Mycophenolate mofetil in children with steroid/cyclophosphamide-resistant nephrotic syndrome. *Pediatr Nephrol* 2010 ; 25 (3) : 453-460).

Mihaela Munteanu

EFECTUL ANTIMICROBIAN AL LASERULUI CU MIEZ DE ERBIUM ASUPRA *STREPTOCOCCUS MUTANS*. STUDIU *IN VIVO*

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THE ANTIMICROBIAL EFFICACY OF AN ERBIUM LASER ON *STREPTOCOCCUS MUTANS*. *IN VIVO* STUDY (Abstract) : This study was conducted in order to demonstrate the bactericidal effect of an erbium laser (Er,Cr : YSGG) on *Streptococcus mutans*, *in vivo*, at different laser power settings, obtaining sterile enamel surfaces with minimal tissue ablation. The practicians use the laser for the cutting effect on hard tissue to eliminate all the infected and affected enamel and dentine, without collateral damage on the pulp and for its bactericidal effect on cariogenic bacteria. **Material and method** : The study group included 132 superficial carious lesions, on 66 patients with ages between 6 and 20 ; a two-year study (January 2006 – November 2008) performed in a private practice. The carious lesions were ablated with an erbium laser with two types of tips. A sample of occlusal plaque was taken using a sterile swab, soaked in normal saline solution (before and after the preparation). **Results** : Before the laser treatment, the percentage with high counts of *Streptococcus mutans was* 70% and low counts 30% ($\chi^2 = 26,16$; $p < 0,01$). At the laser power of 4,5 watts, the zirconium tip (Z6) had a higher number of sterile culture (96,3%). The numbers of sterile cultures were higher at the laser power of 5 watts, with both tips : zirconium and sapphire, the percentage being equal : 91-92%. **Key words** : LASER THERAPY, SUPERFICIAL CARIOUS LESIONS, STREPTOCOCCUS MUTANS

ASSESSMENT OF *IN VITRO* ANTIOXIDANT ACTIVITY OF SOME NEW FERULIC ACID DERIVATIVES

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ASSESSMENT OF *IN VITRO* ANTIOXIDANT ACTIVITY OF SOME NEW FERULIC ACID DERIVATIVES (Abstract): **Aim:** The *in vitro* antioxidant potential of new thiazolidin-4-one derivatives of ferulic acid was evaluated according to the total antioxidant activity and ferric reducing power assays. **Material and methods:** The ferric reducing power assay was based on the reduction of ferricyanide to ferrocyanide, which form in the presence of ferric chloride a Perl Prussian blue color complex. The total antioxidant activity assay was assessed using phosphomolybdenum method. The results were expressed as effective concentration (EC_{50}) values and ascorbic acid was used as positive control. All determinations were performed in triplicate. **Results**: It was found that the activity of the tested compounds is influenced by the substituents on phenyl ring of the thiazolidine-4-one moiety. The most active compound was **1i,** which contains 2,3-diOH as substituent on phenyl ring. **Conclusions:** A total of 10 new thiazolidin-4-one derivatives of ferulic acid were investigated for their *in vitro* antioxidant activity. The most active compound **1i** (R=2,3-diOH) proved to be about 4 times more active than ferulic acid and comparable to ascorbic acid in both antioxidant assays. **Keywords:** FERULIC ACID, THIAZOLIDIN-4-ONES, ANTIOXIDANT ACTIVITY.

Free radicals, chemical species which contain unpaired electrons, could damage the human cells or tissues and may lead to different physiological disorders. They have been implicated in several diseases including diabetes mellitus, inflammation, cancer, cardiovascular and neurological disorders, aging (1). Antioxidants play an important role in free radical scavenging, being involved in the defense mechanism of the human body, thus providing protection against infections and degenerative diseases (1, 2).

Due to its antioxidant effect, ferulic acid could be useful in the prevention and treatment of several oxidative stress-related disorders, including Alzheimer's disease, diabetes mellitus, cancer, hypertension and atherosclerosis (3).

One the other hand one of the most studied classes of heterocyclic compounds is the thiazolidin-4-ones due to their important biological effects: anti-inflammatory (4, 5), antioxidant (6, 7), antimicrobial (4), analgesic (5), antitubercular (8) and antitumor (7) effects.

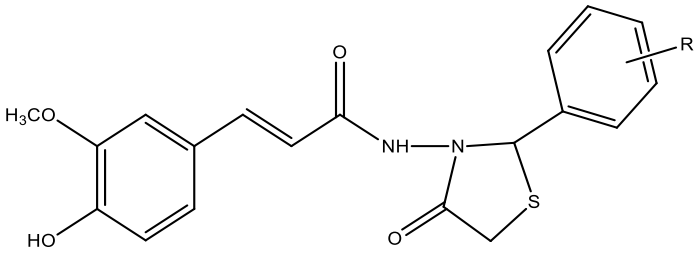
In order to improve the antioxidant effects of ferulic acid, new thiazolidin-4-one derivatives were synthesized. The synthesis of these new derivatives and their spectral characterization have been presented in our previous paper (9). The goal of this study was to evaluate the *in vitro* antioxidant potential of new thiazolidin-4-one derivatives of ferulic acid.

MATERIAL AND METHODS

Chemistry. The structure of the tested compounds, thiazolidin-4-one derivatives of ferulic acid, is presented in tab. I.

TABLE I

Thiazolidine-4-one derivatives of ferulic acid (1a-j)



1a-j

Comp	-R	Comp	-R
1a	-H	**1f**	-OH(2)
1b	-Cl(4)	**1g**	-Cl(2,6)
1c	-F(4)	**1h**	$-N(CH_3)_2(4)$
1d	$-NO_2(4)$	**1i**	-OH(2,3)
1e	$-NO_2(2)$	**1j**	$-OH(4),-OCH_3(3)$

The reagents used for antioxidant assays (ammonium heptamolybdate, potassium ferricyanide, ferric chloride, sodium dihydrogen phosphate, disodium phosphate, trichloroacetic acid, dimethylsulfoxide – DMSO, sulfuric acid) were purchased from Sigma Aldrich Company and Fluka Company.

The total antioxidant activity assay was assessed using phosphomolybdenum method according to the procedure described in the literature (6) with minor modifications. The method is based on the reduction of Mo(VI) to Mo(V) by the tested compounds followed by the formation of green phosphate/Mo(V) complex at acid pH. The samples were dissolved in DMSO in order to obtain a stock solution of 2 mg/mL concentration, from which different volumes (10 µL, 20 µL, 40 µL, 60 µL) were measured and completed with DMSO up to 200 µL. Over these samples 2 mL of the reagent solution (0.6 M sulfuric acid, 28 mM disodium hydrogen phosphate, and 4 mM ammonium heptamolybdate) were added. The samples were incubated at 95°C for 90 min, cooled at room temperature and centrifuged at 4600 rpm for 15 min. The supernatant was separated and the absorbance was measured at 695 nm using a blank (200 µL DMSO and 2000 µL reagent solution) (10). For each sample the effective concen-

tration (EC_{50}) was calculated and expressed in µg/mL. A low EC_{50} value indicate a higher total antioxidant capacity. Ascorbic acid (AA) was used as positive control being processed in a similar manner as the samples. All determinations were performed in triplicate and the values are expressed as mean ± SD (11).

The ferric reducing power assay is based on the reduction of ferricyanide to ferrocyanide in the presence of antioxidants with electron-donating abilities, which form in the presence of ferric chloride a Perl Prussian blue color complex. The compounds were tested at four concentrations (0.25 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL). To 1 mL sample of each concentration 1 mL of phosphate buffer (pH 6.6) was added. The reaction was then initiated by the addition of 1 mL of potassium ferricyanide 1%, after which the samples were incubated at 50°C for 20 min and the reaction was completed by the addition of 1 mL trichloroacetic acid 10%. The samples were centrifuged at 4500 rpm for 15 min. To 1 mL of supernatant 1 mL of double distilled water and 0.2 mL of ferric chloride 0.1% were added. After 5 min, the absorbance was measured at 700 nm using a blank prepared similar to the sample, which contained 1 mL DMSO instead of 1 mL sample (10). The results were expressed as EC_{50} values (mg/mL). A small EC_{50} value indicated a higher reducing power (12, 13). Ascorbic acid (AA) was used as positive control being processed in a similar manner as the samples. All determinations were performed in triplicate and the values were expressed as mean ± SD (11).

RESULTS AND DISCUSSION

Total antioxidant activity. The EC_{50} values of the tested compounds are presented in tab. II. As it can be seen, the most active compound was **1i** (R=2,3-diOH), with EC_{50} = 6.60 ± 0.12 µg/mL, which means that it is about 4 times more active than ferulic acid (EC_{50}=26.64 ± 0.01 µg/mL) and comparable with ascorbic acid (EC_{50}=5.12 ± 0.17 µg/mL) at the same concentration. A good antioxidant activity also showed **1j** (R=4-OH,3-OCH_3, EC_{50}=22.76 ± 0.26 µg/mL) and **1a** (R=-H, EC_{50}=23.06 ± 0.02 µg/mL), their activity being comparable with the activity of ferulic acid.

TABLE II

Total antioxidant activity (EC_{50} µg/mL) of the ferulic acid thiazolidin-4-one derivatives (1a-j)

Comp	EC_{50} µg/mL	Comp	EC_{50} µg/mL	Comp	EC_{50} µg/mL
FA	26.64 ± 0.01	**1d**	39.21 ± 0.04	**1h**	39.39 ± 0.01
1a	23.06 ± 0.02	**1e**	31.17 ± 0.04	**1i**	6.60 ±0.12
1b	25.11 ± 0.03	**1f**	31.00 ± 0.03	**1j**	22.76 ± 0.26
1c	30.37 ± 0.01	**1g**	49.54 ± 0.02	**AA**	5.12± 0.17

FA – ferulic acid; AA –ascorbic acid; Data are mean ± SD (n = 3, p<0.05)

Ferric reducing power. As it can be seen in tab. III, the most active compound was **1i** (R=2,3-diOH), with EC_{50} = 0.0899 ± 0.01 mg/mL, which means that it is about 4.2 times more active than ferulic acid (EC_{50}=0.3812 ± 0.09 mg/mL) and comparable with ascorbic acid (EC_{50}=0.0516 ± 0.01 mg/mL) at the same concentration. A good antioxidant activity also showed **1h** (R=4-$N(CH_3)_2$, EC_{50}=0.2571 ± 0.04 mg/mL), **1d**

(R=4-NO_2, EC_{50}=0.2937 ± 0.02 mg/mL) and **1e** (R=2-NO_2, EC_{50}=0.3357 ± 0.09 mg/mL), their activity being comparable with the activity of ferulic acid.

TABLE III

Ferring reducing power (EC_{50} mg/mL) of the ferulic acid thiazolidin-4-one derivatives (1a-j)

Comp	EC_{50} mg/mL	Comp	EC_{50} mg/mL	Comp	EC_{50} mg/mL
FA	0.3812 ± 0.09	**1d**	0.2937 ± 0.02	**1h**	0.2571 ± 0.04
1a	0.3716 ± 0.03	**1e**	0.3357 ± 0.09	**1i**	0.0899 ±0.01
1b	1.7269 ± 0.15	**1f**	1.2106 ± 0.33	**1j**	0.3710 ± 0.06
1c	0.4228 ± 0.09	**1g**	0.6923 ± 0.13	**AA**	0.0516 ± 0.01

FA – ferulic acid; AA –ascorbic acid; Data are mean ± SD (n=3, p<0.05)

CONCLUSIONS

In this study a total of 10 new thiazolidin-4-one derivatives of ferulic acid were investigated for their *in vitro* antioxidant activity using the total antioxidant activity and the ferric reducing power assays. The most active compound was **1i,** a thiazolidine-4-one derivative of ferulic acid that has 2,3-diOH as substituent on phenyl ring, this compound being more active than ferulic acid in both antioxidant assays. It is also noted that the activity of this compound was comparable to ascorbic acid used as positive control. These data encourage us to continue these *in vitro* and *in vivo* investigations.

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NEWS

RELAPSING POLYCHONDRITIS AND CARDIOVASCULAR INVOLVEMENT

It is well known that autoimmune diseases are characterized by an early atherosclerosis and cardiovascular events. Relapsing polychondritis is a rare inflammatory disorder that affects cartilaginous structures from the nose, ears, larynx and trachea. The cardiovascular involvement appears in 4 to 6% of patients in the form of aortic insufficiency and aortic aneurysm. All the cardiovascular events are associated with an increased mortality. The combination of aortitis and coronary ostial lesion is extremely rare and the decisive role in establishing the diagnosis is owned by the PET-CT scan. (Gavin Sugrue, Laura Durcan, Leann Bell, Ferdia Bolster, Suzanne Donnelly, Eoin Kavanagh. Unsuspected Cardiovascular Involvement in Relapsing Polychondritis A Case of Aortitis With Critical Coronary Artery Stenosis Secondary to Relapsing Polychondritis. *Circ Cardiovasc Imaging*. 2014;7:409-411).

Anca Cardoneanu

IN VITRO MEASUREMENT OF TOTAL ANTIOXIDANT CAPACITY OF *CRATAEGUS MACRACANTHA LODD* LEAVES

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IN VITRO MEASUREMENT OF TOTAL ANTIOXIDANT CAPACITY OF CRATAEGUS MACRACANTHA LODD LEAVES (Abstract): *Crataegus macracantha Lodd*, family *Rosaceae,* is a very rare species in Europe, and unlike *Crataegus monogyna* is less investigated for pharmacologic activity**. Aim:** To analyze the ability of the lyophilisate of extract obtained from leaves of *Crataegus macracantha Lodd* (single plant at the Iaşi Botanical Garden) to capture free radicals *in vitro*. **Material and methods:** The lyophilisate obtained in Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iaşi. The decreased absorbance of chromophore chlorpromazine radical cation in the presence of the lyophilized solutions was studied spectrophotometrically. The indicator radical cation, obtained by oxidation of chlorpromazine by potassium persulfate, has the maximum absorbance at 525 nm. Ascorbic acid was used as a standard antioxidant. **Results:** The absorbance of radical solution was determined after the addition of a certain amount of lyophilisate at different time intervals. The antioxidant activity was calculated using the calibration curve obtained by plotting the variation in radical solution absorbance depending on ascorbic acid concentration. For each ascorbic acid concentration the area under the curve was calculated from plotting the percentage inhibition of the absorbance at two pre-established time intervals. **Conclusions:** The results confirm the antioxidant activity of the leaves of *Crataegus Macracantha Lodd* and by optimizing the proposed analytical methods the antiradical activity can be quickly evaluated with minimal reagent consumption. **Keywords:** ANTIOXIDANT ACTIVITY, CHLORPROMAZINE RADICAL CATION, SPECTROPHOTOMETRIC ANALYSE.

The interest in natural antioxidants, aimed at reducing the harmful action of free radicals on the human body has been steadily increasing. In this context, various methods for assessing the antioxidant activity of plant products in view of their use in pharmaceutical, food, cosmetics industries etc. have been developed (1-4). *Crataegus macracantha Lodd*, *Rosaceae* family, is a very rare species in Europe, and unlike *Crataegus monogyna*, is less studied pharmacologically.

The aim of this study was to analyze the ability of the lyophilisate obtained from leaves of *Crataegus macracantha Lodd* (single plant at the Iaşi Botanical Garden) to capture free radical cations resulting from oxidation of chlorpromazine by potassium persulfate ($CP^{\bullet+}$). These chromophore radicals show maximum absorbance at 525 nm. The color of radical solution is diminished by the presence of antioxidants. Ascorbic acid was used as a standard antioxidant.

MATERIAL AND METHODS

The lyophilisate was obtained at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa" - Iaşi as follows: 10 g of plant product (leaves) was treated with 100 mL of 70% methanol (v/v); the mixture was stirred for one hour (FALC F-30 ST magnetic stirrer), filtered, concentrated (Rotavapor, BUCHI R-210) and lyophilized (UNICRYO TFD 5505 freeze-dryer).

Of the 10g of plant product 2.62g of crude extract were obtained.

To determine the antioxidant activity the decrease in absorbance of pre-formed radical cation solution ($CP^{\bullet+}$) depending on the amount of lyophilisate was measured spectrophotometrically.

The spectrophotometric measurements were made with a Hewlett Packard 8453 UV-VIS spectrophotometer equipped with multi-diode detector.

Preparing the chlorpromazine radical cation. Chlorpromazine (CP) was oxidized in acidic environment (HCl) by potassium persulfate ($K_2S_2O_8$), forming a radical cation ($CP^{\bullet+}$) with maximum absorbance at λ = 525 nm. For the preparation of the radical 1 mL of 0.01 M chlorpromazine maleate solution was treated with 8.5 mL of 1 M hydrochloric acid and 0.5 mL of 0.001M potassium persulfate solution.

The optimum time interval for the use of radical solution was 20-40 minutes after the addition of potassium persulfate. During this time interval the absorbance of radical solution remained constant.

Sample preparation. About 0.05 g lyophilisate (a) was weighted and dissolved in 10 mL of distilled water. After 30 minutes the resulting suspension was filtered and the obtained solution was diluted with distilled water to final volume of 25 mL.

Working method. To 2 mL radical solution was added a certain amount of lyophilisate solution (v) diluted to 1 mL with distilled water. The absorbance of the solution was measured 10 and 20 minutes after the start of reaction, at λ = 525 nm, 1 cm cuvettes, against water.

The antioxidant activity of the analyzed samples was expressed as molar equivalents (micromolar) of ascorbic acid/gram dry plant product. For this, we used the calibration curve obtained by plotting the variation in absorbance of radical solution depending on the concentration of the standard antioxidant - ascorbic acid (fig. 1).

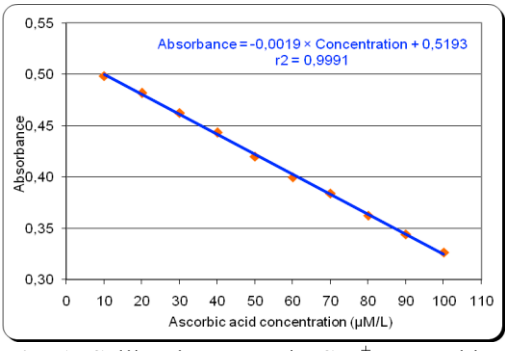

Fig. 1. Calibration curve in $CP^{\bullet+}$ – ascorbic acid system

The method was validated for the linear range of 10-100 μM/L ascorbic acid (5).

RESULTS

For the analyzed samples, the concentration in ascorbic acid was calculated using the straight line equation of calibration depending on the absorbance of freeze-dried solution ($C_{calculated}$). As the final volume of freeze-dried solutions was 1 mL,the concentration of each sample was C = $C_{calculated}$/1000.

Then the result was related to 1 gram dried plant product, taking into account the dilutions made. Measurements were made at 10 minutes (tab. I) and 20 minutes after the start of reaction (tab. II).

TABLE I

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (10 minutes after the reaction)

No. det.	a (g) raw extract	v (mL) lyophilisate	Absorbance	AAE (μM/L lyophilisate)	AAE (μM/ 1 g leaves)
1	0.0552	0.1	0.44543	37.97	45.05
2	0.0552	0.3	0.27479	125.73	49.73
3	0.0324	0.2	0.43309	44.31	44.79
4	0.0324	0.6	0.26442	131.06	44.16
5	0.0264	0.5	0.32683	98.96	49.11
6	0.0264	0.7	0.26485	130.84	46.37
Statistical data				**Mean**	**46.54**
				SD	**2.3549**
				RSD	**5.0605%**

TABLE II

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (20 minutes after the reaction)

No. det.	a (g) raw extract	v (mL) lyophilisate	Absorbance	AAE (μM/L lyophilisate)	AAE (μM/ 1 g leaves)
1	0.0552	0.1	0.42615	47.88	56.81
2	0.0552	0.3	0.25721	134.77	53.31
3	0.0324	0.2	0.41397	54.15	54.73
4	0.0324	0.6	0.22287	152.43	51.36
5	0.0264	0.5	0.29325	116.23	57.67
6	0.0264	0.7	0.21944	154.19	54.65
Statistical data				**Mean**	**54.76**
				SD	**2.2983**
				RSD	**4.1972%**

The values obtained at the two fixed time intervals demonstrate that quenching of radical reaction by antioxidants from natural product is not completed 10

minutes after the start of reaction (fig. 2).

For this reason, measurements at 20 minutes have RSD values within the acceptable range given while measurements at 10 minutes show a slight deviation from the acceptable range.

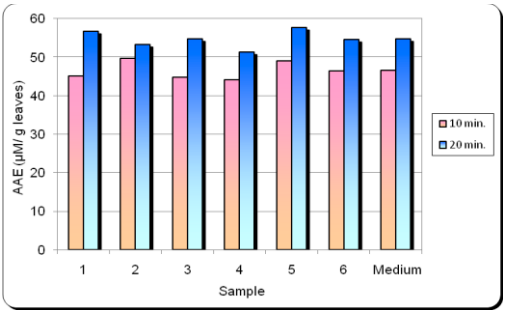

Fig. 2. Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves(10 and 20 minutes after the start of reaction)

In order to eliminate the fluctuations due to changes in absorbance during testing time we used the calibration curve obtained by plotting the area under the curve (AUC) versus ascorbic acid concentration (fig. 3).

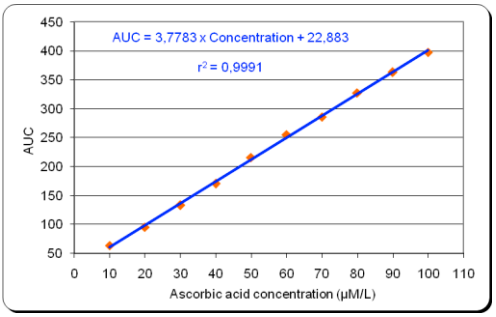

Fig. 3. Area under the curve (AUC) dependent on ascorbic acid concentration

The area under the curve was calculated for each concentration in ascorbic acid (10-100 µM/L) in the graphical representation of the variation of percent inhibition during the fixed time interval (10 minutes and 20 minutes). % Inh = Ar - As / Ar.100, where As–sample absorbance; A_r - radical absorbance (6)

The values obtained by this calculation method for the analyzed samples are given in Table III. The percent inhibition was calculated with Ar = 0.53103.

TABLE III

Micromolar ascorbic acid equivalents (AAE)/1 g *Crataegus macracantha* leaves (values calculated from AUC)

No. det.	Absorbance		Inhibition (%)		AUC	µM / L	µM / g
	10 min.	20 min.	10 min.	20 min.			
1	0.44543	0.42615	16.12	19.75	179.35	41.41	49.14
2	0.27479	0.25721	48.25	51.56	499.09	126.04	49.85
3	0.43309	0.41397	18.44	22.04	202.44	47.52	48.04
4	0.26442	0.22287	50.21	58.03	541.18	137.18	46.22
5	0.32683	0.29325	38.45	44.78	416.15	104.09	51.65
6	0.26485	0.21944	50.13	58.68	544.01	137.93	48.89
Date statistice						**Medie**	**48.96**
						SD	**1.8124**
						RSD %	**3.7016**

DISCUSSION

It has been demonstrated that the radical quenching reactions are influenced by the activity of the antioxidant (its reducing

power), its concentration and the time point selected for carrying out the determinations (7-9).

Thus, antioxidants with a higher reducing power will react faster with the radicals, while in case of a decreased reducing power the reaction rate is slower and the time required to complete the reaction of radical quenching will be longer.

The variations in the antioxidant activity of the leaves of *Crataegus macracantha Lodd* within 10-20 minutes after the start of reaction is explained by the presence in the lyophilisate of some compounds with a weaker reducing power, acting on radicals with lower speed.

The values obtained when evaluating the antioxidant activity from the calibration curves AUC- standard antioxidant concentration are situated between the two limit values (from 10 and 20 minutes respectively), the error of determinations being also below the RSD value of the method.

Determination of AUC is a more accurate criterion for the assessment of total antioxidant activity, because it considers both the antioxidant concentration and reaction time, being a parameter for measuring the ability of the antioxidant to capture free radicals, compared to a standard antioxidant, during a fixed time interval.

CONCLUSIONS

Our results confirm the antioxidant activity of the leaves of *Crataegus macracantha Lodd* and by optimizing the proposed analysis methods the antiradical ability of this less studied species can be measured with minimum reagent consumption and in a short period of time. Its antioxidant properties justify a more thorough research aimed at making full use of this plant in therapy.

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IMPLICATIONS OF THE RANK/RANKL AXIS IN THE BONE PATHOLOGY

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IMPLICATIONS OF THE RANK/RANKL AXIS IN THE BONE PATHOLOGY (Abstract): The receptor activator of NF-κB (RANKL) is a member of the tumor necrosis factor (TNF) super-family. Its receptor, RANK, is expressed on the membrane surface of the dendritic cells (DC). RANKL, expressed by mesenchymal cells on bone tissue level, is a direct regulatory factor of osteoclasts formation and of the bone turnover. T and B lymphocytes express RANKL following their activation. On biologic level, various bone disease are mediated by RANKL. Moreover, the proinflammatory cytokines such as interleukines (IL-1, IL-6, TNFα) present high levels in periodontal impaired tissues. The current literature describes an interaction between these cytokines and RANK/RANKL/OPG system. High levels of IL-1 and TNFα determine high levels of RANKL and, consequently, initiate the osteoclastogenesis. Current studies are focused on the research of the inhibitory effects for RANKL in patients with post-menopausal osteoporosis, rheumatoid arthritis, prostate cancer, breast cancer and multiple myeloma. **Keywords**: RANK, RANKL, OSTEOPOROSIS, PERIODONTITIS, INHIBITORY THERAPY.

The receptor activator of NF-κB (RANKL) was cloned in 1997 as a member of the tumor necrosis factor (TNF) superfamily (1). Its receptor, RANK, is expressed on the membrane surface of the dendritic cells (DC). The main role of RANKL, as an osteoclastogenetic factor, was discovered in 1999 (2). RANKL, expressed by mesenchymal cells on bone tissue level, is a direct regulatory factor of osteoclasts formation and of the bone turnover. Furthermore, T and B lymphocytes express RANKL following their activation (3, 4). Therefore, the activation of the immune system can induce abnormal bone loss.

Signaling pathways of RANK in the osteoclastic differentiation. Since the discovery of the RANK/RANKL axis, the signaling pathways stimulated by RANKL were extensively studied. RANK, which is coded by the *Tnfrsf11a* gene, is induced by M-CSF on the osteoclastic precursor cells, stimulating their proliferation. The binding of RANKL initiates the trimerization of RANK and activates the intracellular signaling (5). The RANK/RANKL signaling is inhibited by osteoprotegerin (OPG), coded

by *Tnfrsf11b* gene; OPG binds to RANKL and prevents its interaction to RANK (6). OPG is expressed on various bone and immune cells (osteoblasts, LyB, DC) (7). The RANK-RANKL binding determines, besides the trimerization of the receptor, also a rapid recruitment of the associated tumor necrosis factor 6 (TRAF6). RANK interacts with different TRAF molecules (1-3, 5, 6) but only TRAF6 is essential for the osteoclastic function (8).

RANK activation leads immediately to the phosphorylation of the ITAM immune receptor in DAP12 protein and in the Fc subunit of the c receptor (FcRc). DAP12 and FcRc are intracellular adaptor molecules which interact with immunoglobulin-like receptors (such as osteoclast associated receptor – OSCAR), with the immunoglobulin-like paired receptor (PIR-A) and with the trigger receptor expressed by myeloid cells 2 (TREM2), which are expressed on the cellular membranes of the osteoclastic precursors (9). These are known as co-stimulatory receptors due to the fact that they are essential for the osteoclastogenesis.

The osteoclast differentiation and their activation is a complex process which depends on a sustained and coordinated activation of the transcription factors, among which we can find c-Fos, NF-κB şi NFATc1 (10). A large part of the regulatory mechanisms and of the pathways which orchestrate this complex cascade was initially identified in immune cells. Therefore, it extremely clear that the immune and bone cells have in common various molecules, such as cytokines, transcription factors and protein kinases.

Particularities in osteoporosis impairment. Osteoporosis is a chronic and frequent disease, the result of a lack of balance between the bone formation and bone resorption, associated to a high risk of pathologic bone fractures and an impaired quality of life. One third of the female patients and one fifth of the male patients, with the age beyond 50 years old would suffer osteoporotic fractures (11). The associated risk for hip, forearm and vertebral fractures is approximately of 40%, similar to the risk for cardiovascular diseases (12).

The incidence of osteoporosis is higher for the female patients (80%), when compared to male patients (20%). There are two phases of primary bone loss in females. The first step includes the trabecular bone loss as a result of the estrogen deficiency initiated by the menopause onset, which lasts 4-8 years and which is characterized by high bone loss and a reduction in the bone formation. The second step implicates a slower trabecular and cortical bone loss, as a result of a diminished bone tissue formation. In males, the bone loss is produced in a slower rate, as a result of the low levels of bioavailable testosterone and estrogen.

B lymphocytes were associated to low concentrations of sexual hormones and bone loss. The ovariectomy stimulated the formation of LyB precursors on medullar level (13). The estrogen therapy in ovariectomized mice prevented the stimulation of the B lymphopoiesis, completely blocking the bone loss. The ovariectomy does not stimulate an exaggerated expression of RANKL on LyB but, more precisely, there is a correlation between the bone loss and a high number of LyB which express abnormal RANKL levels (14).

RANKL expression in case of periodontal impairment. The periodontal disease is an infectious and inflammatory disease. The presence of the bacteria on the root surfaces generates a specific and non-specific immune response of the host. The

relationship between the defense cells and bone cells offers an immunological image of the periodontitis pathogeneity. The pro-inflammatory cytokines (such IL-1, IL-6, TNFα) present high levels in periodontal impaired tissues. The current literature describes an interaction between these cytokines and RANK/RANKL/OPG system. High levels of IL-1 and TNFα determine high levels of RANKL, and consequently initiate the osteoclastogenesis (15).

It was demonstrated that RANKL mRNA is very high in inflammatory cells in patients with severe periodontitis, when compared to the levels of moderate periodontitis or healthy patients, indicating a possible involvement of RANKL in alveolar bone destruction. Immune histochemistry techniques demonstrated a significant expression of RANKL protein in periodontal tissues, associated to macrophages and lymphocytes in chronic periodontitis patients (16). LyT and LyB present in the inflammatory sites have an essential role as a trigger of bone resorption by RANKL-dependent mechanisms. Lymphocytes, especially LyT subsets, are involved in stimulations or inhibitions of RANKL mediated bone resorption; type 1 T helper cells will favor the bone resorption, while regulatory LyT will suppress LyTh1 mediated bone loss.

A possible connection between systemic osteoporosis and alveolar bone loss was suggested by the fact that osteoporosis will determine a stimulation of pro-resorptive cytokines on bone and medullar level, higher levels of circulatory Il-1, Il-6 or TNF-α. Even small risings in the levels of these cytokines will initiate the cytokine-prostaglandine cascade (prostaglandins – matriceal metalloproteinases – RANKL) on periodontal level (17). In a study conducted on mice with osteoporosis and ligature induced periodontitis it was demonstrated this cumulative effect of these two chronic diseases; the mice presenting both osteoporosis and periodontal disease manifested significantly higher osteoclastic activity and RANKL than those with only one of the diseases (18).

New therapeutic approaches – RANKL inhibition. Denosumab is a monoclonal antibody of IhH2 with high affinity and specificity which binds human RANKL, thus inhibiting its interaction with RANKL and interfering with the osteoclastogenesis steps. The single dose denosumab determined rapid, profound and sustained inhibitions of the RANKL mediated resorption (up to 80%), reflected by reduced biochemical markers of the bone resorption (N-terminal seric telopeptide of collagen I – NTx and NTx/urinary creatinine) (19). This rapid suppression of osteoclasts suggests that mature and active osteoclasts are immediately influenced by denosumab. The inhibition of resorption by denosumab is dose-dependent, maximal at one month and sustained for 6-9 months for doses higher than 1mg/bkg.

On biologic level, various bone disease are mediated by RANKL. Current studies are focused on the research of the inhibitory effects for RANKL in patients with postmenopausal osteoporosis, rheumatoid arthritis, prostate cancer, breast cancer and multiple myeloma (20).

The RANKL inhibition presents a series of advantages when compared to the anti-resorptive therapy with bisphosphonates. The RANKL inhibition can block the bone lesions which, in practice, are refractory to bisphosphonates (like arthritic bone erosions). The RANKL inhibition can prove to be more active, more efficient and less

expensive than the bisphosphonates.

Regarding the limitations of RANKL inhibition therapy, there are a few aspects which need to be taken into account:

- The adynamic bone disease – is a process in which the profound inhibition of the bone remodelling can lead to microfractures and high bone fragility. Unusual fractures were reported (i.e. on femoral neck) after the prolonged therapy with alendronate (21, 22);

- Immunosuppression: RANK/RANKL signaling was initially identified as a co-stimulatory pathway during the activation of LyT (2). Even though the short term studies failed to demonstrate adverse effects, the possible effects on the immune system must be considered in patients with long term RANKL inhibition therapy (20).

The evolution of the osteoimmunology concept offered the possibility for the development of new therapy approaches, focused on the RANK/RANKL interaction. High affinity monoclonal antibodies (like denosumab) are the treatment of choice in RANKL inhibition. Short term data suggest that such therapy is safer and more efficient in cases of post-menopausal osteoporosis, even more in cases which are refractory to bisphosphonates.

Regarding the particularities of the cases with periodontal disease, the new therapy means must address, above the classical periodontal debridement therapy, also to the immune contribution to the bone resorption. When having a deep knowledge of the host immune response to the bacterial aggression, a re-evaluation of the therapy measures is necessary, with new therapy strategies focused on the cell mediated destruction. Specifically, the approaches of the RANKL production can include the interference with co-stimulatory molecules, which are necessary for the RANKL production, the control of the signaling pathways or the inhibition of RANKL expression on lymphocytes level by controlling the cytokine balance (23). The classical mechanical debridement with scaling and root planning remains the therapy gold standard when confronting with a periodontal patient but the cases must be addressed to a more complex level, with adjunctive therapies which could facilitate the therapy success or even the prophylaxis of the periodontal tissue losses.

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HYPOGLYCEMIA INDUCED BY ANTIDIABETIC SULFONYLUREAS

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HYPOGLYCEMIA INDUCED BY ANTIDIABETIC SULFONYLUREAS (Abstract): Diabetes mellitus is a major health problem due to its increasing prevalence and life-threatening complications. Antidiabetic sulfonylureas represent the first-line drugs in type 2 diabetes even though the most common associated risk is pharmacologically-induced hypoglycemia. In the development of this side effect are involved several factors including the pharmacokinetic and pharmacodynamic profile of the drug, patient age and behavior, hepatic or renal dysfunctions, or other drugs associated with a high risk of interactions. If all these are controlled, the risk-benefit balance can be equal to other oral antidiabetic drugs. **Keywords**: TYPE 2 DIABETES MELLITUS, SULFONYLUREAS, HYPOGLYCEMIA, SUSCEPTIBILITY FACTORS.

Nowadays diabetes mellitus (DM) has become one of the most challenging health problems due to its remarkable increase in incidence in both developed and developing countries (1, 2). The International Diabetes Federation stated that in 2013 382 million people had diabetes and this number is expected to rise to 582 million in 2035 (3).

At the same time, for researchers DM is still an open subject, both in terms of understanding and elucidating its pathophysiological mechanisms and in developing new drugs for type 2 diabetes with a more advantageous pharmacotoxicological profile.

In terms of biochemistry and pathophysiology, DM is a chronic metabolic disorder characterized by disturbances of carbohydrate, lipid and protein metabolism. This disorder appears to be due to deficiency in insulin secretion by pancreatic β cells, associated or not with insulin resistance in peripheral tissues, leading to elevated blood glucose levels (2, 4, 5).

DM is a silent disease and if undiagnosed or untreated for a long time it can lead to serious macrovascular and microvascular complications. It can be stated that DM has become a life-threatening disease due to its complications, being the fourth leading cause of death in humans (2, 6).

Taking into consideration the fact that an intensive glycemic control is essential in reducing the development and progression of diabetes complications, the pressure to discover new antidiabetic agents has increased and at the moment nine classes of

medications are available for type 2 DM. (4, 7-9). The different mechanisms of action of the various classes of hypoglycemic drugs make combined therapy feasible and give different metabolic and adverse effect profiles (10).

THE PLACE OF SULFONYLUREAS IN THE TREATMENT OF TYPE 2 DIABETES

Hypoglycemic sulfonamides were the first oral antidiabetic agents used in the treatment of type 2 DM (11, 12); they are the first-line medication in patients with moderate diabetes when diet, physical exercise and weight loss do not manage to keep an optimal glycemic control (9, 13).

All these drugs have in common the phenyl-sulfonyl-urea sequence which gives them the hypoglycemic properties; the R and R_1 radicals modulate the pharmacokinetic and pharmacotoxicological profile (14) (fig. 1).

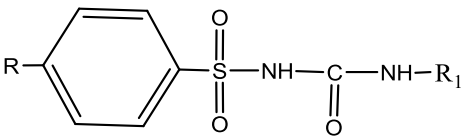

Fig. 1. General chemical structure of hypoglycemic sulfonylureas

Hypoglycemic sulfonylureas act by closing ATP-sensitive K^+ channels with reduction of K^+ efflux and depolarization of β-cells membrane. Consequently, voltage dependent Ca^{2+} channels are opened, Ca^{2+} influx is increased and phosphorylation reactions are stimulated, leading to the release of insulin from pancreatic β-cells (2, 5, 10, 14, 15).

Despite the extensive use of these drugs, recommendations in official guidelines and pathophysiologic plausibility, there are concerns regarding their safety profile (16); the most troubling adverse effects are hypoglycemia (2, 10, 14) and weight gain (2, 10); other risks are hepatotoxicity (4, 10, 17), hematologic dyscrasias (10, 14), myocardial infarction (10, 13, 15, 18), allergic reactions (2, 10), and gastrointestinal disturbances (10).

SULFONYLUREAS-INDUCED HYPOGLYCEMIA

Pharmacologically-induced hypoglycemia is the major risk in patients using sulfonylureas for the treatment of type 2 DM and a major barrier in maintaining a good long-term glycemic control (19). If weight gain is a side effect generally accepted and considered „the price to be paid for normalization of blood glucose" (10), hypoglycemia caused by sulfonylureas is a major concern because of its severe consequences.

It is known that an optimal glycemic control plays an essential role in preventing chronic microvascular complications; achieving this objective without the risk of hypoglycemia is a priority in the early stages of diabetes (8).

Current recommendations advise individualizing the objective of glycemic control based on patient features and diabetes evolution; in young patients without longstanding diabetes and associated comorbidities a strict glycemic control is recommended. This control refers to an HbA1c level less than 6.5%. In older patients with cardiovascular diseases and microvascular complications an HbA1c level of 7% - 8% is considered adequate in order to prevent hypoglycemia (8).

Hypoglycemia can be defined as a condition in which the low blood glucose level exposes the individual to serious damages (8). The most accepted value to define

hypoglycemia is 70 mg/dl because this is the threshold for activation of counter regulatory mechanism in non-diabetic individuals and the superior limit at which the counter regulatory response to hypoglycemia changes (20) (tab. I).

TABLE I
Classification of hypoglycemia depending on severity (8, 20)

Type of hypoglycemia	Characteristics
Severe hypoglycemia	Requires external assistance for recovery
Documented symptomatic hypoglycemia	Typical symptoms and a measured capillary glucose level < 70 mg/dl
Asymptomatic hypoglycemia	Capillary glucose level < 70 mg/dl without typical symptoms
Probable symptomatic hypoglycemia	Typical symptoms of hypoglycemia not accompanied by measurement of capillary glycemia
Pseudohypoglycemia	Symptoms of hypoglycemia with capillary glycemia values > 70 mg/dl

Symptoms. The symptoms of hypoglycemia vary between individuals and they are generated by activation of the adrenergic system and by the direct effect of glucose deprivation on the brain. The release of counter regulatory hormones (glucagon and adrenaline), the adrenergic symptoms and the development of central nervous system dysfunctions are triggered at specific levels of blood glucose. There are 11 most common symptoms experienced during hypoglycemic episodes: sweating, palpitations, shaking, hunger, confusion, drowsiness, strange behavior, difficulty in speaking, incoordination, headache, nausea (19, 21).

Incidence. The incidence of hypoglycemic events in type 2 DM depends on several factors and increases with disease progression, degree of insulin deficiency, as well as in cases of treatment with sulfonylureas and insulin (8). Sulfonylureas cause much more hypoglycemia than it is recognized, with an annual prevalence of severe episodes of 7% (19). Studies reported that up to 20% of sulfonylurea-treated patients experienced symptoms suggestive of hypoglycemia. During the first year of United Kingdom Prospective Diabetes Study at least 30% of glibenclamide-treated patients experienced hypoglycemia. After a follow-up period of 10 years the annual incidence of patients with at least one hypoglycemic event was 11% with chlorpropramide, 17.7% with glibenclamide, and 36.5 % with insulin therapy (16). However, severe hypoglycemic episodes appear to be more prolonged and associated with greater mortality when induced by sulfonylureas than by insulin (22).

Despite differences between the studies, sulfonylurea-induced hypoglycemia has a significant incidence and it is a major concern because of its considerable economic impact on health care system.

Susceptibility factors. Sulfonylurea-induced hypoglycemia is a multifactorial adverse effect where drug features; patient features and patient behavior are intermingled. It is difficult to establish in which proportion each factor leading to hypoglycemia is involved (5).

Regarding drug characteristics, the pharmacokinetic profile of sulfonylureas is relevant, especially their duration of action

which is linked to long-lasting hypoglycemia (23).

It is interesting to note that there is not always a relationship between the duration of action and blood concentrations; sulfonylureas may make the cells more sensitive to insulin and when the concentration is falling, stimulation of insulin secretion can continue and it is difficult to predict for how long the hypoglycemic effect will last (10).

The majority of reported cases of hypoglycemia were on chlorpropamide or glibenclamide (12, 16) which are long-acting sulfonylureas whose metabolism can lead to accumulation. Glipizide, gliclazide and gliquidone are less likely to cause hypoglycemia because they are short-acting drugs and their metabolites are inactive or have minimal hypoglycemic effect (2). Although glimepiride has a long duration of action, there were fewer hypoglycemic reactions compared with glibenclamide, probably due to a better modulation of insulin release (16) (tab. II).

TABLE II

Phamacokinetic profile of some sulfonylureas (10, 14, 23)

Drug	Half-life (h)	Duration of effect (h)	Active metabolites
Tolbutamide	3-5	6-10	-
Chlorpropamide	24-48	27-72	+
Glibenclamide	2-4	20-24	+
Gliclazide	6-15	10-15	-
Glipizide	3-5	6-12	-
Gliquidone	6-10	6-12	-

As to patient features, there are several susceptibility factors leading to sulfonylurea-induced hypoglycemia including age, liver, renal and cognitive impairment (5, 24). The risk of hypoglycemia among elderly patients treated with sulfonylureas is 36% greater than in younger adults; in this group of patients glimepiride and glipizide are preferred due to their less hypoglycemic potential (25). In a study of 57 patients with type 2 DM who experienced glibencamide-induced hypoglycemia, more than 90% were older than 60 years and more than 70% above 70 years (16).

In the elderly patients the elimination half-life is longer for most sulfonylureas and patients older than 60 years are particularly at risk. These drugs are metabolized in the liver and eliminated from the circulation via the kidneys (12); thus, renal and hepatic failures are important contributing factors depending on the drug involved (23).

Hypoglycemia may be difficult to recognize in old patients because the characteristic warning symptoms could be absent or not well interpreted but also in patients on β-adrenergic blockers (10).

Taking these into consideration, the most frequent contraindication for sulfonylureas is the elderly diabetic patient with renal or kidney disease; if prescribed, there are preferred short-acting drugs, with a moderate hypoglycemic effect and without active metabolites (26).

Not only the sulfonylurea is responsible for hypoglycemia, but also the circumstances of drug administrations; reduced food intake, restricted carbohydrate intake, severe or prolonged physical exercise and alcohol consumption are the most common predisposing factors (10, 23).

Finally, another cause of hypoglycemia which cannot be overlooked is represented by drug interactions. Most drugs have been reported to interact with sulfonylureas; these interactions result from changes in the pharmacokinetic profile of sulfonylureas (absorption, displacement from plasma proteins, alterations in their metabolism) or are caused by drugs that affect blood glucose levels (23, 27).

CONCLUSIONS

The treatment of type 2 diabetes mellitus remains a challenge given the necessity to maintain an optimal glycemic control without the risk of hypoglycemia. Despite their increased risk of hypoglycemia, sulfonylureas still occupy a central position in the recommendations of many guidelines.

However, when choosing a specific treatment for diabetes, it is necessary to take account of patient preferences and comorbidities as well as all conditions that may be associated with a sulfonylurea-induced hypoglycemia. If all these procedures are being followed the risk-benefit balance for sulfonylureas is not worse than for others oral hypoglycemic agents.

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ASSESSMENT OF THE MICROBIAL LOAD OF SOME MEDICINAL PLANTS COMMONLY USED IN ROMANIA

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ASSESSMENT OF THE MICROBIAL LOAD OF SOME MEDICINAL PLANTS COMMONLY USED IN ROMANIA (Abstract): **Aim**: This study aims to assess the microbial load of some medicinal plants (*Matricaria chamomilla, Achillea millefolium, Ocimum basilicum, Calendula officinalis, Tilia cordata, Hypericum perforatum*) commonly used as medicinal teas. **Material and methods**: A total of 24 samples collected from the local market, as well as from the spontaneous flora of Galati County, were analyzed in terms of the total number of germs, the content of coliform bacteria, yeasts and molds, and the presence of specific pathogenic bacteria (*Escherichia coli, Bacillus cereus, Coagulase-positive Staphylococcus, Salmonella* spp.). **Results:** The values obtained for total number of germs, yeasts and molds were in accordance with the limits set by the *European Pharmacopoeia* for plant products to which hot water is added before use. In the case of specific pathogenic bacteria, the presence of *E. coli* was confirmed in 41.66% of the samples, the presence of *Coagulase-positive Staphylococcus* in 16.66% of the samples, and the presence of *Bacillus cereus* in 33.33% of the samples. *Salmonella* spp. was absent in all samples. **Conclusions:** The study highlights a certain microbial load of medicinal plants analyzed, emphasizing the importance of quality control in all stages of production. **Keywords:** MICROBIAL CONTAMINATION, MEDICINAL PLANTS, QUALITY STANDARDS

Medicinal plants have been used since ancient times, modern science today proving many of their therapeutic benefits. Worldwide, there is an increasing interest for products from medicinal plants, so their use is rapidly rising. In these conditions, the quality and safety of the phyto preparations is a major concern for health authorities and consumers (1). Microorganisms from the environment (soil, air, water) can contaminate medicinal and aromatic plants, the process being influenced by various factors: temperature, humidity, but also by the harvesting, storage, processing and marketing conditions. The increase in the degree of contamination during the stages of harvesting, production, transportation or storage may have significant consequences on the appearance, physical characteristics, pharmacological action of the active principles, or the stability of preparations obtained from medicinal plants, but also on the health of

consumers (2, 3, 4, 5). *European Pharmacopoeia* (6) provide specific requirements regarding the presence and the level of microbial contaminants in medicinal plants and phytomedicines. This study aims to assess the quality of some medicinal and aromatic plants, commonly used as medicinal teas in terms of the presence of microbial contaminants: total number of bacteria, the content of coliform bacteria, yeasts and molds, as well as some specific pathogenic bacteria (*Escherichia coli, Bacillus cereus, Coagulase-positive Staphylococcus, Salmonella* spp.).

MATERIAL AND METHODS

Plant material. Six common medicinal species were selected for the study: *Matricaria chamomilla, Achillea millefolium, Ocimum basilicum, Calendula officinalis, Tilia cordata, Hypericum perforatum.* They are common plants, easily available and frequently used in Romania as medicinal tea. For each species three commercial samples from different distributors (A, B and C), and a sample collected from the spontaneous flora in the Galati County were analyzed (tab. I). The commercial samples were purchased in bulk packaged form in September 2012, and the harvesting was done in the period May-September 2012.

TABLE I
Plant material samples

Plant material		Source			
		A	B	C	Spontaneous flora
Flowers	*Matricaria chamomilla*, Asteraceae, chamomille	MC1	MC2	MC3	MC4
	Calendula officinalis, Asteraceae, marigold	CO1	CO2	CO3	CO4
	Tilia cordata, Tiliaceae, linden	TC1	TC2	TC3	TC4
Flowering aerial parts	*Achillea millefolium*, Asteraceae, yarrow	AM1	AM2	AM3	AM4
	Ocimum basilicum, Lamiaceae, basil	OB1	OB2	OB3	OB4
	Hypericum perforatum, Hypericaceae, St. John's Wort	HP1	HP2	HP3	HP4

Sample preparation. The plant material was dried at 600°C in 24 h, and then it was pulverized by grinding and then it was properly labeled (7). For each type of plant product fragments were taken out from different bags, out of which 10 g of triturated sample were weighed, with an equal amount of sterile sand. The test solution was obtained by gradually adding the dilution liquid in an amount 9 times greater than the triturated sample (primary dilution 1/10). If necessary, decimal dilutions were performed successively (the difference between two successive dilutions was 10 times) depending on the degree of assumed contamination.

Assessment of the total number of germs. It was done by using the spread-plate technique. Thus, 0.1 mL of the prepared dilutions were spread on the culture medium (agar, Oxoid, United Kingdom) fused and solidified at ambient temperature. After 48 h of incubation at 30-37ºC, the colonies from the Petri dishes were counted and the total

number of bacteria per g/mL (N) was calculated using the formula:

$$N = \frac{EC}{(n1 - 0.1\ x\ n2) x\ d}$$

where: EC=the sum of colonies counted in all retained plates; n1=the number of plates where between 10 and 300 colonies were counted on the first dilution; n2=the number of plates where between 10 and 300 colonies were counted on the second dilution; d=dilution factor corresponding to the first dilution (7).

Estimation of the total number of coliform bacteria. It was performed using the end point dilution technique (MPN=most probable number). 10 mL of liquid culture medium (Lauryl Tryptose Broth, Oxoid, United Kingdom) was distributed in test tubes with Durham tubes. 1 mL homogenate of each dilution (three successive dilutions were used) was inoculated into each Durham tube. After spreading, they were incubated at 35°C for 48 h. On the basis of the number of positive tubes (gas accumulation in the at least 1/10 of the collector tube height) from the dilutions taken into account, estimation of total coliforms was achieved using the standard MPN table (7).

Estimation of the most probable number of Escherichia coli. It was performed by passing the content of the test tubes considered to be positive for coliform bacteria into two test tubes (one with Lauryl Tryptose Broth and one with Peptone Water), preheated to 44ºC. A strip impregnated with Kovasc reagent was added to determine the presence of the indole. After incubation at 44ºC for 48 h, were considered to be positive those test tubes from the presumptive test corresponding to the test tube with Lauryl Tryptose Broth where gas was produced, and the one where the paper strip inserted in Peptone Water (Oxoid, United Kingdom) turned red. The most probable number was calculated on the basis of the number of tubes in which both reactions were positive (7).

The Bacillus cereus number was determined by using the colony-counting technique. 0.1 mL of each dilution (prepared by successive dilutions of the 10^{-1} dilution inactivated on a water bath, 45 min at 65°C) were spread on the surface of Petri plates with agar (Oxoid, United Kingdom) and defibrinated sheep blood 5% (Cantacuzino Institute, Bucharest, Romania). After 48 h incubation at 30-37ºC, 5 colonies were selected in each plate and the Gram coloration was analyzed, the macroscopic aspect and the biochemical characteristics (the presence of hemolysin) (7).

Determination of the Coagulase-positive Staphylococcus number was performed by using the end point dilution technique (MPN=most probable number). For each sample three successive dilutions were used, and from each dilution (1 mL) series of three test tubes were brought in Chapman liquid medium (Mannitol Salt Broth, Himedia, India), and then incubated for 48 h at 30-37ºC. From each test tube passes were made to the solid Chapman medium (Mannitol Salt Agar, Oxoid. LTD, United Kingdom) distributed into Petri dishes and they were incubated for 24 h at 30-37ºC (7).

Estimation of Salmonella spp. count. 1 mL of incubated pre-enrichment medium (25 g dried plant material mixed with 225 mL broth in an Erlenmeyer flask and incubated to 35°C for 24 h) was inoculated into a tube with 10 mL enrichment medium (Selenite Broth Base, Oxoid, United Kingdom), and it was incubated at 35ºC for 48 h. From the enrichment medium passes were made to the selective medium (Desoxycholate Citrate Lactose Agar, Cantacuz-

ino Institute, Bucharest, Romania), distributed in Petri dishes and they were incubated for 24 h at 35ºC. The suspicious or typical colonies were biochemically tested (7).

Determination of yeasts and molds. The degree of contamination was determined by incorporating a certain quantity of sample (1 mL) in a solid medium (Czapek Dox, Oxoid, United Kingdom) and incubating at room temperature (22-24ºC) for 5 days. The colonies were counted (CFU=colony-forming units/g or mL) (7).

RESULTS AND DISCUSSION

The values of the microbiological load of vegetable samples analyzed are shown in tab. II. The total number of germs varies between $1.0x10^2$-$7.5x10^5$ CFU/g, the highest value being determined in a sample of *Calendula officinalis* ($CO2=7.5x10^5$ CFU/g). In accordance with the limits set by the *European Pharmacopoeia* for plant products to which hot water is added before use (10^7 CFU/g) (6), the values obtained were adequate.

TABLE II

Microbial contaminants values (CFU/g) present in the plant samples

Plant material samples	Total number of bacteria	Coliforms	Yeasts and molds	*E. coli*	*Coagulase-positive Staphylococcus*	*B. cereus*	*Salmonella*
MC1	$3.3x10^4$	**$2.6x10^2$**	$1.1x10^3$	0	2.1x10	0	0
MC2	**$3.5x10^5$**	4.3x10	$2.5x10^3$	9.0x10	0	0.2x10	0
MC3	$6.0x10^4$	0.9x10	$2.5x10^3$	0	0	0	0
MC4	$3.0x10^4$	0	**$1.3x10^4$**	0	0	1.2x10	0
CO1	$3.0x10^3$	2.1x10	2.1x10	0	0.9x10	1.1x10	0
CO2	**$7.5x10^5$**	**$2.4x10^2$**	**$1.1x10^4$**	9.3x10	0	0	0
CO3	$5.0x10^4$	**$2.4x10^2$**	$4.5x10^2$	9.3x10	0	0	0
CO4	$9.0x10^3$	**$2.4x10^2$**	$5.5x10^3$	**$2.4x10^2$**	0	1.5x10	0
TC1	$2.1x10^4$	**$2.6x10^2$**	**$1.0x10^4$**	0	**2.4x10**	**6.1x10**	0
TC2	$4.0x10^2$	0	$1.3x10^2$	0	0	0	0
TC3	**$1.2x10^5$**	**$2.4x10^2$**	**$1.1x10^4$**	9.3x10	0	0	0
TC4	$1.1x10^4$	0	$8.2x10^3$	0	0	0	0
AM1	$5.0x10^3$	2.4x10	**$1.1x10^4$**	0	0	**2.4x10**	0
AM2	$5.0x10^5$	**$2.4x10^2$**	$6.0x10^2$	9.2x10	0	0	0
AM3	$9.0x10^2$	0	$1.5x10^2$	0	0.2x10	1.2x10	0
AM4	$4.0x10^2$	**$2.4x10^2$**	$5.0x10^3$	**$2.4x10^2$**	0	0	0
OB1	$9.0x10^3$	2.4x10	**$1.0x10^4$**	0	0	0	0
OB2	$2.5x10^5$	4.3x10	$1.8x10^3$	0.9x10	0	0	0
OB3	$8.0x10^4$	3.2x10	$3.0x10^3$	0	0	0	0
OB4	$1.1x10^5$	9.0x10	**$1.2x10^4$**	0.4x10	0	0	0
HP1	$1.0x10^2$	0	1.7x10	0	0	0.2x10	0
HP2	$2.0x10^4$	0	$4.0x10^2$	0	0	0	0
HP3	$1.1x10^3$	0	$8.0x10^2$	0	0	0	0
HP4	$1.1x10^3$	0.4x10	8.0x10	0.4x10	0	0	0

Coliform bacteria, considered to be health indicators because of the fact that their presence indicates contamination with faecal bacteria, are not pathogenic bacteria, they are ubiquitous, but a large number of them may affect product quality and hence

consumer's health. The highest values were determined in some samples of *Matricaria chamomilla* (MC1=$2.6x10^2$ CFU/g) and *Tilia cordata* (TC1=$2.6x10^2$ CFU/g).

Yeasts and molds, widely distributed in the atmosphere, can provide information on the extent of contamination at all stages of production. In addition, under favorable conditions, certain fungi can synthesize highly toxic metabolites of the aflatoxin type (8, 9). The content of yeast and molds of the samples were adequate in relation to *European Pharmacopoeia* for plant products to which hot water is added before use (10^5 CFU/g) (6).

Escherichia coli is a commensal bacterium with a high capacity for adaptation and one of the main contaminants incriminated for food poisoning due to medicinal tea consumption (10). In the present study, *E. coli* was determined in 41.66% of the samples (0.4x10-$2.4x10^2$ CFU/g), predominantly in *Calendula officinalis* (75% of the samples). According to the *European Pharmacopoeia* requirements (6), only 8.33% of the samples had values ($2.4x10^2$ CFU/g) above the permissible limit (10^2 CFU/g or mL), that were harvested from the spontaneous flora and that belonged to the *Achillea millefolium* and *Calendula officinalis* species.

Coagulase-positive staphylococci are not common contaminants of medicinal teas. However, in 16.66% of the samples (*Achillea millefolium, Calendula officinalis, Matricaria chamomilla* and *Tilia cordata*) these pathogens were determined (0.2-2.4x10 CFU/g). The presence of these pathogenic bacteria in samples of packed medicinal teas indicates a contamination of human nature.

Bacillus cereus is a bacterium that multiplies by spores and it is involved in the occurrence of food poisoning caused by two distinct metabolites (enterotoxins), but maximum limits allowed for medicinal teas are still not set. Its presence was identified in 33.33% of the investigated samples (0.2-6.1x10 CFU/g), and it is found especially in *Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla*. *Salmonella* spp. was absent in the all plant samples.

CONCLUSIONS

This study highlights some aspects regarding the quality of some medicinal plants commonly used in Romania as medicinal teas. Determinations made revealed the presence of specific pathogens (*Escherichia coli, Coagulase-positive Staphylococcus, Bacillus cereus*) in most of the samples investigated. The lowest prevalence of contamination was found in *Hypericum perforatum* samples. In order to ensure the quality and safety of medicinal plants consumption it is required to fully comply with the complete quality criteria so as to avoid microbial contamination in all stages of production. It is also necessary to identify the causes which favor the presence of the main etiological agents, as well as determining their role in generating disease in humans in order to prevent and decrease overall morbidity.

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NEWS

THE USE OF LIPIDS AS TASTE-MASKING AGENTS IN SOME PHARMACEUTICAL FORMS CONTAINING PARACETAMOL

Paracetamol is a fever reducer and a pain reliever used in many health conditions such as: fever, toothaches, headaches and even arthritis. However, the bitter taste of paracetamol represents a problem in pharmaceutical products for oral administration such as: tablets, chewable tablets, syrups or effervescent powders. In some patient caregories (children, elderly or patients with swallowing disorders) the use of these dosage forms is indispensable. Therefore, new methods for masking the unpleasant taste of some substances is needed. Lipids can be successfully used to coat the taste buds, covering the bitter taste of the drug and imparting a good mouth feel without delaying the release of the active substance. El-Refaie *et al.* investigated a new technique for paracetamol taste masking using lipid coating. The study aimed the investigation of some lipids (glyceryl palmitostearate, stearic acid and cetyl alcohol) potential to reduce the bitterness of paracetamol. Different ratios of paracetamol:lipid granulations were analyzed. The obtained granules were formulated as chewable tablets and sachets. Furthermore, the formulations were evaluated in terms of performance during storage. The characterization of the paracetamol:lipids formulations was performed using scaning electron microscopy and differential scanning calorimetry. In case of sachets, glyceryl palmitostearate showed a better masking of the taste, compared with the other two lipids, while in case of chewable tablets, two of the three analyzed formulations showed similar performance. Regarding the stability, the sachets proved to be stable for approximately 6 months, while for chewable tablets, only the formulations with cetyl alcohol presented taste masking changes during storage (El-Rafaie WM, El-Massik MA, Abdallah OY, Khalafallah NM. Formulation and evaluation of taste-masked paracetamol-lipid sachets and chewable tablets. *J Pharm Invest* 2014; 44(6): 431-442)

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A SOFTWARE TOOL USED IN 3D EVALUATION OF THE ALVEOLAR BONE DEFECT IN BILATERAL CLEFT LIP AND PALATE PATIENTS

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A SOFTWARE TOOL USED IN 3D EVALUATION OF THE ALVEOLAR BONE DEFECT IN BILATERAL CLEFT LIP AND PALATE PATIENTS (Abstract): Evaluation of the cleft size and the assessment of an estimative volume required for bone grafting provide useful information to the surgeon performing secondary alveolar bone grafting. **Aim:** To use a software tool to evaluate the estimative volume of the bone defect in the alveolar cleft area, based on cone beam computed tomography (CBCT) data. **Material and methods:** Three patients with bilateral cleft lip and palate (BCLP) were randomly selected in the order of referral to treatment at "Saint Mary" Emergency Hospital for Children. CBCT examinations were performed prior to secondary alveolar bone grafting procedure. The preoperative estimative volume (PEV) of the alveolar bone defect was evaluated by three observers with different areas of expertise (a dentomaxillofacial radiologist, a maxillofacial surgeon, and a Ph.D. radiology student), using Romexis 3.0.1 software program. The inter-observer fidelity was assessed using the Interclass Correlation Coefficient (ICC), with an optimal value of 0.837 for all observers. **Results:** The mean PEV for all BCLP patients was 1.84 ± 0.16 cm³. **Conclusions:** The results suggest that measuring the preoperative estimative volume of the alveolar bone defect in CLP patients using Romexis 3.0.1 software program, with CBCT scan data is beneficial. A proper protocol should be established, in order to provide useful information for the surgeon performing the secondary alveolar bone grafting. **Keywords:** BILATERAL CLEFT LIP AND PALATE, CONE BEAM COMPUTED TOMOGRAPHY, PREOPERATIVE, ALVEOLAR BONE DEFECT, SOFTWARE

Cleft lip and palate (CLP) consist in a heterogeneous group of pathologies that are among the most common malformations in the head and neck region and may determine various functional, aesthetic and social disorders (1). Previous European and US studies suggest that unilateral CLP is more frequent, and within CLP, only 20% are bilateral (2).

With the advancement of maxillofacial imaging, cone-beam computed tomography (CBCT) has become the procedure of choice when 3D imaging is necessary during CLP management, offering a lower dosage of radiation exposure compared to the classic CT (3-5), thus respecting the

ALARA (As Low As Reasonably Achievable) principle (6,7). CBCT examination brings essential information in the preoperative assessment, as well as in the outcome evaluation during different stages of the CLP surgical and orthodontic treatment, allowing accurate linear and three-dimensional measurements of the bone defect areas (8-10).

Evaluation of the cleft size and the assessment of an estimative volume required for bone grafting provide useful information to the surgeon performing secondary alveolar bone grafting, influencing the choice of an optimal donor site. The aim of this study was to use a software tool to calculate the estimative volume of the bone defect in the alveolar cleft area, based on the CBCT data collected from three patients presenting bilateral cleft lip and palate (BCLP), prior to secondary alveolar bone grafting.

MATERIAL AND METHODS

From all the CLP patients presented to pursue surgical treatment at "Saint Mary" Emergency Hospital for Children, between 1 January 2014 and 30 June 2014, three BCLP patients were randomly selected in the order of referral to treatment.

Neither of the selected patients had undergone secondary alveolar bone grafting, or followed any orthodontic treatment. Patients presenting other congenital malformations or syndromes were excluded from the study. An informed consent for research, approved by the Ethics Committee, was obtained from all patients' parents/tutors.

TABLE I

Patients' characteristics and protocol used during CBCT data collection

Patient	Cleft type	Age	Sex	Voxel (m)	DAP* (mGy x cm²)	Tube current (mA)	Tube voltage (kV)	Scan Time (s)
C.A-E.	BCLP**	14	F	400	2490	10.0	90	27
C.I.	BCLP**	9	M	400	1992	8.0	90	27.5
G.M.M.	BCLP**	11	M	400	2242	9.0	90	28

* Dose-Area-Product

** Bilateral Cleft Lip and Palate

CBCT examinations were performed prior to secondary alveolar bone grafting procedure, in a private radiology clinic. A Planmeca Promax 3D MID (*Planmeca Oy, Helsinki, Finland*) unit was used to collect CBCT data, with the patient in upright sitting position, Frankfort horizontal plane being parallel to the floor. Technical parameters for obtaining CBCT data were selected based on the patient's age and body structure (tab. I).

The scans were reconstructed using three - dimensional imaging software Romexis 3.0.1 (*Planmeca OY, Helsinki, Finland*) (fig.1). In order to achieve coronal, sagittal and axial views, sections were established with a thickness of 1 mm and at a distance of 1 mm.

All CBCT scans were screened independently by three observers with different

areas of expertise (a dentomaxillofacial radiologist, a maxillofacial surgeon, and a Ph.D. radiology student). Prior to the assessment of the alveolar bone defect volume, all observers agreed on defining the following limits of the region of interest: the superior limit was set to be the nasal floor plane, defined as the plane parallel to the Frankfort horizontal plane that passed through the anterior and posterior nasal spine; the inferior limit was considered to be the inferior margin of the alveolar bone adjacent to the cleft; the lateral boundaries were represented by the limits of the alveolar bone surrounding the teeth adjacent to the cleft (fig. 2).

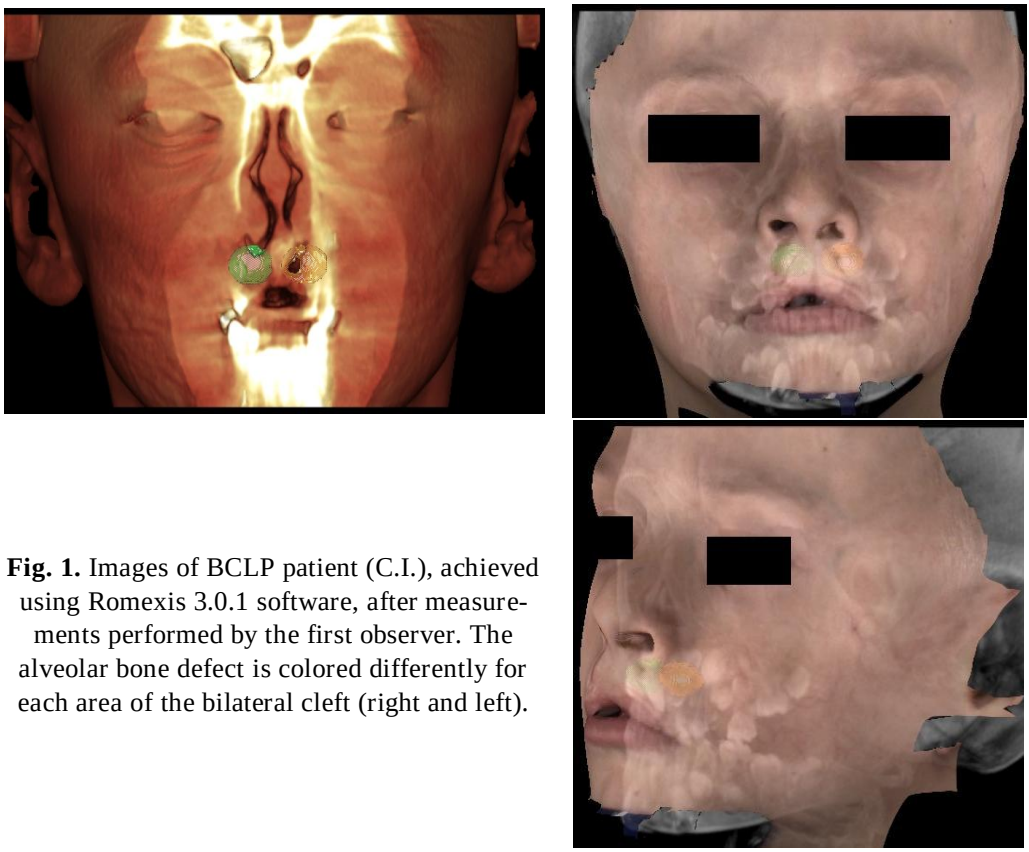

Fig. 1. Images of BCLP patient (C.I.), achieved using Romexis 3.0.1 software, after measurements performed by the first observer. The alveolar bone defect is colored differently for each area of the bilateral cleft (right and left).

The alveolar bone defect was traced by selecting Volume rendering option in the Tools panel, then by using the elliptical cursor to establish the limits of the cleft aria, in both sides separately (fig. 2). The established limits were evaluated by every observer separately, in all CBCT sections in coronal, sagittal and axial planes.

Statistical analysis was performed using SPSS version 20.0 (*SPSS Inc, Chicago, IL*). The Intraclass Correlation Coefficient (ICC) was used to evaluate the inter-observer reliability in the assessment of the alveolar bone defect volume using Romexis 3.0.1 software program. The results showed no significant difference between the inter-

observer measurements, with a ICC value of 0.837 for all three observers. The ICC value for average measures was calculated with a 95% confidence interval. The resulting value indicates an optimal inter-observer reliability.

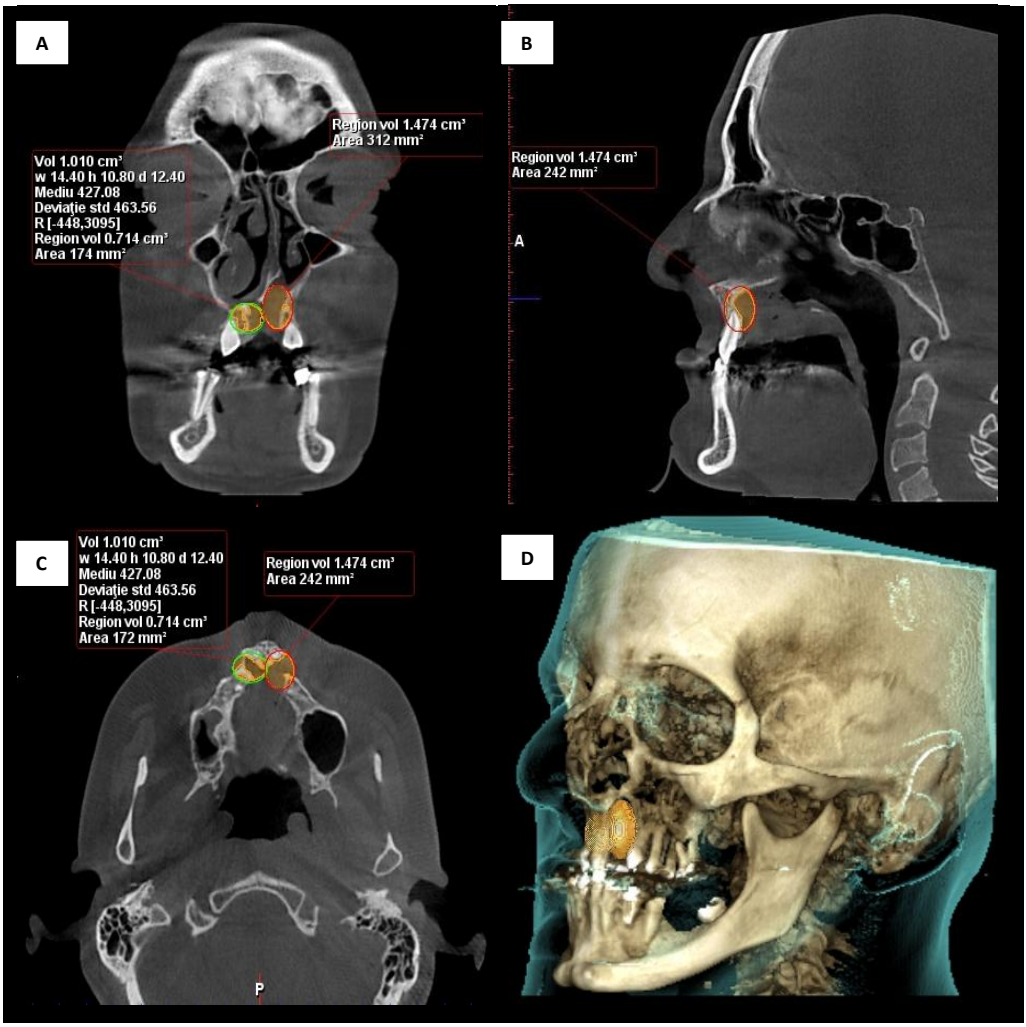

Fig. 2. Assessment of alveolar bone defect in BCLP patient (C.A-E.); A – coronal view; B – sagittal view; C – axial view; D – three-dimensional view, soft and hard tissues.

RESULTS

The results of the measurements performed by all three observers are presented in Table II. The mean value for the right and the left area of each cleft region resulted from the values obtained by all the observers. The combined average volume for both sides of the bilateral clefts resulted in the overall preoperative estimated volume (PEV) for each BCLP patient (tab. II). The mean PEV for all BCLP patients was 1.84 ± 0.16 cm³.

TABLE II

Estimative bone defect volume in the alveolar cleft area, calculated by all observers

Patient	Cleft area	Measurements O1* (cm^3)	Measurements O2** (cm^3)	Measurements O3*** (cm^3)	Mean value (cm^3)	Overall PEV**** (cm^3)
C. A-E.	Right	0.727	0.714	0.688	0.710	1.992
	Left	1.136	1.474	1.236	1.282	
C.I.	Right	1.272	0.740	0.963	0.991	1.860
	Left	0.909	0.790	0.908	0.869	
G.M.M.	Right	0.663	0.884	0.709	0.752	1.668
	Left	0.904	0.932	0.911	0.916	

*O1: Observer 1 (maxillofacial surgeon); **O2: Observer 2 (dentomaxillofacial radiologist); ***O3: Observer 3 (Ph.D. radiology student); ****PEV: Preoperative estimative volume

DISCUSSION

The preoperative volumetric assessment of the alveolar bone defect provides useful information for the secondary alveolar bone grafting, which is considered the gold standard in the surgical reconstruction of the cleft region (4, 10-12). The accurate evaluation of the alveolar bone defect helps determining the quantity of bone necessary to the bone grafting procedure, thus influencing the choice of the optimal donor site and avoiding inadequate bone harvesting (11, 12).

Previous studies have reported a significant correlation between the size of the alveolar bone defect in the cleft area and the success of the secondary alveolar bone grafting procedure (13-15).

Most studies evaluating PEV of the alveolar bone defect have been conducted on unilateral CLP (UCLP) patients. A possible explanation may be the lower prevalence of BCLP compared to UCLP (2).

The PEV values reported by previous studies conducted among BCLP patients vary widely. Choi et al. reported findings slightly lower than ours ($1.4\pm0.4cm^3$) (16). Oberoi et al. reported even lower values: 0.82cm3 (4). Shirota et al. study included only two BCLP patients and declared higher findings compared to ours: $3cm^3$ and, respectively, $3.8cm^3$ (14).

There may be various explanations for the differences found among these studies. Different software programs were used to assess the PEV of the alveolar bone defect in CLP patients. Meanwhile, the absence of a specialized multidisciplinary center for the treatment of CLP patients in Romania, along with the unavailability of an adequate protocol for the CLP management, leads to an incomplete CLP management for many patients. All the patients involved in this study had not undergone orthodontic treatment, which must begin prior to secondary alveolar bone grafting (2). The absence of maxillary expansion may explain our findings being higher than those reported by other studies.

CONCLUSIONS

The results suggest that measuring the preoperative estimative volume of the alveolar bone defect in CLP patients using

Romexis 3.0.1 software program, with CBCT scan data is beneficial. A proper protocol should be established, in order to provide useful information for the surgeon performing the secondary alveolar bone grafting.

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IN VITRO SCREENING OF *CRATAEGUS SUCCULENTA* EXTRACTS FOR FREE RADICAL SCAVENGING AND 15-LIPOXYGENASE INHIBITORY ACTIVITIES

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IN VITRO SCREENING OF *CRATAEGUS SUCCULENTA* EXTRACTS FOR FREE RADICAL SCAVENGING AND 15-LIPOXYGENASE INHIBITORY ACTIVITIES (Abstract): *Crataegus succulenta* Schrad. ex Link is widely spread in North America. A literature survey revealed no studies on the chemical composition and biological effects of this species. **Aim**: The aim of the present study was to investigate the phenolic content, free radical scavenging and 15-lipoxygenase inhibitory effects of *Crataegus succulenta* leaf and flower extracts. **Material and methods**: Total phenolic, flavonoid and proanthocyanidin contents were quantified by spectrophotometric methods. Both extracts were evaluated for their ability to scavenge DPPH, superoxide anion and hydroxyl radicals and to inhibit 15-lipoxygenase activity. **Results**: There were noticed no striking differences in the total phenolic, flavonoid and proanthocyanidin contents between leaf and flower extracts. Both extracts showed similar 15-lipoxygenase inhibitory effects. Flower extract scavenged more effectively DPPH and superoxide radicals while leave extract was more active against hydroxyl radical. In superoxide anion radical scavenging assay, both extracts were more active than (+)-catechin. In hydroxyl radical scavenging and 15-lipoxygenase inhibition assays, the extracts were only 4-5 times less active than (+)-catechin. **Conclusions**: The high antioxidant potential of *Crataegus succulenta* extracts suggest a possible use as ingredients in functional foods for the prevention of oxidative stress-related diseases.
Keywords: *CRATAEGUS SUCCULENTA*, SUPEROXIDE ANION RADICAL, HYDROXYL RADICAL, 15-LIPOXYGENASE

The genus *Crataegus* (Rosaceae) consists of approximately 300 species widely spread in the northern hemisphere. Many of these species have been used in folk medicine in the treatment of gastrointestinal, urinary and cardiovascular disorders. Flavonoids (vitexin, orientin and their derivatives, hyperoside, rutin) and oligomeric procyanidins are the main phytochemicals that have been identified in the leaves, flowers and berries of many *Crataegus* species. *Crataegus* extracts have beneficial effects on the cardiovascular system exhibiting antioxidant, antiatherosclerotic, hypo-

tensive, positive inotropic, coronary dilatation and antiarrhythmic effects (1, 2).

Crataegus succulenta Schrad. ex Link (syn. *Crataegus macracantha* Loudon, fleshy hawthorn, succulent hawthorn) is widely spread in North America (3). The species has not been studied regarding its chemical composition and possible biological effects. In this respect, the aim of the present work was to investigate the phenolic content, free radical scavenging and 15-lipoxygenase inhibitory effects of leaf and flower extracts.

MATERIAL AND METHODS

Chemicals.

Tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid (EDTA), pyrogallol, Folin-Ciocalteu's phenol reagent, linoleic acid, lipoxydase from soybean were from Fluka (Steinheim, Germany). (+)-Catechin hydrate, gallic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, iron (II) sulfate heptahydrate, hydrogen peroxide, sodium salicylate were from Sigma-Aldrich (Steinheim, Germany). Ammonium iron (III) sulfate dodecahydrate and sodium nitrite were obtained from Riedel-de Haën (Seelze, Germany). All other solvents and reagents were of analytical grade.

Plant material. Leaves and flowers of *Crataegus succulenta* Schrad. ex Link were collected in the Botanical Garden "Anastasie Fatu", Iasi in May 2010. The identity of the plant material was confirmed by taxonomists from Botanical Garden "Anastasie Fatu". Leaves and flowers were dried in dark at room temperature. Voucher specimens have been deposited in the Discipline of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa"-Iasi.

Extraction. Dried and powdered leaves and flowers (50 g each) were extracted with 500 mL of 70% aqueous methanol by stirring for 6 h at room temperature. The extraction was repeated twice. The combined extracts were concentrated under reduced pressure at 40°C and then freeze-dried.

Total phenolic content. Total phenolic content was quantified by Folin-Ciocalteu method as previously described (4). Electron-donating compounds such as phenols react with phosphomolybdic/ phosphotungstic acid complexes in Folin-Ciocalteu reagent and form a blue complex. The results were expressed as g gallic acid equivalents (GAE)/100 g extract using the following equation based on the calibration curve: $y=0.1165\times+0.0269$, $R^2=0.9987$.

Flavonoid content. Flavonoids were quantified by a spectrophotometric method based on their ability to generate pink coloured products in the presence of sodium nitrite and aluminium chloride, in alkaline solution (5). The results were expressed as g catechin equivalents/100 g extract using the following equation based on the calibration curve: $y=0.0759\times - 0.0038$, $R^2=0.9984$.

Proanthocyanidin content. The assay, described by Porter *et al*., is based on the conversion of proanthocyanidins to anthocyanidins in *n*-butanol-HCl and in the presence of ferric salts (6). The results were expressed as cyanidin equivalents (g cyanidin/100 g extract) using the molar extinction coefficient and molecular mass of cyanidin (ε=17,360 $L\cdot mol^{-1}\cdot cm^{-1}$ and M=287.24, respectively) (7).

DPPH radical scavenging assay. Antioxidants reduce DPPH radical (purple) to diphenylpicrylhydrazine DPPH-H (yellow) leading to a decrease in absorbance at 517

nm (4). (+)-Catechin hydrate was used as positive control. DPPH scavenging activity (%) was calculated as:

$100 \times (A_{start}\text{-}A_{end}/A_{start})$ where A_{start} and A_{end} are the absorbances of DPPH radical solution before and 5 min after adding the samples, respectively.

Superoxide anion radical scavenging assay. Superoxide anion radical, generated by the auto-oxidation of pyrogallol, oxidizes the latter to quinones that are quantified spectrophotometrically (8). (+)-Catechin hydrate was used as positive control. Superoxide anion radical scavenging activity (%) was calculated as follows: $100 \times (slope_{control}\text{-}slope_{sample}/slope_{control})$ where $slope_{control}$ and $slope_{sample}$ are the slopes of the plots of absorbance *vs.* time for control and samples, respectively.

Hydroxyl radical scavenging assay. The assay is based on the ability of hydroxyl radical, generated by Fenton reaction, to produce a violet product in the presence of sodium salicylate and ferric ions (9). (+)-Catechin hydrate was the positive control. Hydroxyl radical scavenging activity (%) was calculated as: $100 \times (A_{control}\text{-}A_{sample}/A_{control})$ where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance in the presence of extracts/(+)-catechin.

15-Lipoxygenase inhibition assay. In the presence of 15-lipoxygenase, linoleic acid is oxidized to conjugated dienes which strongly absorb at 234 nm. The assay was performed as described by Wangensteen *et al*. with minor changes (4). (+)-Catechin hydrate was used as positive control. 15-Lipoxygenase inhibition (%) was determined on the basis of the absorbance of the control and samples after 30 and 90 s reaction time as follows:

$$100 \times [(A_{control,90}\text{-}A_{control,30})\text{-}(A_{sample,90}\text{-}A_{sample,30})/(A_{control,90}\text{-}A_{control,30})].$$

Statistical analysis. All experiments were carried out in triplicate. The results were expressed as means±standard deviations. The EC_{50} values were calculated by linear interpolation between values above and below 50% activity.

RESULTS

Extraction. The extraction afforded 13.3 g of leave extract (S-l, yield: 26.6%) and 13.1 g of flower extract (S-f, yield: 26.2%).

Total phenolic, flavonoid and proanthocyanidin contents. S-l and S-f contained similar amounts of total phenolics, flavonoids and proanthocyanidins (tab. I).

TABLE I
Total phenolic, flavonoid and proanthocyanidin contents in *Crataegus succulenta* extracts

Extract	Total phenolic content (%)	Flavonoid content (%)	Proanthocyanidin content (%)
S-l	15.33 ± 0.40	5.25 ± 0.42	3.5 ± 0.0
S-f	16.15 ± 0.23	5.16 ± 0.30	3.65 ± 0.17

S-l – leave extract; S-f – flower extract

DPPH radical scavenging assay. DPPH radical scavenging effects of S-l and S-f were found to increase in a dose-dependent manner from 11.96±0.14 and 13.31±0.24%, respectively at 10.41 µg/mL to 89.08±0.31 and 92.62±0.04%, respectively at 166.66 µg/mL. At the same concentrations, (+)-catechin exhibited scavenging effects of

84.49±0.50 and 95.37±0.47%, respectively (fig. 1). An additional dilution (5.20 µg/mL) was tested in order to determine the EC_{50} value of (+)-catechin; at this dilution, a scavenging activity of 46.98±0.11% was registered. According to the EC_{50} values, S-f (46.7±0.2 µg/mL) was more active than S-l (52.0±0.5 µg/mL). The EC_{50} value of (+)-catechin was found to be 5.5±0.0 µg/mL suggesting a higher DPPH radical scavenging ability than *Crataegus succulenta* extracts (tab. II).

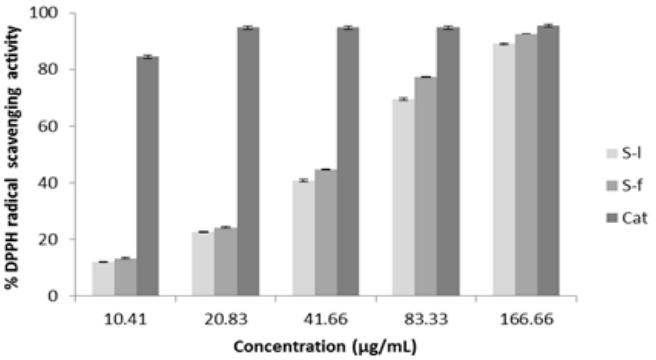

Fig. 1. DPPH radical scavenging activity of *Crataegus succulenta* extracts (S-l – leave extract; S-f – flower extract; Cat – (+)-catechin)

Superoxide anion radical scavenging assay. Superoxide anion radical scavenging activity was dose dependent for both extracts. In the concentration range of 0.65-0.87 mg/mL, (+)-catechin exhibited only 20.15-24.02% scavenging activity (fig. 2). Therefore, in order to calculate the EC_{50} value of (+)-catechin, higher concentrations (1.61, 2.26 and 2.58 mg/mL) were tested. At these concentrations, (+)-catechin scavenged superoxide anion radical by 40.15±1.31, 48.83±0.00 and 52.70±1.33%, respectively. Within this assay, both extracts were more active than (+)-catechin; the EC_{50} value of S-f (0.65±0.01 mg/mL) was lower than that of S-l (0.73±0.00 mg/mL) suggesting stronger scavenging properties for the former (tab. II).

Hydroxyl radical scavenging assay. In this assay, both extracts showed concentration-dependent scavenging effects but they were less active than (+)-catechin. At 1.87 mg/mL, the scavenging effects of S-l, S-f and (+)-catechin were found to be 56.57±0.79, 66.76±0.80 and 90.01±1.81%, respectively (fig. 3). With regard to the EC_{50} values, S-l (1.26±0.05 mg/mL) and S-f (1.6±0.0 mg/mL) were only 4-5 times less active than (+)-catechin (0.3±0.0 mg/mL) (tab. II).

15-Lipoxygenase inhibition assay. *Crataegus succulenta* extracts inhibited 15-lipoxygenase in a concentration-dependent manner with similar EC_{50} values (S-l: 154.6±0.6 µg/mL, S-f: 154.86±0.35 µg/mL) (fig. 4, tab. II). In the range of tested concentrations (100-166.66 µg/mL), (+)-catechin exhibited high activity (95.22±0.76-99.99±0.01%);therefore, lower concentrations (20.83, 41.66 and 83.33 µg/mL) were tested in order to determine its EC_{50} value; at these concentrations, catechin exhibited 31.65±0.53, 67.05±0.16 and 87.07±0.26% scavenging activity, respectively. (+)-Catechin inhibited 15-

lipoxygenase activity with an EC_{50} value of 29.2±0.2 µg/mL. With regard to the EC_{50} values, both extracts were 5 times less active than (+)-catechin (tab. II).

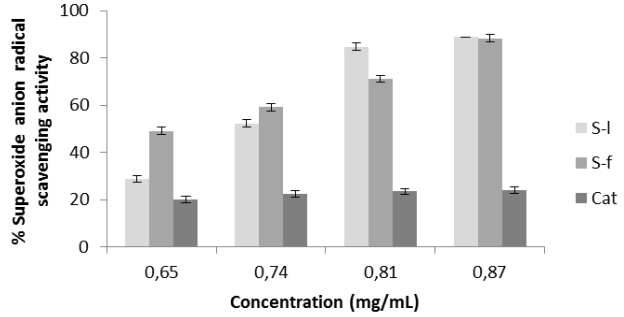

Fig. 2. Superoxide anion radical scavenging activity of *Crataegus succulenta* extracts (S-l – leave extract; S-f – flower extract; Cat – (+)-catechin)

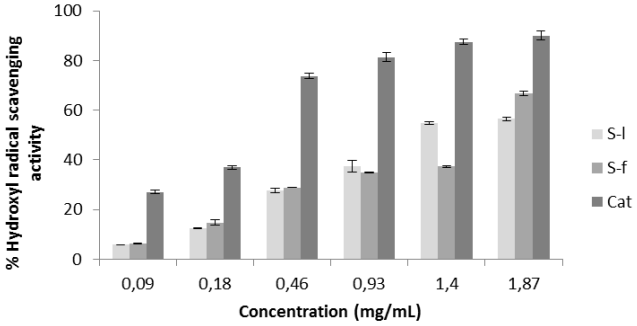

Fig. 3. Hydroxyl radical scavenging activity of *Crataegus succulenta* extracts (S-l – leave extract; S-f – flower extract; Cat – (+)-catechin)

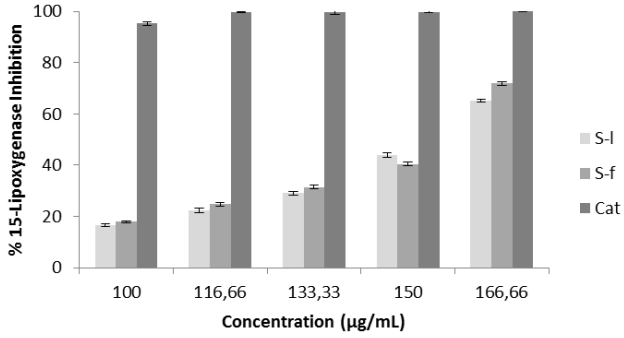

Fig. 4. 15-Lipoxygenase inhibitory activity of *Crataegus succulenta* extracts (S-l – leave extract; S-f – flower extract; Cat – (+)-catechin)

TABLE II

Free radical scavenging and 15-lipoxygenase inhibitory activities of *Crataegus succulenta* extracts

Extract/ Positive control	EC_{50}			
	DPPH radical scavenging activity	Superoxide anion radical scavenging activity	Hydroxyl radical scavenging activity	15-Lipoxygenase inhibitory activity
S-l	$52.0 \pm 0.5^{*}$	$0.73 \pm 0.00^{**}$	$1.26 \pm 0.05^{**}$	$154.6 \pm 0.6^{*}$
S-f	$46.7 \pm 0.2^{*}$	$0.65 \pm 0.01^{**}$	$1.6 \pm 0.0^{**}$	$154.86 \pm 0.35^{*}$
(+)-Catechin	$5.5 \pm 0.0^{*}$	$2.36 \pm 0.03^{**}$	$0.3 \pm 0.0^{**}$	$29.2 \pm 0.2^{*}$

S-l – leave extract; S-f – flower extract

* results expressed in µg/mL; ** results expressed in mg/mL

DISCUSSION

This *in vitro* screening compared the free radical scavenging and 15-lipoxygenase inhibitory effects of *Crataegus succulenta* extracts with those of (+)-catechin. Due to the *o*-dihydroxy moiety, catechin is one of the most potent free radical scavengers (10, 11). In addition, catechin has been reported to be an efficient inhibitor of 15-lipoxygenase (12). It is worthy to note that, in superoxide anion radical scavenging assay, both extracts were more active than (+)-catechin. In hydroxyl radical scavenging and 15-lipoxygenase inhibition assays, the extracts were only 4-5 times less active than (+)-catechin. In light of these data, it is obvious that *Crataegus succulenta* extracts are efficient free radical scavengers and 15-lipoxygenase inhibitors. These effects suggest potential therapeutic applications for *Crataegus succulenta* extracts. Reactive oxygen species such as superoxide anion and hydroxyl radicals are involved in the development of many chronic diseases (cardiovascular, neurodegenerative and malignant diseases) (5). 15-Lipoxygenase is a prooxidant enzyme; it catalyzes the oxidation of low-density lipoproteins (LDL) being a source of free radicals responsible for non-enzymatic oxidative processes. 15-Lipoxygenase is involved in the development of atherosclerosis, prostate cancer, diabetes, Alzheimer's and Parkinson's diseases (13).

This study revealed no striking differences in total phenolic, flavonoid and proanthocyanidin contents between *Crataegus succulenta* extracts. According to the EC_{50} values, both extracts showed similar 15-lipoxygenase inhibitory activities but different free radical scavenging effects. S-f scavenged more effectively DPPH and superoxide radicals while S-l was more active against hydroxyl radical. A lack of concordance between the antioxidant effects of *Crataegus succulenta* extracts is noticeable. Such a lack of concordance between the antioxidant properties evaluated by different assays has already been mentioned in literature and might have two possible explanations: first, the different chemical profile of the antioxidant constituents in plant extracts that significantly affects the antioxidant activity and second, the complexity of the mechanisms involved in antioxidant activity (14).

CONCLUSIONS

The results of this study suggest the possibility that extracts from the leaves and

flowers of *Crataegus succulenta* could be used as ingredients in functional foods for the prevention of oxidative stress-related diseases. Further research is required to investigate the *in vivo* efficacy and safety of the extracts.

ACKNOWLEDGEMENTS

The authors are grateful to Professor Catalin Tanase (Faculty of Biology, "Alexandru Ioan Cuza" University, Iasi) for providing the vegetal material.

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DIFFERENTIAL EQUATIONS WITH APPLICATIONS IN CANCER DISEASES

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DIFFERENTIAL EQUATIONS WITH APPLICATIONS IN CANCER DISEASES (Abstract): Mathematical modeling is a process by which a real world problem is described by a mathematical formulation. The cancer modeling is a highly challenging problem at the frontier of applied mathematics. A variety of modeling strategies have been developed, each focusing on one or more aspects of cancer. **Material and methods:** The vast majority of mathematical models in cancer diseases biology are formulated in terms of differential equations. We propose an original mathematical model with small parameter for the interactions between these two cancer cell sub-populations and the mathematical model of a vascular tumor. We work on the assumption that, the quiescent cells' nutrient consumption is long .One the equations system includes small parameter ε . The smallness of ε is relative to the size of the solution domain. **Results**: MATLAB simulations obtained for transition rate from the quiescent cells' nutrient consumption is long, we show a similar asymptotic behavior for two solutions of the perturbed problem .In this system, the small parameter is an asymptotic variable, different from the independent variable. The graphical output for a mathematical model of a vascular tumor shows the differences in the evolution of the tumor populations of proliferating, quiescent and necrotic cells. The nutrient concentration decreases sharply through the viable rim and tends to a constant level in the core due to the nearly complete necrosis in this region. **Conclusions**: Many mathematical models can be quantitatively characterized by ordinary differential equations or partial differential equations. The use of MATLAB in this article illustrates the important role of informatics in research in mathematical modeling. The study of avascular tumor growth cells is an exciting and important topic in cancer research and will profit considerably from theoretical input. Interpret these results to be a permanent collaboration between math's and medical oncologists. **Keywords**: STEM CELLS, VASCULAR TUMOR, DIFFERENTIAL EQUATIONS, NUMERICAL MODELING.

Cancer is one of the main causes of morbidity and mortality in the world. Mathematical modeling plays an important role especially in cancer stem cells and tumor growth. The mathematical exploration of cancer was initiated in the 1950s with a study of the age dependent incidence curves of human cancers. Mathematical modeling and computational approaches have become more accepted by clinicians as contributing to new understandings of complicated cancer mechanisms and tissue physiology. Basic models represented in this article by two or three equations allowing a qualitative examination. In recent years, cancer stem cells have become the subject of theoretical investigations as well, and studies were performed to elucidate the

biology and dynamics of colorectal cancer stem cells, breast cancer stem cells, hematologic malignancies, and the role of stem cells in the evolution of drug resistance. Proliferating cells can divide with constant rate or lose their division ability and therefore transit to the resting phase. Resting cells can either return to the proliferating state or die with constant rate. His interactions between these two cancer cell subpopulations are described in this article by the original singularly perturbed system of ordinary differential equations. It is additionally assumed that the transition from the quiescent to the proliferating state depends on the number of proliferating cells only. The majority of mathematical models of solid tumor growth seem to have appeared in the literature since 1990 .In recent years, applied mathematicians and oncologists have collaborated to develop models and simulations that characterize a vascular tumor (1).Tumors appear when cells lack of growth control. Uncontrolled proliferation can be the result of different alterations of normal cells properties. To proliferate, cells need nutrients and oxygen coming from existing vascular vessels surrounding the tissue where the tumor grows.

MATERIAL AND METHODS

We analyze an original singularly perturbed system of ordinary differential equations for the model with form:

$$(P_\varepsilon)\begin{cases}\varepsilon\dfrac{dx}{dt}=\varepsilon a_1 x - x - a_2 y\\ \varepsilon\dfrac{dy}{dt}=x + a_2 y - \varepsilon a_3 y\end{cases}$$

where $x(t)$ and quiescent cancer cell $y(t)$ populations, based on experimental data. Proliferating cells can divide with constant rate a_1 or lose their division ability and therefore transit to the resting phase. Resting cells can either return to the proliferating state or die with constant rate a_3, and a_2 describe the proliferating cells' nutrient consumption (2). The interactions between these two cancer cell subpopulations are described by the following system of ordinary differential equations with small parameter. It is additionally assumed that the transition from the quiescent to the proliferating state depends on the number of proliferating cells only. We work on the assumption that $\frac{1}{\varepsilon}$, the quiescent cells' nutrient consumption is long, where ε is the small parameter. The use of a small parameter here is simply for definiteness. Should mention this model system differential equations with small parameter are experimental and must be validated in future theoretical results. Mathematical modeling of a vascular tumor can be seen as the first step in building models for tumors in later stages. There are several different stages in the growth of a tumor before it becomes so large that it causes the patient to die or reduces permanently their quality of life. The factors that trigger these mutations are unknown, but are thought to include both environmental and hereditary effects. The most significant turning point in cancer, however, is the establishment of metastasis. The metastatic spread of tumor cells is the predominant cause of cancer deaths. Metastasis signifies that a tumor is malignant and this is in fact what makes cancer so lethal. Nonlinear differential equations modeling has been performed to study the effects of morphology instabilities on both avascular and vascular solid tumor growth (3) .This mathematical mod-

els (is based on a set of partial differential equations) provide a powerful analysis tool to dissect the biological mechanisms and the role of tumor growth in angiogenesis and the consequences of its inhibition.

$$(2)\begin{cases}\dfrac{da}{dt}=\dfrac{\partial}{\partial x}\left(\dfrac{a}{a+b}\dfrac{\partial(a+b)}{\partial x}\right)+0.4e^{0.4u}a(1-a-b-c)-\dfrac{(1-e^{-\frac{u}{2}})}{2}a\\ \dfrac{db}{dt}=\dfrac{\partial}{\partial x}\left(\dfrac{b}{a+b}\dfrac{\partial(a+b)}{\partial x}\right)+\dfrac{(1-e^{-\frac{u}{2}})}{2}a-\dfrac{(1-e^{-\frac{u}{2}})}{4}b\\ \dfrac{dc}{dt}=\dfrac{(1-e^{-\frac{u}{2}})}{4}b\end{cases}$$

Where are denoted by:

$a(x,t),b(x,t),c(x,t),u(x,t)$ the cell densities for the proliferating, quiescent, necrotic cells and concentration of some nutrients. The nonlinearity of this model is their most important property: it reflects mathematically the openness of biological systems and their state beyond thermodynamic equilibrium.

RESULTS

We investigate, using numerical computation, the behavior of the models in various small parameter regimes. In Figure 1-5 we present numerical solutions of the differential equations systems (P_ε), (2) for different parameter values .The choice of a small parameter is connected with the order of the solution and the asymptotic sequence which describes the behavior of the solution as $\varepsilon \to 0$. The overall procedure for work is: develop an appropriate scaling for the equations, identify the small parameter, writing regular series, writing boundary function series. The simulation of a single set of equation can be performed on a single workstation because the numerical integration of a system (P_ε) not very time consuming. The parameter values have highs impact on the accuracy of the model in representing real biological systems but these values are difficult to estimate experimentally (4). Using the program MATLAB, I did various simulations for different values of biological parameters presented in the models studied. MATLAB simulations obtained for transition rate from the quiescent cells' nutrient consumption is long, we show a similar asymptotic behavior for two solutions of the perturbed problem (5) .The choice of a small parameter is connected with the order of the solution and the asymptotic sequence which describes the behavior of the solution as $\varepsilon \to 0$.

The growth dynamics for vascular tumors are of particular interest because vascular tumors, having their own blood supply, have nearly limitless provisions of nutrient. The graphical output in this article shows the differences in the evolution of the tumor populations of proliferating, quiescent and necrotic cells. Graphical observation is that cancer cells need a high nutrient environment to proliferate, tumor grow is influenced by cell movements as well as other cell growth factors. Necrotic

cells are dead while proliferating and quiescent cells can move. The nutrient concentration decreases sharply through the viable rim and tends to a constant level in the core due to the nearly complete necrosis in this region (6). Necrotic cells can be the result of different alterations of normal cells properties.

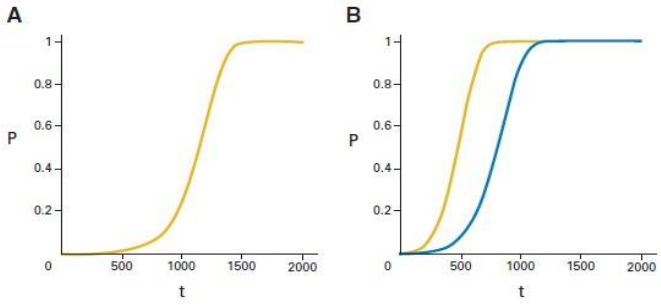

Fig. 1. (A) The curve is the same for patients receiving imagine therapy and for untreated patients, because imagines cannot deplete cancer stem cells. The higher growth rate for the normal cells increases the region of acceptable drug regimens. (B) The right curve shows the probability of blast crisis when the patient is treated, and the left curve shows the probability of blast crisis when there is no treatment.

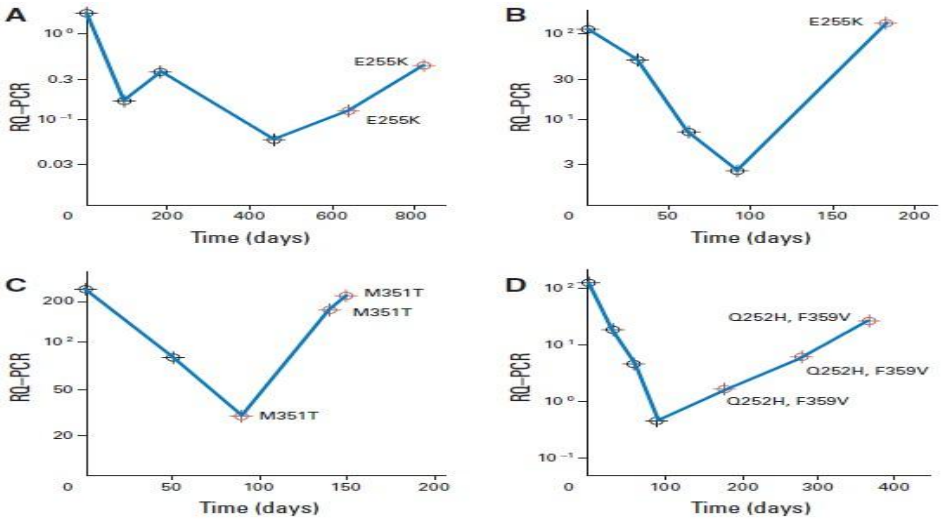

Fig. 2. A numerical simulation shows the dynamics resistances mutations lead to relapse of cancer stem cells for different values of parameter a_1, a_2, a_3. Parameter estimation adjusts this model to reproduce the experimental results in the best possible way for a set of experimental data. For these parameters, solutions can change on a time scale that is very short compared to the interval of integration, but the solutions of interest changes on a much longer time scale.

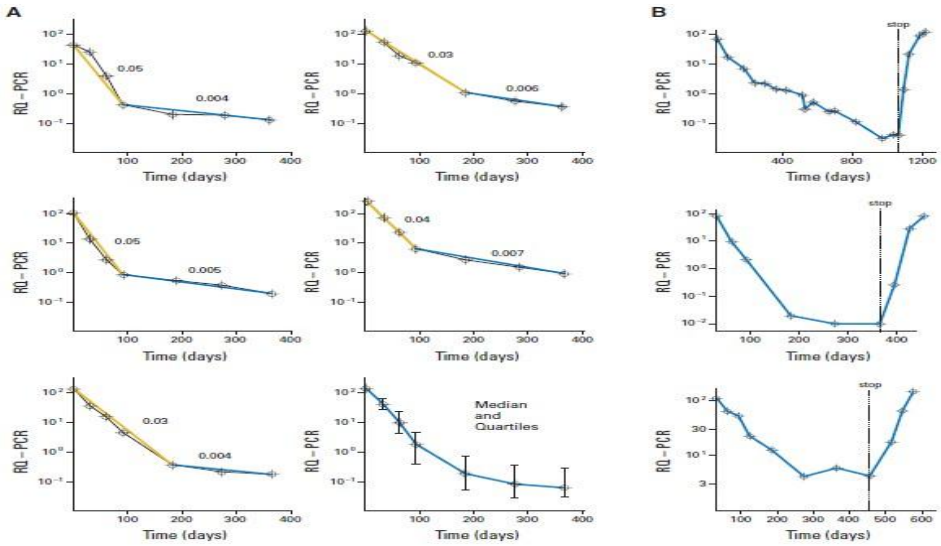

Fig. 3. (A) Drugs lead to a biphasic decline of cancer cells. The sixth figure shows the median with quartiles taken over all patients who do not evolve resistance mutations. (B) Discontinuation of drugs therapy leads to a rapid increase of cancer cells to levels at or beyond pretreatment baseline. Therefore, cancer stem cells are not depleted by drugs during this time period.

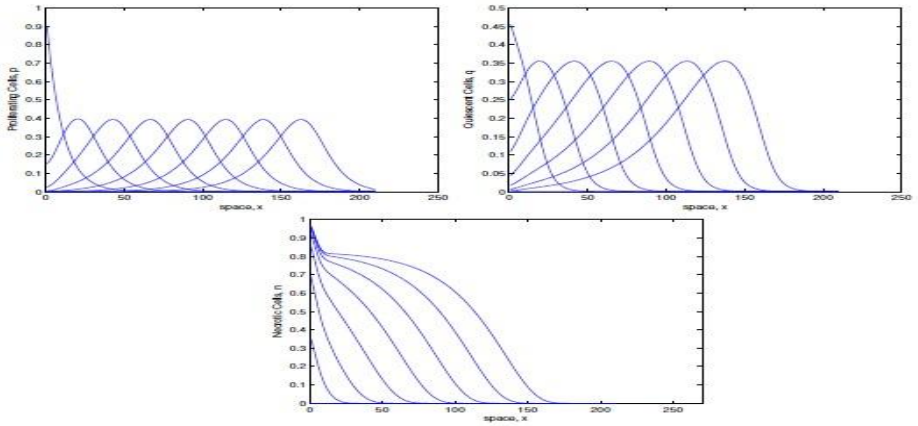

Fig. 4. The numerical simulations shows the cells densities of proliferating, quiescent, and necrotic cells plotted as a function of space of time, and curves moves from left to right as time increases. The surviving quiescent cells can thus proliferate afterwards because of the renewed nutrient supply, and the tumor may then recover. Necrotic cells formation is also a very poorly-understood aspect of tumor development.

Generally the estimation of the kinetic parameters is performed by fitting the experimental by computing a number of ordinary differential equations systems with different parameters and verifying the best solution.

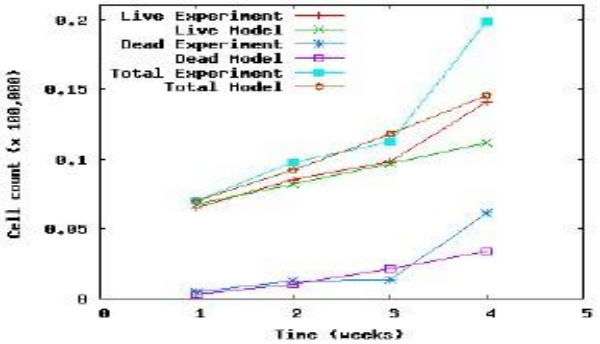

Fig. 5. Cell counts between calibrated model and experimental data. Calibration model should be included in probabilistic sensitivity analyses .The parameter values have highs impact on the accuracy of this model in representing real biological systems but these values are difficult to estimate experimentally. Adjustments may need to be made to account for temporal shifts in data.

CONCLUSIONS

Modeling cancer has become one of the active areas within the mathematical, oncologists and theoretical biology communities. This model is essentially based on differential equations and they can describe abundances kinetics and binding affinities of pathway components and their interactions. Validating a model is essentially examining whether it is good enough in relation to its intended purpose. A valid model is one that has successfully passed through the validation process. Proper validation of the above models will require input from statisticians. In this article, the models above ignore stochastic effects. The complexity of this biological process is revealed every time a mathematical simulation of the processes is carried out. The models are solved numerically using the software MATLAB. The use of MATLAB in this article illustrates the important role of informatics in research in mathematical modeling. Should mention these differential equations systems with small parameter are experimental and must be validated in future theoretical results. The lacunae in our understanding of tumors growth and invasion necessitate a sustained input by mathematicians into current and future investigations. Future work will include thereby formulating a more comprehensive model for vascular tumors growth.

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REMOTE RESPIRATION MONITORING SYSTEM FOR SLEEP APNEA DETECTION

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REMOTE RESPIRATION MONITORING SYSTEM FOR SLEEP APNEA DETECTION (Abstract): Sleep is a dynamic physiological process, its primary function being the restoration of the central nervous system. Because sleep disorders affect a significant part of the population the dynamic long term monitoring of human respiration plays a very important role in diagnosis and treatment. **Material and methods:** We describe a remote monitoring system designed for in-hospital or at-home detection with a high degree of accuracy of sleep-related disorders in patients experiencing episodes of obstructive sleep apnea. Patient's respiratory rate is continuously measured by using wireless devices and then transfered to a central monitoring station via a wireless sensor network. The central monitoring station runs a respiration monitoring application that receives the patient's respiratory rate from the network and activates an alert upon detection of a sleep apnea episode. **Results:** A user-friendly graphical user interface was designed for the respiration monitoring application that displays patient's respiratory rates and sleep apnea alerts. A prototype of the system has been developed, implemented and tested. **Conclusions:** The described system allows persons with respiratory diseases or elderly to be monitored at their homes, as an alternative to medical supervision in hospitals. **Keywords:** APNEA DETECTION, REMOTE MONITORING, WIRELESS SENSOR NETWORKS.

Sleep is a naturally recurring state of rest for the mind and body, a third of life being spent. During sleep the whole central nervous system is restored. Therefore, good sleep is an important component of a healthy lifestyle. However, sleep disorders affect a significant part of the population. According to the American Academy of Sleep Medicine approximately 20% of the population in modern societies suffers from sleep disorders. For instance, in Europe the prevalence of obstructive sleep apnea is estimated at 2% of women and 4% of men, having a direct impact on their quality of life (1).

The remote sleep monitoring devices, integrated into a system, allow patients to be monitored from a distance, for example at their homes, the recorded signals being transmitted to a monitoring centre and allowing sleep specialists to analyze the evolution of the patient.

The long term monitoring of human respiratory dynamics plays a very im-

portant role in the diagnosis and treatment of a number of medical conditions such as hypertension, circadian rhythm analysis, atherosclerosis, stroke, or even sudden death syndrome. Long term respiratory monitoring is a widely used method in the diagnosis of obstructive sleep apnea (OSA).

Respiratory rate (RR) is a physiological parameter used by some wireless monitoring systems as it allows the assessment of OSA, as RR can be recorded promptly and normal and abnormal variations can be easily differentiated.

A widely used method for the diagnosis of sleep-related diseases is polysomnography that records a number of physiologic variables including brain and heart activity, patient motion, RR, or oxygen saturation. However, polysomnography test is expensive, requires well trained medical personnel and keeps the patient in hospital overnight with many electrodes and sensors attached to him. To overcome these problems, numerous systems for home sleep monitoring are already in use. For example, some respiratory activity monitoring methods use thermistor based sensors (2), others use piezoelectric elastic bands placed around the chest (3), dual frequency microwave radars (4) or respiratory inductive plethysmography (5). Other methods use the ECG signals to extract the RR (6). All these, although acceptable for shorter periods of time, involve sensors attached to patient, connected by some unwieldy wires to monitoring devices that are not very comfortable for the sleeping patient. In order to avoid this situation, our system uses low power wireless devices and radio transceivers.

Many available home systems are not adequate for monitoring as they require the patients to be in conscious state able to press the alarm button. Other systems record the sleep data on a memory card and process the signals the next day, making them improper for real time operation. To solve this problem, we propose a wireless sensor system for real-time sleep apnea detection which enables remote monitoring while the patient is at home.

This paper describes a remote respiratory monitoring system for the detection of sleep disorders based on wireless sensor nodes. The sensor nodes contain commercially available modules that are connected together, perform the measurements, compute the results and transmit them to a central monitoring station. The central monitoring station runs a patient RR monitoring application that displays the RR in real-time and activates the alarms when an OSA episode is detected.

MATERIAL AND METHODS

The proposed remote monitoring system for sleep disorder detection (fig.1) consists of a wireless sensors network (WSN) used to measure the RR and transmit it to a central monitoring station running a patient RR monitoring application. Each sensor node in the WSN contains a wireless low power device attached to the sleeping patient used to compute the RR. There are also several repeater nodes distributed in WSN at fixed location, their number and density depending on the specific coverage requirements. An access point acting as a WSN coordinator is connected to the monitoring station through a USB connection. The central monitoring station running a patient RR monitoring application that receives the RR data from WSN, displays it as temporal waveforms, and activates the alarms when an OSA is detected.

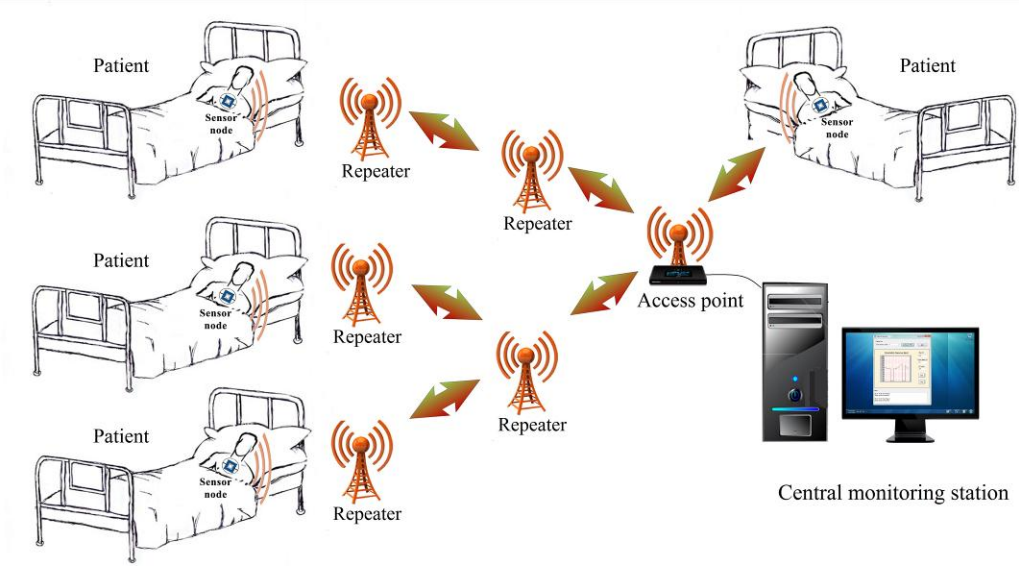

Fig. 1. Remote respiratory monitoring system – network architecture

Each sensor node contains an eZ430RF2500 circuit (Texas Instruments) (7). The eZ430-RF2500 is a complete wireless development tool for the MSP430F2274 microcontroller and CC2500 wireless transceiver that includes all the hardware and software required to develop a WSN in a convenient USB stick. MSP430F2274 is a 16-bit RISC ultra-low-power mixed signal microcontroller, has a memory structure that includes a 32 kb flash memory and 1kb on-chip SRAM. Other features of the MSP430 include: two built-in 16-bit timers, a fast 10 bit 200 kbps A/D converter with internal reference, enhanced universal serial synchronous/asynchronous communication interfaces USART, I2C, SPI. The internal digitally controlled oscillator allows wake-up from low-power modes to active mode very fast, facility that is used to extend batteries life.

The CC2500 wireless transceiver is a low-cost 2.4 GHz transceiver designed for very low-power wireless applications. The circuit uses for data transmission the 2400 MHz ISM (Industrial, Scientific and Medical) and SRD (Short Range Device) frequency bands, supports various modulation formats, has a configurable data rate up to 500 kBaud, and consumes less than 21.2 mA in transmission mode at 0 dBm output power and 17.0 mA in receiving mode.

We used the SimpliciTi protocol from Texas Instruments to transfer data from sensor node to central monitoring station. SimpliciTI is a proprietary, low-power radio-frequency network protocol used with small-scale WSNs that requires less memory than the widely used ZigBee protocol. This network contains battery-operated devices which require long battery life, low data rate, and have a limited number of nodes talking directly to each other. SimpliciTI network protocol supports End Devices (ED) in a peer-to-peer network topology, an Access Point (AP) to store and forward the data, and Range Extenders

(RE) to extend the range of the network.

Airflow detection using a nasal thermistor is one of the most frequently used methods to sense breathing pattern. Thermistors are electric circuit elements made of semiconductor materials that are characterized by a high negative temperature coefficient (NTC) or a high positive temperature coefficient (PTC). A NTC thermistor acts like a resistor, with temperature coefficients of typically –3% to –5%/ºC. The high sensitivity allows the thermistor circuit to detect minute changes in temperature, which could not be observed with other temperature detectors such as a thermocouple circuit. Thermistors placed in front of a nose detect the inspiratory and expiratory temperature fluctuations between ambient temperature (inhalation) and lung temperature (exhalation). It is known that the difference in temperature between the inhaled and exhaled air is about 10°C. We use two 0603 SMD type thermistors (fig. 3) with the following characteristics: R_{nom} = 10 kΩ at 25°C, B = 3380, 5% tolerance, connected as in fig. 2.

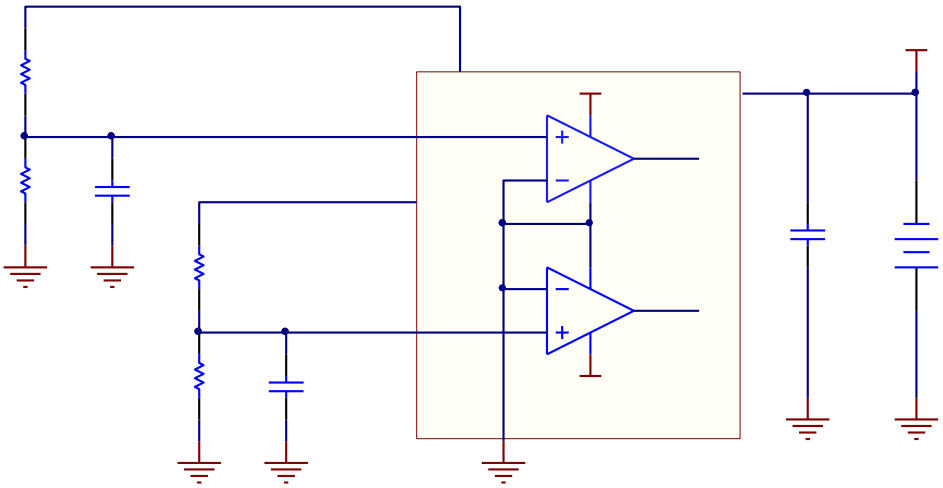

Fig. 2. Thermistors to MSP430F2274 microcontroller interface

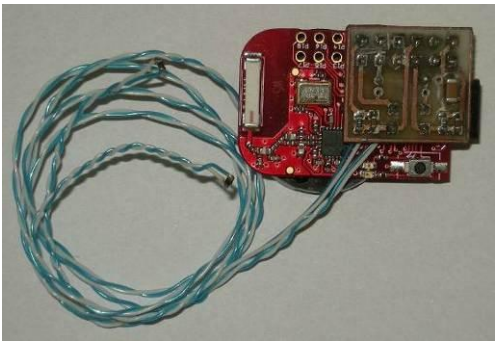

Fig. 3. Thermistor

Fig. 4. Chest belt

Another wireless device uses Pneumotrace II chest belt respiration transducer. The chest belt is a rugged, piezo-electric respiration transducer, which linearly generates a high level signal in response to changes in thoracic circumference associated with respiration. It generates 20 to 200 mV into a 1 MΩ load. Practically, for signal acquiring we use the same device, replacing the thermistor with the chest belt (fig. 4).

Power supply to the devices in fig. 3 and fig. 4 is provided by a CR2032 lithium battery and the A/D sampling frequency is 20 Hz.

A third wireless device uses a small accelerometer to detect the chest movements associated with respiration. It is a three-axis accelerometer (ADXL330 – fig. 5) with buffered outputs, connected to the 10 bit A/D inputs of the MSP430F2274. ADXL330 has the following technical specifications: 3-axis sensing, small, low power consumption: 200 μA at VS = 2.0 V (typical), single-supply operation: 2.0 V to 3.6 V, excellent temperature stability. The typical sensitivity shift over temperature for supply voltages of 3 V is typically better than ±1% over the −25°C to +70°C temperature range. To detect the RR, accelerations signals from the three axes are acquired at a sample rate of 20 Hz, 10 bit/sample, then the microcontroller identifies the peaks in the filtered acceleration signal $a = \sqrt{a_x^2 + a_y^2 + a_z^2}$, where a_x, a_y and a_z are accelerometer outputs (fig. 6).

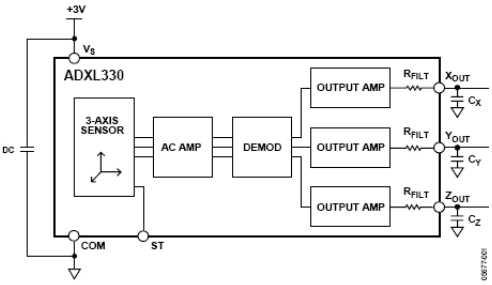

Fig. 5. Functional block

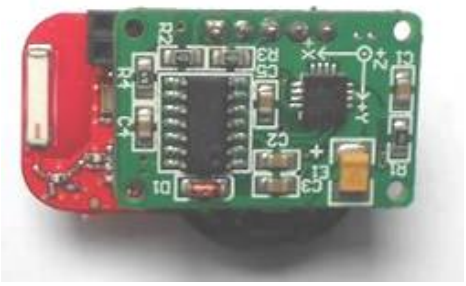

Fig. 6. Accelerometer diagram for ADXL330

RESULTS AND DISCUSSION

The flowchart of the software running on MSP430F2274 microcontroller in each node of the sensor network, including radio communication, is presented in fig. 7. After initializing, authentication and a START command, the sensor node wakes up to read and process the analogue signals. To minimize the energy waste, since an important power consumer is the radio transceiver, CC2500 enters a low power mode after each transmission cycle.

A user-friendly Graphical User Interface (GUI), running on the central monitoring station, was developed for real-time RR monitoring and apnea alert. GUI was developed by using LabWindows/CVI (fig. 8). On the GUI, temporal waveform of RR signal for selected patient are displayed, and the status of each sensor node (the battery voltage and the measured power from the received radio signal, used as

estimation for the distance between the wireless pacemaker and nearest Range Extender or Access Point).

The prototype of the above described system has been implemented and tested. The RR of three patients was computed by the wireless devices described above, and transmitted via WSN to the central monitoring station. The GUI running on the monitoring station displayed the RR correctly. Then, some apnea events (pauses in breathing lasting at least 10 seconds) were simulated, and the interface also displayed these alerts correctly.

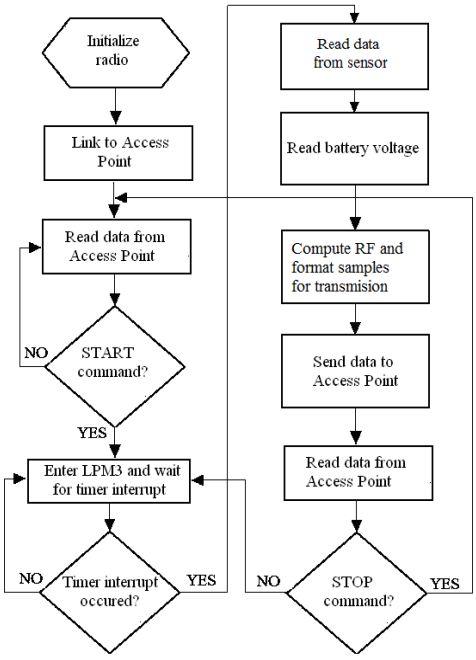

Fig. 7. Flowchart of the software

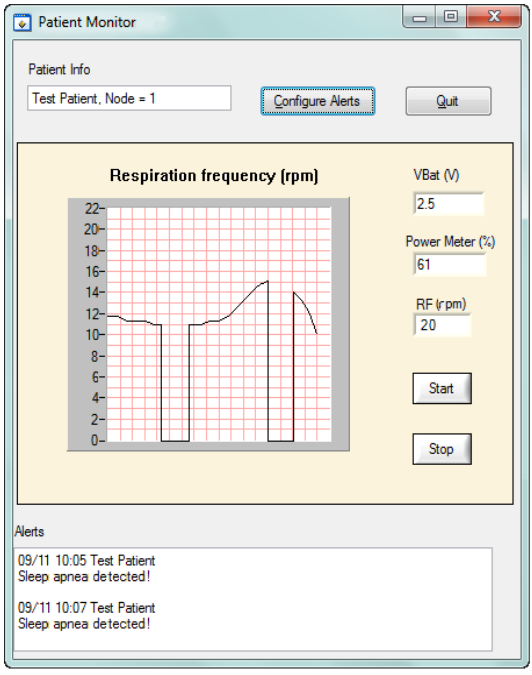

Fig. 8. Graphical user interface (GUI) running on MSP430F2274

Finally we measured the power consumption of the each sensor node. Knowing that CC2500 wireless transceiver is the largest contributor to overall power consumption, the average per-node estimate of power consumption is 0.44 mA. Calculating the battery life span (lithium battery 2240 mAh) ideally assuming that batteries maintain constant terminal voltage throughout life span, we obtained a value of approximately 23 days.

CONCLUSIONS

The prototype of a flexible, scalable and cost-effective medical remote monitoring system for the detection of OSA has been developed, implemented and tested. The system is suitable for continuous long-time monitoring of human respiration for a number of medical conditions requiring analysis of respiratory rate, sleep-related respiratory disorder or ischemic heart disease.

The described system allows persons with respiratory diseases or elderly to be

monitored at home, as an alternative to medical supervision in hospitals.

ACKNOWLEDGEMENT

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NEWS

CD35 PROTEIN AND EPSTEIN-BARR VIRUS

Epstein-Barr virus (EBV) is member in the human herpesvirus family. EBV affects nine out of 10 people and can cause infectious mononucleosis. Also it was the first human virus found to be associated with several types of cancer and with autoimmune disorders. To enter into the host cell, EBV attaches to the receptor CD21 of the primary B-cell membrane complementary with envelope glycoprotein EBV gp350/220. A recent study conducted by Fingeroth and Ogembo, show that the EBV glycoprotein gp350/220 is able to bind also a second receptor represented by the CD35 protein. This protein, which exists only in humans, was identified as a malaria receptor and as target in autoimmune diseases. Researchers showed that the CD35 receptor has a role in primary infection and reveals the importance of EBVgp350/220 for the development of a vaccine able to prevent or reduce the risk of infectious mononucleosis and EBV-associated pathology (Ogembo JG, Kannan L, Ghiran et al. Human Complement Receptor Type 1/CD35 Is an Epstein-Barr Virus Receptor. *Cell Reports*, 2013; 3 : 371-85).

Cătălina Luncă

PROPROSTHETIC INTERVENTIONS IN THE POSTERIOR MAXILLARY IMPLANT THERAPY

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PROPROSTHETIC INTERVENTIONS IN THE POSTERIOR MAXILLARY IMPLANT THERAPY (Abstract): **Aim**: The purpose of this study is to evaluate the necessary number of pro-prosthetic interventions in view of the implanto-prosthetic rehabilitation of the defective prosthetic fields. **Materials and methods**: We investigated a lot that included 230 patients aged between 18 and 82 who benefited from implant-prosthetic rehabilitation, the type of graft used, the types of membranes selected as well as the various remodeling and elevation techniques at the maxillary level dictated by the particularity of the clinical case**. Results and discussions**: The edentulous posterior maxillary region presents in implantology a more difficult situation than any other region of the maxillary. The absence of teeth often triggers a progressive reduction of the alveolar process volume, a reduction that aims particularly at the bony vertical dimension, namely the region between the top of the alveolar ridge and the floor of the maxillary sinus, called the sub-sinus Misch vertical dimension. The presence of the maxillary sinus, combined with the reduction of height of available bone in the terminal maxillary region poses serious problems for implant insertion. **Conclusions**: The success of the implant therapy depends on the absence of radio-transparent areas, while the per-implant bone resorption must be limited to the cervical region and should not involve the implant threads or its osteo-integrated area. The implant should also be fix, pain free, with no local infection. **Keywords**: IMPLANTS, PREIMPLANT SURGERY, AUGMENTATION, ORAL REHABILITATION.

The main success factors of the implant therapy are related to the preimplant augmentation surgery, The three-dimensional optimum and precise positioning of the implant, The Management of peri-implant soft tissues, Implant axis compatible with prosthetic options, The quality of prosthetic restorations (1).

Pre-implant surgery of augmentation is absolutely necessary to ensure a good osteo-integration, as well as a good stability over time of the implant (2).

The purpose of this study is to evaluate the necessary number of pro-prosthetic interventions in view of the implanto-prosthetic rehabilitation of the defective prosthetic fields.

MATERIAL AND METHODS

We investigated a lot that included 230 patients aged between 18 and 82 who benefited from implant-prosthetic rehabilitation, the type of graft used, the types of membranes selected as well as the various re-

modeling and elevation techniques at the maxillary level dictated by the particularity of the clinical case.

RESULTS AND DISCUSSION

The edentulous posterior maxillary region presents in implantology a more difficult situation than any other region of the maxillary. The absence of teeth often triggers a progressive reduction of the alveolar process volume, a reduction that aims particularly at the bony vertical dimension, namely the region between the top of the alveolar ridge and the floor of the maxillary sinus, called the sub-sinus Misch vertical dimension. The presence of the maxillary sinus, combined with the reduction of height of available bone in the terminal maxillary region poses serious problems for implant insertion (3).

The bone structure of the sinus walls is mainly composed of compact tissue. However, in certain areas there is a spongy tissue that fills in the gaps between the corticals, especially at the level of inferior areas (the floor of the sinus maxillary) (4).

According to Misch's bone density, it can be argued that in most cases the bone tissue in this area is mainly of D2-D3 class and, especially in the distal areas of the edentulous alveolar processes, D4 class (4).

Howell and Cawood's experimental studies showed that in the presence of teeth there is a centrifugal type circulation: the blood comes mostly from the arteries inside the bone tissue and partly from the blood vessels from the periosteum.

The edentulous alveolar process suffers from an ongoing resorption and its circulatory pattern becomes centripetal; most of the blood comes from the periosteum, while the intrinsic vascular component is greatly reduced. In agreement with the theory of Moss' oro-functional conformations, the loss of maxillary volume that follows the tooth loss is attributed to the following factors:

- the reduction of the centrifugal blood contribution through the atrophy of the vessels of the superior dental plexus;
- the pneumatization of the maxillary sinus towards the alveolar process, which is no longer hindered by the functional bone remodeling of mastication;
- the reduction of the centripetal blood contribution caused by the sufferance of the soft tissue in the case of removable dentures carriers.

The mucous membrane of the maxillary sinus is similar to that of paramaxillary sinuses, but less vascularized.

The thickness of the membrane is variable, generally ranging between 0.3 - 0.8m.

The maxillary sinus is linked to the nasal fossa through the ostium, infundibulum and middle meatus. These structures are called the osteomeatal complex. Of a particular importance is the fact that this opening is situated in the upper part of the maxillary sinus.

The adequate reflection and lifting of the sinus membrane and the insertion of the bone graft is possible without affecting the sinus drainage and improve the quality of life (5).

Until the first half or 1970, the maxillary sinus was considered an inviolable anatomic limit in implantology. With the rapid development of implantology, the need for surgical techniques to allow the augmentation of the atrophied alveolar process emerged, especially in the distal region of the upper alveolar maxillary arch.For exemplification, we show patient A.C, 43 years old, who had the following condition (fig.1).

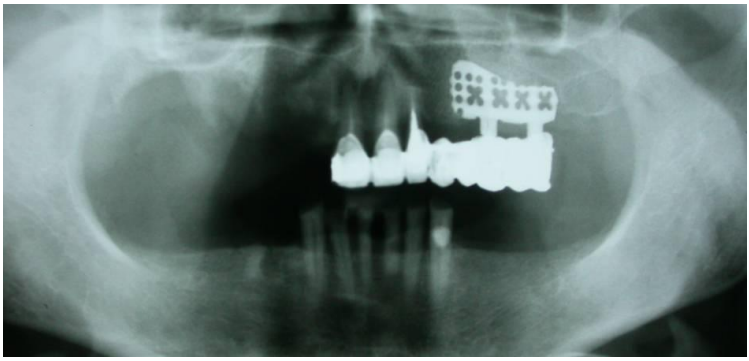


Fig. 1. Paraclinic OPT examination

After removing the existent implants, the case was evaluated again and the fix implant therapy was decided as a therapeutic solution, thus recreating the integrity and functionality of the stomatognathic system. Because the prosthetic field was defective from the odonto-periodontal and mucous-bone point of view, surgical interventions were necessary to readjust the two supports, in order to ensure the viability of the solution (fig. 2).

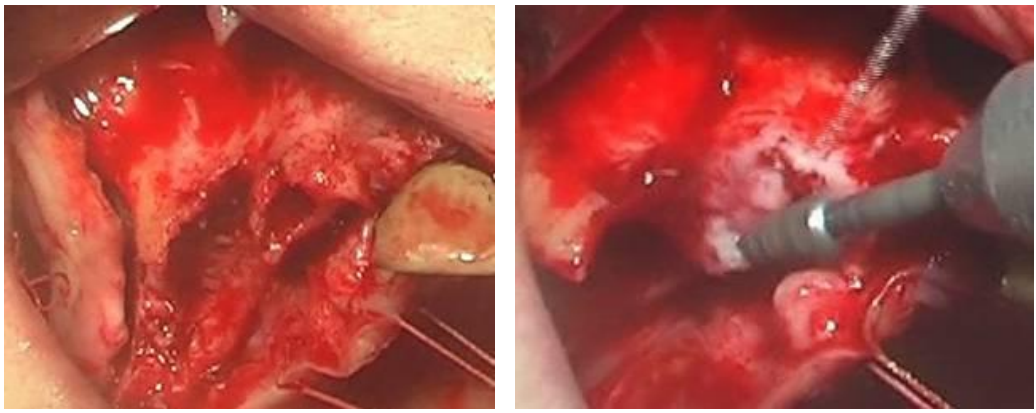
Thus, in a first stage, the regularization of the edentulous ridge in quadrant I was necessary, as well as the proposthetic preparation in view of the implant (fig. 3 a,b). The regularization of the alveolar ridge involved the realization of a mucous-periostal flap, the evidencing of the bone support and the removal of bone irregularities with the bone drill, followed by the suture of the alveolar ridge.

a.

b.

c.

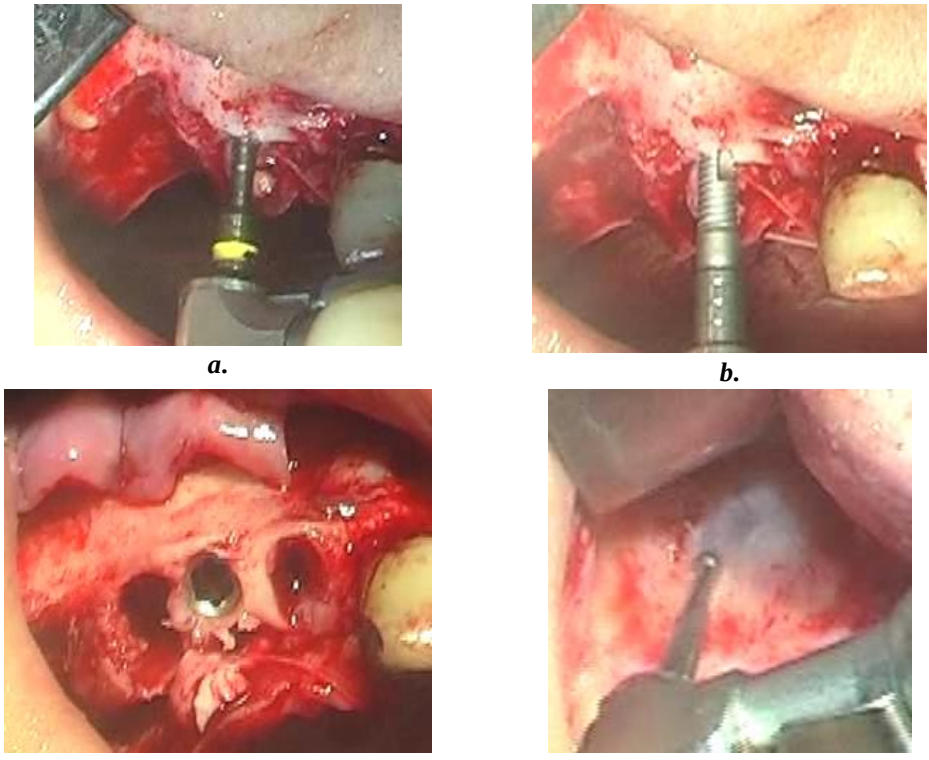
Fig.2 (a,b,c). Removal of existent implants



a. ***b.***

Fig. 3 (a,b). Regularization of the edentulous ridge

In a next stage the implants sites were created and the implants of the teeth corresponding to spaces 13, 15, 12.At the level of second premolar 13 a tuareg swell implant was chosen, with the following dimensions: 3.3x10 (fig.4 a,b,c,d).



a. ***b.***

c. ***d.***

Fig. 4(a,b,c,d). Tuareg swell implant

For 15a tuareg sp implant was selected 4,2x11 (Fig. 5 a,b,c,d,e)

Tooth 12 was substituted by means of a tuareg s implant 3,5x10 (Fig. 6 a,b,c,d)

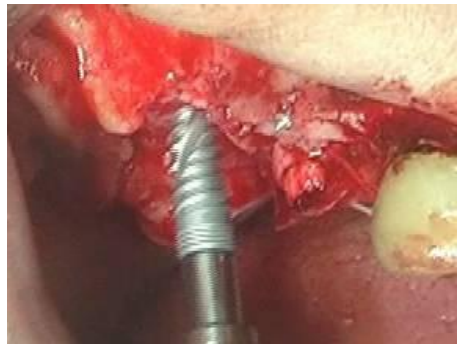
Because the bone support was deficient, we opted for a bone addition of Bio-Oss, as well as for the placement of a membrane, augmentation materials which will remedy the deficit of hard bone, contributing to a primary and secondary stability of the implants (Fig. 7 a,b,c,d,e, f,g).

The pro-prosthetic treatment included the apical resection at level 11.

a.

b.

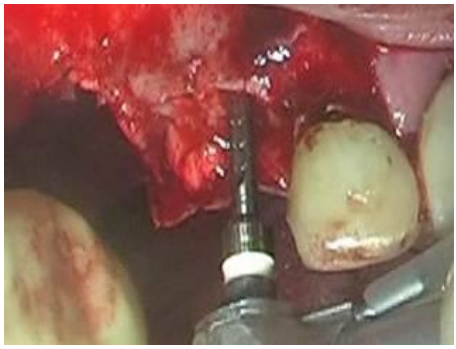
c.



d.

e.

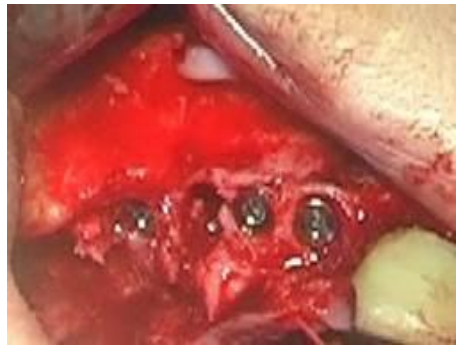
Fig. 5 (a,b,c,d,e). Tuareg sp implant 4,2x11



a.

b.

c.



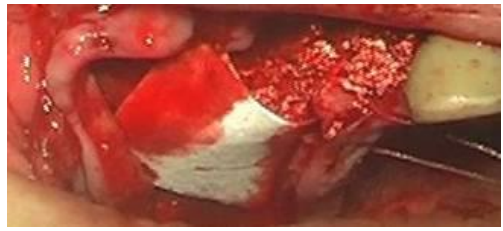
d.

Fig. 6 (a,b,c,d). Tuareg` s implant 3,5x10

a.

b.

c.



d.

e.

f.

g.

Fig. 7 (a,b,c,d,e,f,g). Bio-Oss addition, as well as placement of a membrane

The prosthetic treatment aimed at an aggregate metallo-ceramic reconstitution at the level of implants 13, 12, 15 (Fig.8 a,b,c,d,e).

a.

b.

c.

d.

e.

Fig. 8 (a,b,c,d,e). The final implanto-prosthetic rehabilitation at level :13, 12, 15

Another case is that of a 36 years old patient, for whom the final prosthetic solution was the implant (Fig. 9).

At level 24 we opted for the placement of a Bio Com Wide implant 4,70X13, as well as for the reinforcement of the bone support with Bio Oss and Mesh Titan **(**Fig. 10 a,b,c,d,e).

The prosthesis aimed at recreating the integrity of the edentulous arch by means of a fix metallo-ceramic denture Fig. 11 (a,b,c,d,e) placed at the level of molar 2 as well as of the two premolars from quadrant II (Fig. 12 a,b,c).

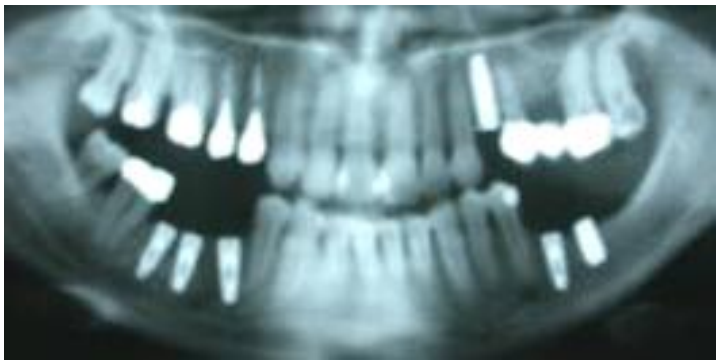


Fig. 9. Paraclinic examination

a.

b.

c.

d.

e.

Fig. 10 (a,b,c,d,e). Bio Com Wide implant 4,70X13, as well as reinforcement of the bone support with Bio Oss and Mesh Titan

a.

b.

c.

d.

e.

Fig. 11 (a,b,c,d,e). The realization of the metallo-ceramic crowns

a.

b.

c.

Fig. 12 (a,b,c). The final aspect of implanto-prosthetic rehabilitation

CONCLUSIONS

The success of the implant therapy depends on the absence of radio-transparent areas, while the per-implant bone resorption must be limited to the cervical region and should not involve the implant threads or its osteo-integrated area. The implant should also be fix, pain free, with no local infection.

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CURRENT MICROBIOLOGICAL DATA ON LOWER RESPIRATORY TRACT INFECTION IN CYSTIC FIBROSIS PART II: RECOMMENDATIONS FOR MICROBIOLOGICAL DIAGNOSIS IN CYSTIC FIBROSIS

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CURRENT MICROBIOLOGICAL DATA ON LOWER RESPIRATORY TRACT INFECTION IN CYSTIC FIBROSIS. PART II: RECOMMENDATIONS FOR MICROBIOLOGICAL DIAGNOSIS IN CYSTIC FIBROSIS (Abstract): Current standards of sputum examination in respiratory infections with opportunistic bacteria cannot be applied to patients with cystic fibrosis due to their particularities. In 2010, a working group from Great Britain established standards for microbiological processing of samples from patients with cystic fibrosis. Recommendations on sample collection, transportation, storage, and processing, identification of bacterial isolates and *in vitro* antibiotic susceptibility are made. Also recommended is that the multidisciplinary team monitoring patients with cystic fibrosis to include a microbiologist. **Key words**: CYSTIC FIBROSIS, LOWER RESPIRATORY TRACT INFECTION, OPTIMIZATION OF MICROBIOLOGICAL DIAGNOSIS.

Current standards of sputum examination in respiratory infections with opportunistic bacteria (1, 2, 3) cannot be applied to patients with cystic fibrosis (CF) due to the previously detailed particularities (4): onset of clinical manifestations in the first year of life, diverse pathogen population with different degrees of implication in the worsening of respiratory insufficiency, difficulties in identifying some of them to species level by conventional techniques.

In these circumstances, expert groups from North America and Europe became concerned with the optimization of microbiological evaluation of samples from lower respiratory tract (LRT) in CF patients as the therapeutic decision and infection control depend on it (5, 6, 7). The main recommendations in the latest guidelines drafted by a working group from United Kingdom in September 2010 (8) are presented, being of interest for all microbiologists dealing with such cases. Quality of care for these patients depends on the introduction of these rules in current practice.

Sample collection, transport, and storage

Sputum is the recommended specimen for routine investigation in patients, both at each hospital visit and during exacerbations. Sputum is of equal value as specimens taken during surgery or by bronchoscopic bronchoalveolar lavage (the golden standard for the investigation of LRT infections). In infants and small children hypo pharyngeal aspirate is recommended as it is a simple and non-invasive method. Pharyngeal exudate is not indicated because of false-positive results.

For best results, the samples have to be processed as soon as possible after collection. Any delay in processing with storage at room temperature can cause bacterial overgrowth which could mask the true pathogens. On the other hand, refrigeration at 4˚C for 2-3 hours does not significant negative consequences. Thus, if processing is delayed, refrigeration is preferable to storage at ambient temperature. .

Sample processing

Homogenization. Numerous studies recommend sputum homogenization of by mechanic disruption with glass beads or by mucolytic agents. However, the British expert group believes that there is insufficient evidence to support the requirement of using homogenization in CF.

Sputum Gram stains. Generally, this is a very important method which allows rejection of unrepresentative samples, and for the representative ones it gives quick information about morphology and staining characteristics of bacteria associated with inflammatory cells, and guides etiolology and empirical antibiotic therapy. In CF there is insufficient evidence to recommend it for routine use as a method for assessing specimen quality and to predict culture results.

Conventional culture. Unlike other respiratory infection, in CF the use of selective medium in addition to usual culture medium (blood agar, chocolate agar) is mandatory; prolonged incubation time leads to higher detection rates of three major pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex (BCC). Their presence should be reported irrespective of quantity.

For *S. aureus*, Chapman medium incubated at 35-37˚C in air and examined next day and after at least other 24 hours is recommended. *P. aeruginosa* can be isolated in good conditions on MacConkey agar incubated at 35-37^{0}C in air and examined next day and after at least other 24 hours. This medium did not give satisfactory results in the isolation of BCC. BCSA medium (***B**urkholderia **C**epacia **S**elective **A**gar*) with polymyxin, gentamicin and vancomycin has a high sensitivity and specificity for BCC as compared to other selective media (9, 10), being thus recommended. To maximize sensitivity, it should be incubated at 35-37˚C in air for 5 days, with daily culture examination.

Haemophilus influenzae is a frequent commensal in the upper airways that may be a sputum contaminant, reason why quantitative evaluations are recommended. Growth on cefsulodin chocolate agar favors the isolation by inhibitory effect against *P. aeruginosa*. Incubation 35-37^{0}C in CO_2 at 35-37˚C for 24-48 hours.

According to the guidelines, the use of selective medium for *Stenotrophomonas*

maltophilia and other Gram-negative bacilli should be considered by laboratories according to their particular clinical needs.

Culture and identification of atypical mycobacteria is a matter for specialized laboratories.

For the isolation of filamentous fungi Sabouraud medium with additional antibiotic (ciprofloxacin, amikacin, ceftazidime or polymyxin) to inhibit *P.aeruginosa* should be used. Plate should be incubated at 35-37˚C for 24-48 hours, then prolonging cultures up to 7 days at room temperature (22^0C).

There is not sufficient evidence to support the use of molecular assays for the detection of microbial agents in CF in addition to routine culture.

Identification of bacterial isolates

Phenotypic identification to species level of isolates from LRT in CF is particular challenging for non-fermentative Gram-negative bacilli. *P. aeruginosa* strains can be accurately identified by the presence of three phenotypic characters: colonies with blue-green pigmentation (pyocyanin), positive oxidase test and growth at 42˚C. Growing strains displaying unusual characters can be misidentified as other Gram-negative non-fermentative bacilli: *Achromobacter* spp, BCC, *Burkholderia gladioli, Pandoraea* spp, *Ralstonia* spp, *S. maltophilia,* with important clinical and therapeutic consequences. An "excellent" identification from API 20NE kit (BioMerieux) in conjunction with colonial appearance, oxidase test, and colistin susceptibility can be used to confirm *P. aeruginosa, S. maltophilia, and Achromobacter* spp. This does not apply to a "very good", "good", or "acceptable" result due to a significant percentage of false-positive results; in these cases molecular techniques should be used by referring laboratory. However, BCC strains suspected by phenotypic methods should be immediately reported to the clinic, and immediate measures for preventing contact with other non-infected patients should be taken.

Additional molecular typing is recommended to identify strains with epidemiogenic potential.

Susceptibility testing guidelines

Antibiotic susceptibility of strains with clinical significance should be assessed by a standard method (CLSI, EUCAST); disc diffusion provides results comparable with broth dilution method (reference method). Automated testing is not recommended for Gram-negative non-fermentative bacilli due to possible major errors.

A good correlation between susceptibility findings and clinical response was found in patients intermittently infected with *P. aeruginosa.* In patients with initial infection, early treatment with appropriate antibiotics resulted in a much slower progression to chronic infection. In chronic infections, the presence of glycocalyx containing slow-multiplication bacteria makes that antibiotic therapy guidance by *in vitro* tests to be unsatisfactory. Currently there are no specific breakpoints for this condition.

Multidisciplinary team

The microbiologist, as laboratory service provider and infection prevention adviser, should participate in the meetings of the multidisciplinary team monitoring CF patients whenever particular situations are identified.

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ESTIMATION OF DIETARY INTAKE OF CADMIUM AND LEAD THROUGH FOOD CONSUMPTION

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ESTIMATION OF DIETARY INTAKE OF CADMIUM AND LEAD THROUGH FOOD CONSUMPTION (Abstract): Cadmium and lead are toxic metals occurring in the environment naturally and from anthropogenic activities and can lead to chemical contamination of products entering in the human food chain. The consumption of polluted food is the main source of lead and cadmium intake in the non-smoking population. Lead is a heavy metal that can affect different organs and systems in humans including the peripheral and central nervous system, the gastrointestinal tract, muscles, kidneys, and the hemopoetic system. Neurological symptoms can range from fatigue, headache, and lethargy to peripheral neuropathy, severe convulsions, encephalopathy, and even coma. The direct neurotoxic actions of lead include apoptosis, excitotoxicity. Lead has been associated with impaired neurobehavioral functioning in children, decrements in intelligence quotient (IQ) while the critical effect of long-term exposure to cadmium is renal tubular dysfunction, which is irreversible; chronic renal failure is the final and severe endpoint. Cadmium is able to induce bone damage (*Itai-Itai*). The body burden of cadmium and lead depends mostly on the dietary intake of these elements. This paper aims to present a brief overview of cadmium and lead contents present in foodstuffs from different countries and the estimated dietary intake of cadmium and lead through food consumption. It has been shown that in some countries the concentrations of cadmium and lead contained in foodstuffs are higher than normal therefore the health of the people consuming them is at threat. **Key words**: CADMIUM, LEAD, DIETARY INTAKE

Toxic metals can be found as environmental contaminants, both through natural occurrence and as a result of human activities (1, 2, 3). Overmuch accumulation of toxic metals in agricultural soils from various sources, may not only result in soil contamination, but also may lead to high toxic metal uptake by crops, and thus affect food quality and safety (2, 3).

Diet is the main route of exposure to these heavy metals in the case of non-smoking population (3) therefore, Codex Committee for Food Additives and Contaminants suggested that dietary intakes of toxic metals with high public concern must be kept under surveillance and updated to identify recent dietary intakes of toxic metals in developing countries. Despite the fact that metals can change their chemical form, they cannot be degraded nor destroyed, thus, the risk evaluation of these elements through dietary intake is an important problem (4).

DIETARY EXPOSURE TO CADMIUM AND LEAD – HEALTH EFFECTS

Cadmium and lead, especially the inorganic forms, are highly toxic to humans. A large number of studies have shown that exposure through food to these toxic metals can have negative health consequences which can include anemia, renal dysfunctions, bone damage and neurobehavioral problems (1, 2).

CADMIUM. Cadmium absorption from the gastrointestinal tract is less than 5%, however the absorption can get higher if the body stores of iron, calcium or zinc are low (3, 4). Following absorption, cadmium binds to high molecular weight proteins in the blood and is transported to the liver, where it is bound to metallothionein (MT-low molecular weight, cysteine-rich protein) forming a Cd-MT complex (5).

The complex is reabsorbed in the kidney tubuli after glomerular filtration and then is stored in the kidney with a half-time of 10-30 years (2, 5). The first manifestation of cadmium nephrotoxicity is renal tubular dysfunction. The resorption function of the proximal tubules is affected, manifesting as an increase in the urinary excretion of low-molecular-weight proteins (tubular proteinuria), calciuria, glycosuria (4), and it has been detected at concentrations greater than or equal to 50 μg/g wet weight kidney cortex (6). The most suitable biomarkers for renal pathology are: urinary β2-microglobulin, α1-microglobulin and retinol-binding protein (RBP) levels.

It is also known that the ingestion of cadmium over a long period of time can cause bone damage (*Itai-Itai*). The disease is characterized by symptoms similar to those found in osteomalacia and osteoporosis combined with kidney damage (5). International Agency for Research on Cancer (IARC) has classified cadmium as being carcinogenic to human (group 1). Recently, it has been found that there is a significant positive association between cadmium intake from food and the risk of postmenopausal endometrial cancer (7). Also, it is known that cadmium can induce testicular injury because cadmium-induced oxidative stress may be responsible for some reproductive dysfunctions (reduced sperm count, reduced semen quality) (8).

LEAD. Lead is also a toxic metal which affects multiple organs and systems, including the central and peripheral nervous system, the red blood cells, the cardiovascular system and the male and female reproductive system (2, 4). Cereals, sea products and vegetables are the main sources of lead intake from foodstuffs for adults and children (9). The absorption of lead is about 3-10% in adults and up to 50% in children. The absorbed lead is transported by red blood cells, bound to thiol groups and other ligands in proteins (δ-aminolevulinic acid - the main binding ligand). Lead is transferred to soft tissues such as liver and kidney and to bone tissue, where it accumulates. In humans, the half-life of lead is approximately 30 days in blood and 10–30 years in bone (2). For children, there are a lot of studies which sustain the association between blood lead levels and impaired neurodevelopment (specifically reduction of intelligence quotient) (10) or neuropsychiatric disorders such as attention deficit hyperactivity disorder and antisocial behavior (11) at very low blood lead levels (BLLs) (2-10 μg/dl). For adults, BLLs higher than 1.9 μg/dl but lower than 10 μg/dl have been associated with increased risk for hypertension and increased cardiovascular mortality (2, 12).

Lead is known to interfere with heme synthesis inhibiting the activity of δ-aminolevulinic acid dehydratase and fer-

rochelatase, thus producing hematological effects (2). *In vitro* studies have shown that the inhibition of these 2 vital enzymes interferes with porphyrin synthesis leading to anemia (2, 13).

Cadmium and lead are known to affect the sexual maturation in children. Recently, Gollenberg et al. (14) have associated low blood lead and cadmium levels with delayed onset and progression of puberty in young girls, thus highlighting the importance of Pb and Cd as reproductive toxicants.

Therefore it should be noticed that the difference between the dietary intake of cadmium and lead by the general population and the concentrations at which toxic effects occur is very small and could be smaller for smokers (2, 3).

CADMIUM AND LEAD IN FOODSTUFFS – POLLUTION SOURCES

Both cadmium and lead are ubiquitous environmental pollutants. The most important sources of food contamination with cadmium and lead include chemical fertilizers, food processing equipment, exhaust fumes from vehicles, industrial gases, wastewater used for irrigation. Cereals, fruits and vegetables cultivated on soils polluted with toxic metals retain large enough quantities to constitute a major source of exposure to these toxics (2, 3).

CADMIUM AND LEAD CONTENT IN FOODSTUFFS

In a recent study, a committee from WHO (World Health Organization) estimated the content of cadmium and lead in some foodstuffs based on the data submitted by 30 countries. Mean concentrations of cadmium ranged between ND (not detected) and 4800 µg/kg (in shellfish and mollusks). Higher concentrations of cadmium were also found in dried vegetables, poultry offal and meat offal. High lead concentrations were determined in stimulants (coffee, tea and cocoas), vegetables, dried fruits and alcoholic beverages (4) (tab. I).

TABLE I

Cadmium and lead content in some foodstuffs (4)

Food category	No.of samples Cd / Pb	Concentration	
		Cd (µg/kg)	Pb (µg/kg)
Rice	2295 / 85	4-20	< LOD-4
Baked goods	55 / 203	ND-20	1-230
Cereals/grains, others	12637 / 5027	ND-30	< LOD-29
Roots and tubers	2319 / 1255	6-40	1-65
Fruits	6314 / 7480	1-7	< LOD-130
Dried fruits	79 / 282	3-9	6-340
Vegetables	18183 / 13402	6-100	< LOD-400
Dried vegetables	348 / -	90-1000	-
Shellfish / Mollusks	7403 / 765	10-4800	10-190
Meat and poultry muscle	20154 / 40313	8-40	3-21
Poultry offal	1224 / -	100	-
Meat offal	1406 / 73	30-500	6-42
Dairy products	9208 / 3833	ND-4	1–13
Stimulants (coffee, tea and cocoas)	3505 / 764	0,1-1800	< LOD-1030
Drinking water (bottled and tap)	21472 / -	ND-0,4	-

ND- Not Determined ; LOD – Limit of Detection

Toxic metal contents in foodstuffs cover a wide range (tab. II). The mean concentration of cadmium varies from 0.014 µg/kg wet weight in milk from Spain (15) and 650 µg/kg dry weights in Lavash bread from Iran (16). Seemingly, the mean concentrations of lead ranges from 8 µg/kg wet weight in meat (pork, beef) from Greece (17) and 746 µg/kg in sweets (candies and chewing gum) from Turkey (18). The sweets present high concentrations of cadmium and lead, therefore it represents a hazard for human health, especially for children due to high consumption of candies and chewing gum.

TABLE II

Cadmium and Lead content in foodstuffs from different countries

Country	Food category	No.of samples	Concentration		References
			Pb (µg/kg)	Cd (µg/kg)	
Turkey	Sweets	17	746	296	18, 19
	Nettle	10	249 ± 230	1 ± 1	
Iran	Bread (Lavash)	6	420[a]	650[a]	16
Greece	Rice	5	19.9 ± 12.6	9.7 ± 5.1	17
	Pork meat	4	<8.0	<1.0	
	Beef meat	3	<8.0	<1.0	
Spain	Milk	20	-	0.014±0.01	15
	Dairy products	20	-	0.61±0.2	
	Potatoes	20	-	5.77±0.10	
Egypt	Apple	6	190 ± 30[a]	50 ± 20[a]	20
	Peach	6	380 ±80[a]	10 ± 0.00[a]	
	Lettuce	6	580 ±70[a]	70± 0.00[a]	
Romania	Mushrooms	-	559	594	21, 22, 23
	Potatoes	-	5272	55	
	Carrots	120	100	30	
	Apples	-	199	8	
	Meat products	870	300	50	
	Cow milk	-	994	<LOD	
	Sheep milk	-	2027	<LOD	
	Pasteurized milk	42	2.70	<LOD	

LOD – Limit of Detection ; [a]-dried weight

ESTIMATION OF DIETARY INTAKE OF CADMIUM AND LEAD

Because of the long half-life of cadmium and lead in the human body WHO decided to withdraw the previous PTWI (provisional tolerable weekly intake) for cadmium (7 µg/kg b.w.) and for lead (25 µg/kg b.w.) and proposed that the dietary intake of cadmium should be measured over a period of one month and a provisional tolerable monthly intake (PTMI) was established at 25 µg/kg body weight (4).

Winter-Sorkina et al. (24) calculated the dietary intake of cadmium and lead in Dutch population. Mean concentrations of dietary intake were 0.14 µg /kg b.w. (body weight)/day cadmium and 0.05 µg /kg b.w./day lead in general population. Jarup et al. (5) have estimated the average dietary intake of cadmium at 15 µg/day (0.22 µg/kg b.w./day) for the Swedish population. For the Korean children, Moon et al. (25) has estimated the dietary intake of cadmium from boiled rice at 2.22±0.84 µg/day and for lead at 0.79±0.3 µg/day (tab. III).

TABLE III

Daily dietary intake of cadmium and lead in different countries

Country	Food category	Concentration (µg/day)		References
		Pb	Cd	
Egypt	Fruits	21	1.47	20
	Vegetables	30	4.67	
Turkey	Fruits	1.957	0	19
	Vegetables	43	2.785	
Iran	Bread (Lavash)	0.06	0.09	16, 26
	Vegetables	2960	70	
Spain	Milk	1.3[a]	0.43[a]	27
	Fruits	3.01 [a]	0.22 [a]	
	Vegetables	3.68[a]	1.13[a]	
	Cereals	4.94[a]	6.8[a]	
France	Bread	1.96[b]	0.16[b]	28
	Vegetables	1.18[b]	0.65[b]	
	Fruits	1.36[b]	0.1[b]	

a- dietary intake of toxic metals for adult males;

b- estimated daily intakes for adults over 15 years and more

WHO has presented a report regarding the estimation of dietary intake of cadmium and lead (table IV).

TABLE IV

Estimation of dietary intake of cadmium and lead in the world (4)

Country/ region	µg Cd/kg b.w./month	µg Pb/kg b.w./day	Country/ region	µg Cd/kg b.w./month	µg Pb/kg b.w./day
Australia	2,2 – 6,9	0,02 - 0,93	Japan	12	-
Chile	9	3 - 9	Korea	7,7	-
China	9,9	0,9 - 3,1	U.S.A	4,6	0,03 - 0,13
Europe	9,1	0.36 - 3,1			

b.w.- body weight ;

CONCLUSIONS

After pointing out cadmium and lead concentrations in many foods, different values of toxic metals have been found, depending on the food category. The data reports submitted by FAO / WHO presented that cadmium levels in food are under PTMI. However, there are some countries where dietary intake of cadmium may endanger human health. Moreover, in countries where exposure to lead through food consumption is close to the maximum tolerable limit, the main sources of pollution should be identified and various methods to reduce exposure to these toxics should be applied.

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NOUTĂȚI

HIPERTENSIUNEA ARTERIALĂ PULMONARĂ INDUSĂ DE DASATINIB

Registrul francez de hipertensiune pulmonară a permis semnalarea unor cauze noi, apărute în context iatrogen. De la aprobarea dasatinibului pentru tratamentul leucemiei cronice mieloide, în noiembrie 2006, şi până pe 30 septembrie 2010, au fost descoperite 9 cazuri de hipertensiune pulmonară precapilară, la pacienţi trataţi cu dasatinib. La patru săptămâni de la întreruperea tratamentului, s-a obţinut ameliorarea clinică, funcţională şi hemodinamică la toate cazurile, cu o singură excepţie. 2 pacienţi au necesitat tratament cu antagonişti ai receptorilor de endotelină şi unul cu blocanţi ai canalelor de calciu. După o urmărire medie de 9 luni (3 – 36 luni) pacienţii nu au obţinut remisiunea clinică şi hemodinamică: deşi ameliorarea a fost semnificativă, la nici unul dintre pacienţii urmăriţi presiunea arterială pulmonară medie nu a scăzut sub 20 mm Hg. În acest interval de urmărire, doi pacienţi au decedat, unul cu moarte subită şi celălalt cu sepsis şi insuficienţă cardiacă. Studiul atrage atenţia asupra efectului specific pe care administrarea de dasatinib l-ar putea avea asupra circulaţiei pulmonare, determinând hipertensiune pulmonară precapilară. (Montani D, Bergot E et al. Pulmonary arterial hypertension in patients treated by Dasatinib, *Circulation 2012*, 125: 2128-2137)

Corina Dima-Cozma

STUDIUL STĂRII DE NUTRIȚIE ŞI A COMPORTAMENTULUI ALIMENTAR A UNUI LOT DE PACIENȚI DIN CADRUL MEDICINII PRIMARE

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STUDY OF THE NUTRITIONAL STATUS AND EATING HABITS ON A GROUP OF PATIENTS IN PRIMARY HEALTH CARE (Abstract) **Aim**: Evaluation of medical and biological status of the study group; analyzing the nutritional status and the way of eating by food investigation; and evaluation of the knowledge about eating behavior and the role of physical activity in health. **Material and methods**: The study was conducted from 2010-2011 on a sample of 375 patients aged 18-74, patients within a family medicine cabinet in our town. The questionnaire included demographic data (age, education, social, financial situation, the belonging religious) and questions about nutrition on food groups and questions about rational knowledge about nutrition, eating behavior and the role of physical activity. **Results**: In the study group by gender distribution was 225 women (60%) and 150 men (40%) with a ratio F/M= 1.5/1 Evaluation of the nutritional status and somatometric exam revealed the following issues: 105 cases (28%) with,103 cases (27.46%), 52 cases (13.86%) 72 (19.2%) and 43 cases (11.46%).Most patients were overweight normo and 27.46% respectively 28% and is followed by those with grade II obesity (19.2%), obesity grade I (13.86%) and final grade III obesity(11.46%).The food investigation on food groups showed no significant differences between the two years of study configuring same eating behavior. **Conclusions**: Metabolic and nutritional diseases became increasingly aggressive in recent decades and obesity is a widespread disease in the population with a multifactorial etiology and with a chronic evolution. The family doctor been in the forefront of the health system can contribute to increasing the quality of life that heis monitoring the implementation of educational programs related to food andeating behavior.
Key words: RATIONAL DIET, OBESITY, EATING BEHAVIOR, EXAMINATION SOMATOMETRIC, FOOD INVESTIGATION.

Alimentația ca factor fundamental de mediu, reprezintă ştiința relațiilor biologico-medicale dintre alimente şi om, în toată complexitatea lor. Alimentația este socotită de unii autori ca fiind cel mai important factor de mediu întrucât are o influență considerabilă asupra organismului, iar efectele ei nu se observă întotdeauna imediat, ci cumulativ în timp. Viața nu poate fi concepută fără un aport exogen de nutrienți pe baza produselor alimentare ingerate, ceea ce permite încorporarea substanțelor nutritive în organism, constituind baza desfăşurării proceselor vitale. Asimilarea şi dezasimilarea continuă, prin transformarea şi metabolizarea substanțelor nutritive, oferă

materiei vii atât energia necesară întreținerii vieții în condiții de repaus şi efort, cât şi elementele materiale necesare refacerii structurilor celulare uzuale (1). Pentru desfăşurarea activității în condiții bune, organismul uman trebuie să primească zilnic o cantitate de energie şi factori nutritivi.

Alimentația rațională ca ştiință caută nu numai să apere organismul de efectele unui aport alimentar neadecvat, ci îi menține şi creşte vigoarea, performanța fizică şi intelectuală, fertilitatea, longevitatea, capacitatea de reacție şi de adaptare (2). Ingestia de alimente trebuie să fie întotdeauna suficientă pentru a asigura necesarul metabolic al organismului şi, în acelaşi timp, să nu depăşească nevoile pentru a nu cauza supraponderabilitate sau chiar obezitate. Alimentația rațională înseamnă un aport adecvat cantitativ şi calitativ conform cerințelor fiziologice.

Însuşirea unei alimentații raționale trebuie să urmărească următoarele obiective: asigurarea tuturor trofinelor necesare în cantități optime; asigurarea echilibrului şi proporției optime între trofine; realizarea unei concordanțe între nevoi şi aport; asigurarea unei bune stări de nutriție, realizată prin cunoaşterea valorii nutritive a produselor alimentare disponibile, în vederea alcătuirii unei rații complete şi echilibrate, a influenței proceselor tehnologice de preparare asupra alimentelor, precum şi a gradului de utilizare digestivă a hranei ingerate; asigurarea unei alimentații adecvate vârstei, activității profesionale, stării fiziologice ca şi în vederea creşterii rezistenței antiinfecțioase şi antitoxice a individului; alimentația trebuie să fie diversificată; asigurarea variației regimului alimentar; prevenirea nocivității alimentelor şi evitarea consumării unui produs alimentar insalubru, ceea ce presupune: cunoaşterea sursei agenților biologici şi chimici ce pot fi vehiculați de alimentație, mijloacele de prevenire, respectarea riguroasă a condițiilor igienico-sanitare pe tot circuitul alimentelor; proprietățile organoleptice ale alimentelor să corespundă deprinderilor consumatorilor; repartizarea rațională a rației alimentare în cursul zilei; asigurarea rației cu material fibros (efect enterokinetic) şi a unui volum corespunzător fiecărei mese; respectarea condițiilor fiziologice generale de alimentare: fixitatea orelor de masă, asigurarea unei ambianțe adecvate, ordinea felurilor de meniu, temperatură potrivită a alimentelor, acordarea unui timp suficient servirii mesei, etc. (4,5).

Termenul de comportament alimentar se defineşte ca un ansamblu de reacții adaptative, observabile obiectiv, prin care organismul privit ca un tot unitar, produce un răspuns la stimulii din mediu, el reprezintă procesul determinant al prizei alimentare spontane a unui individ (6,7).

Studiul nostru a avut urmatoarele obiective (i) evaluarea statusului biologico - medical al lotului de studiu (ii) analizarea starii de nutritie şi a modului de alimentație prin ancheta alimentara şi (iii) evaluarea cunoştințelor despre comportamentul alimentar şi rolul activitatii fizice in starea de sanatate.

MATERIAL ŞI METODĂ

În studiu au fost incluşi 375(28,2%) de pacienti din cei 1327 în vârstă de peste 18 ani, aflați în evidență ai cabinetului de medicina familiei care s-au prezentat la controlul medical periodic sau cu diferite acuze în perioada 1 februarie 2010 - 30 martie 2011. După semnarea consimțământului informat, subiecții au completat un chestionar şi au fost supusi unui examen clinic general.

Chestionarul include date demografice (vârstă, studii, mediul de proveniență, situație financiară, apartenănță religioasă) şi întrebări privind modul de alimentație cantita-

tiv şi calitativ, pe grupuri de alimente şi principii nutritive, şi cele referitoare la cunoştinţele legate de alimentaţia raţională, comportament alimentar şi rolul activităţii fizice. Pe lângă datele obţinute din completarea chestionarului, s-au luat în consideraţie: greutatea corporală, talia şi circumferinţa acesteia, pentru calcularea indicelui de masă corporală (IMC). În cadrul examenului clinic s-au analizat semnele şi simptomele pacienţilor, însoţit de evaluarea stării aparatelor şi sistemelor, înregistrându-se datele antropometrice şi valoarea tensiunii arteriale.

Prelucrarea datelor s-a efectuat cu ajutorul programului SPSS 16.0

REZULTATE ŞI DISCUŢII

1. *Caracteristicile lotului de studiu*. Lotul de 375 de pacienti cu o distributie pe sex de 225 (60%) femei şi 150 (40%) bărbaţi cu un raport F/M= 1,5/1. Au predominat cazurile de sex feminin provenite din mediul urban (48%), fara diferenţa semnificativă faţă de cazurile sex masculin (32%) comparativ cu distribuţia cazurilor din mediul rural (F=12% vs M=8%) (χ^2=0,09; GL=1; p=0,768).

Diferenţele procentuale în relaţie cu nivelul de instrucţie a evidenţiat: lotul de studiu cuprinde semnificativ mai multe femei cu nivel mediu de instrucţie comparativ cu bărbaţii (p=0,008); mediul de proveniență este preponderent urban la persoanele cu nivel mediu de instrucţie (p=0,008); nu s-au înregistrat diferenţe semnificative din punct de vedere statistic pe grupuri de vârstă; activitatea profesională incluzand pensionari, şomeri şi fără activitate cu nivel mediu de instrucţie (p=0,002); condiţiile socio-economice satisfăcătoare cu nivel mediu de instrucţie au fost predominante (p=0,015) (tab.I)

TABELUL 1

Diferenţe statistice în relatie cu nivelul de instrucţie, sex, mediul de proveniență şi grupul de vârstă, activitatea profesionala, conditiile socio-economice la lotul de pacienţi investigaţi

Caracteristici epidemiologice	*Nivel de instrucţie*				*Semnificaţie statistică*
	< 12 clase		*≥ 12 clase [a]*		
	n	*%*	*n*	*%*	
Sex					
Feminin	165	44,0	60	16,0	χ^2=7,0; GL=1;
Masculin	60	16,0	90	24,0	**p=0,008**
Mediu de proveniență					
Urban	31	41,3	29	38,7	χ^2=7,03; GL=1;
Rural	14	18,7	1	1,3	**p=0,008**
Grupa de vârstă					
18- 45 ani	20	26,7	11	14,7	χ^2=0,19; GL=1;
45 -75 ani	25	33,3	19	25,3	p=0,667
Activitate profesională					
Activi profesional	26	34,7	20	26,7	χ^2=9,79; GL=1;
Pensionari, şomeri şi fără activitate	27	36,0	2	2,7	**p=0,002**
Condiţii socio-economice					
Satisfăcătoare	37	49,3	8	10,7	χ^2=5,92; GL=1;
Nesatisfăcătoare	16	21,3	14	18,7	**p=0,015**

[a]pentru valorile mai mici de 10, proportiile sunt orientative

2. *Evaluarea stării de nutriție prin examen somatometric* prezintă o mare importanță pentru aprecierea stării de sănătate. S-au luat în considerare datele antropometrice: greutatea corporală, talia, circumferința taliei şi s-a calculat indicelui de masă corporală (IMC). Datele IMC au servit pentru împărțirea lotului în: normoponderali, supraponderali şi obezi. În paralel cu datele antropometrice s-a măsurat tensiunea arterială în vederea analizei acesteia în funcție de IMC şi s-a calculat circumferința taliei

Pe baza rezultatelor antropometrice obținute la cei 375 de pacienti analizati, şi prin calcularea IMC s-au evidentiat următoarele aspecte: 105 (28%) cazuri cu IMC intre 18,5-24,9 kg⁄m² (limite normale); 103 (27,46%) cu IMC intre 25-30 kg⁄m² (suprapondere); 52 (13, 86%) cu IMC = 30-33 kg⁄m² (obezitate gradul I); 72 (19,2%) cu IMC intre 35-40 kg ⁄ m²(obezitate gradul II) şi 43 (11,46%) cu IMC >40 kg⁄m² (obezitate gradul III) (fig. 1).

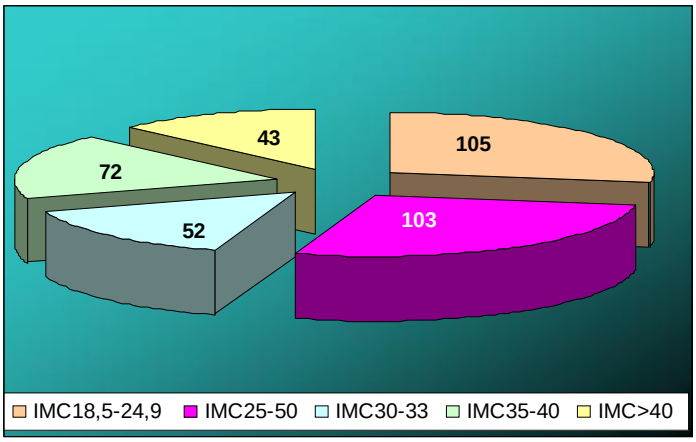

Fig. 1. Valorile IMC a pacienților studiați

Cei mai mulți pacienți sunt normo şi supraponderali, respectiv 28% şi 27,46%, fiind urmați de cei cu obezitate de gradul II (19,2%), de gradul I (13,86%) şi în final de gradul III 11,46%.

4. *Analiza chestionarului de ancheta alimentară* a evidentiat urmatoarele aspecte : ratia alimentara pe grupurile de alimente nu prezinta diferente semnificative in cei doi anii de studiu *Consumul de carne* a rezultat : pe parcursul anului 2010, procentul cel mai mare de persoane (46,40 %) consumă carne roşie de 2-3 ori pe săptămână, fiind urmate de persoanele ce consumă acest aliment o dată pe săptămână (18,60 %) şi de 5-6 ori pe săptămână (12,09 %). În anul 2011, pe primul loc s-a situat, de asemenea, consumul cărnii de 2-3 ori pe săptămână (40,60 %), urmând consumul o dată pe săptămână (22,90 %) şi pe locul 3 s-a situat consumul de 5-6 ori pe săptămână (7,30 %).

Consumul de peşte a fost de 46,40 % în 2010 şi 50,10 % în 2011 ; de 1-3 ori pe lună , urmat de o dată pe săptămână 30,90 % persoane în 2010 şi 31,10 % în 2011. A rezultat ca in lotul examinat au fost persoane care nu au consumat peste 8,80 % în

2010 şi 8,20 % în 2011 şi la polul opus s-au situat cei care au consumat de 2-3 ori pe săptămână (12,90 % în 2010 şi 10,40 % în 2011) şi chiar de 5-6 ori (1 %).

Consumul de lapte şi produse lactate a evidentiat că majoritatea persoanelor au consumat laptele şi produsele lactate o dată pe zi (41,70 % în 2010 şi 49 % în 2011), iar evaluarea consumului acestora pe săptămână arată o frecvenţă asemănătoare a consumului de 5-6 ori pe săptămână, de 2-3 ori pe săptămână şi o dată pe săptămână; în 2010 frecvenţele au fost cuprinse între 14,07 % şi 16,90 % iar în 2011 în jur de 14%.

Persoanele din lot consumă ouă cel mai frecvent o dată pe săptămână (41,20 % în 2010 şi 41,10 % în 2011), urmând consumul de 2-3 ori pe săptămână (20,40 % în 2010 şi 26,70 % în 2011), iar consumul de 5-6 ori pe săptămână se întâlneşte mai rar (1,60 %). De asemenea, nu poate fi omis faptul că există persoane care nu consumă "aproape niciodată" ouă (11,30 % în 2010 şi 7,90 % în 2011).

Consumul de fructe proaspete este deosebit de important la toate vârstele,si s-a evidentiat o frecvenţă crescută a consumului de 2-3 ori pe săptămână (33,60 % în 2010 şi 28,80 % în 2011) şi o dată pe săptămână (31,40 % în 2010 şi 31,50 % în 2011), urmând consumul de 5-6 ori pe săptămână (11,90 % în 2010 şi 10,80 % în 2011). Consumul de 1-3 ori pe lună se întâlneşte la 20,80 % dintre persoane în 2010 şi 25,80 % în 2011.

Prin evaluarea consumului de legume proaspete se constată că majoritatea persoanelor din lot consumă legume de 2-3 ori pe săptămână (32,60 % în 2010 şi 36,20 % în 2011) şi o dată pe săptămână (32,30 % în 2010 şi 28,90 % în 2011), situaţie întâlnită şi în cazul fructelor. Un procent relativ crescut se întâlneşte şi în cazul persoanelor care consumă legume proaspete de 1-3 ori pe lună (14,40 % în 2010 şi 13 % în 2011) şi un procent mai mare decât în cazul consumului de fructe se întâlneşte pentru cei care nu consumă "aproape niciodată" legume proaspete (9,70 % în 2010 şi 4 % în 2011).

S-a constatat că procente însemnate a persoanelor din lot sunt consumatoare de băuturi alcoolice de 5-6 ori pe săptămână şi de 2-3 ori pe săptămână, ceea ce reprezintă un factor de risc pentru multe boli cronice. Trebuie subliniat faptul că este foarte importantă cantitatea consumată de aceste persoane.

5. *Modul de manifestare al comportamentului alimentar* cuprinde una sau mai multe din urmatoarele forme: mese copioase care realizează un aport caloric mult mărit, pe seama tuturor principiilor nutritive, asociat de regulă cu consumul de alcool; persoane preocupate de hrană, mâncând rapid şi lacom; abuz preferenţial de lipide şi glucide; mese rare pe zi (de două ori pe zi), dar copioase. S-a invocat mai multe cauze pentru explicarea fenomenului de ingestie abuzivă a alimentelor, cele mai importante sunt: dereglarea apetitului, apariţia unei pofte de mâncare exagerate (hiperfagie), cauzată de traumatisme psihice, boli psihice sau ale sistemului nervos care afectează centrul saţietăţii din hipotalamus; ofertele crescute de hrană în cadrul unui meniu culinar familial; disponibilităţile crescute de hrană ca o consecinţă a bunăstării materiale; existenţa unui dezechilibru afectiv, cauzat de insuccese profesionale, de stări conflictuale, de lipsa satisfacţiilor emoţionale, plăcerea de "a gusta" în permanenţă "câte ceva". În aceste cazuri, hiperfagia este o modalitate de refulare; factorii hormonali care apar în circumstanţe speciale (în sarcină şi menopauză), deter-

minând creşterea apetitului (1,7).

6. *Evaluarea cunoştinţelor* de alimentație şi importanța activității fizice a evidențiat pentru pacienții incluşi în studiu că principiile unei alimentații raționale sunt insuficient cunoscute iar practicarea activității fizice este complet neglijată de pacienții din lotul de studiu.

CONCLUZII

În cercetările noastre a rezultat că există categorii populaționale care au comportamente nutriționale nesanogene. Astfel din cele 375 (28,2%) persoane aflate în asistența medicului de familie au beneficiat de un control medical periodic, cu ocazia căruia s-au realizat intervenții educaționale privind ritmul consumului săptămânal de carne, peşte, lapte şi derivate, ouă, fructe şi legume şi băuturi alcoolice.

Evaluarea cunoştinţelor de alimentație rațională a evidențiat pentru lotul inclus în studiu că principiile unei alimentații raționale, sanogene sunt insuficient cunoscute.

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PROCALCITONINA MARKER RAPID AL INFECȚIEI NEONATALE

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PROCALCITONINE - EARLY MARKER OF NEONATAL INFECTION (Abstract). Neonatal infection represents the third most important cause of mortality in Neonatal Intensive Care Units (NICU), following perinatal asphyxia and respiratory distress syndrome. The incidence varies according to the level of care, between 5% in level II neonatal wards and 20% in the NICUs. The lack of specific early markers for infection could be partially responsible for the lack of antibiotic treatment or unnecessary treatment. In this context, many variables were examined as markers for sepsis. Large concentrations of C-reactive proteine (CRP) and procalcitonine (PCT) were positively correlated with the severity of the infection and generally indicated a poor outcome. **Aim**: The aim of this study is to demonstrate the utility of PCT as a fast, early and routinely used marker of neonatal infection, correlated with CRP, clinical symptoms and blood cultures. **Materials and methods:** this is a retrospective-prospective study on 2 lots of newborns from two neonatal wards of different levels of care (Lot I – 32 newborns from the Cuza-Voda NICU in Iasi, born in 2010, Lot II – 127 newborns from the Buna Vestire Maternity in Galati, born during 2008-2009). PCT was assessed before the beginning of the antibiotic treatment in newborns with high risk of sepsis or newborns with proven sepsis as corroborated with complete blood counts, CRP, fibrinogen, clinical symptoms and blood cultures. **Results**: Among risk factors for neonatal infection, those found more often in newborns with proven sepsis are: ruptured membranes over 18 hours ($p=0.043$), Gram-negative bacilli colonization ($p=0.003$), respiratory distress syndrome ($p=0.0008$), abdominal distention ($p=0.0042$), oedema ($p<<0.05$) and necrotizing enterocolitis ($p<<0.05$). High values for PCT in premature newborns show the high risk for infection for this category of newborns, due to immunological imaturity and the presence of risk factors. High sensitivity and specificity of PCT (specificity =88.98%, sensitivity=87.5%) showed that the accuracy of PCT assessment in studied lots was 88.68% during the 24-72 hours of life time frame. **Conclusion**: High serum levels of PCT in studied lots plead for the usage of PCT for the fast and early diagnostis of neonatal infection. In those cases with positive PCT and negative blood cultures or positive CRP and clinical symptoms of infection, PCT would be a useful tool, aiding in the initiation or termination of antibiotic treatment, which would ultimately lead to lowering costs. PCT as a screening tool for cases with risk factors for infection is still to be analysed in terms of costs versus benefits and the longterm implications of neonatal sepsis and antibiotic treatment. **Key words**: NEWBORN, NEONATAL INFECTION, SEPSIS, PROCALCITONINE, C REACTIVE PROTEINE, BLOOD CULTURES

Diagnosticul infecției bacteriene la nounăscut este dificil de stabilit mai ales că din punct de vedere clinic simptomatologia este nespecifică şi se asociază de obicei cu o altă

patologie dată de factori non-infecţioşi care pot genera răspuns inflamator (asfixia nou-născutului, EHI, malformaţie de cord etc.). Testele diagnostice utilizate pentru identificarea răspunsului inflamator sistemic sunt sărace de asemenea şi confirmarea punctului de plecare a infecţiei în neonatalogie este extrem de greu de precizat.

Lipsa unor markeri precoce specifici pentru infecţie ar putea fi responsabilă parţial pentru întârzierea sau dimpotrivă instituirea fără necesitate a tratamentului antimicrobian. În acest context au fost examinate mai multe variabile ca markeri pentru sepsis. Concentraţiile mari ale CRP, procalcitoninei (PCT) au fost corelate pozitiv cu severitatea procesului infecţios indicând în general un prognostic nefavorabil.

Procalcitonina (PCT) creşte marcat în condiţii de sepsis şi pare a fi un bun indicator al severităţii infecţiei. Procalcitonina este eliberată în circulaţie în primele 3 ore de la pătrunderea endotoxinelor în organism, atinge platoul la 6h şi rămâne crescută timp de 24 h, face din acest marker un agent promiţător pentru identificarea precoce şi sensibilă a cazurilor infectate [1]. PCT nu este măsurabilă în serul indivizilor normali, însă titrul său creşte în infecţiile microbiene fungice şi parazitare, nu şi în cele virale.

Acurateţea procalcitoninei în diagnosticarea sepsisului precoce la nou-născut (infecţia bacteriană severă apărută în primele 72h de viaţă) este încă un subiect controversat.

PCT a fost propusă ca indicator al infecţiilor severe generalizate (sepsis). A fost documentat faptul că nivelurile PCT cresc odată cu severitatea răspunsului inflamator la infecţie. Mai mult, se pare că nivelul PCT se corelează cu prognosticul pacientului [2]. Scopul studiului este de a demonstra utilitatea PCT ca marker rapid precoce şi de rutină al infecţie neonatale corelat cu CRP-ul, cu simptomatologia clinică şi cu hemocultura.

MATERIAL ŞI METODE

S-a efectuat un studiu prospectiv şi retrospectiv pe 2 loturi de nou-născuţi din două maternităţi de niveluri diferite (Lot I – 32 nou-născuţi din CRTIN – Spitalul Clinic de Obstetrică Ginecologie „Cuza−Vodă" Iaşi în anul 2010, Lot II – 127 nou-născuţi din perioada 2008-2009, în Spitalul Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi). Nou-născuţii incluşi în studiu au prezentat factori de risc pentru sepsis.

În determinarea PCT s-a utilizat testul BRAHMS, test imunocromatografic pentru determinarea semicantitativă a procalcitoninei, utilizat pentru a demonstra şi controla tratamentul infecţiilor severe. Recoltarea probelor de laborator s-au efectuat înainte de tratamentul cu antibiotice, sângele recoltat s-a centrifugat şi serul obţinut (6 picături) s-a plasat pe un strip, după care s-a urmărit modificarea culorii în funcţie de standard. Citirea s-a efectuat după 30 min sau max. 40 min.

Interpretarea rezultatelor. PCT sub 0,5 ng/mL – este posibilă o infecţie bacteriană locală sau recoltarea s-a făcut precoce şi se va repeta după 6-24 ore. [3] Un interval de 0,5 şi mai mic de 2 ng/ml – este posibilă o infecţie sistemică severă, dar pot exista şi alte condiţii care cresc PCT (asfixia, traumatisme obstetricale, malformaţii congenitale de cord etc.). Între 2-10 ng/mL – este probabil o infecţie sistemică şi risc crescut de infecţie spre sepsis şi peste 10 ng/ml risc cu probabilitate înaltă de sepsis. Interpretarea valorilor PCT s-a făcut în coroborarea cu alte probe de laborator şi

semne clinice ale nou-născutului. Determinările au fost făcute în primele 72 ore. [4]

Au fost luați în studiu următorii factori de risc pentru infecție:

Factori de risc materni: MRS>18h, coriamniotita, colonizare cu streptococ de grup B-E. coli (SGB), infecție de tract urinar;

Factori de risc neonatali: naştere prematură (< 37 săptămâni), greutatea mică la naştere (<2500gr.)

Semnele şi simptomele clinice de sepsis au fost:

Semne clinice (+): detresă respiratorie (tahipnee, efort respirator etc.), distensie abdominală, drenaj gastric bilios, edem de perete abdominal, scleredem, EUN;

Semne de suspiciune de laborator: leucopenie (<5000 mm^3), leucocitoză (>30000 mm^3), CRP (>8mg %), Fb (>3,5), acidoză metabolică, icter cu BD;

Semne de certitudine: culturi pozitive din sânge.

PCT a fost determinată în situația de suspiciune clinică de infecție coroborată cu factorii de risc matern şi neonatali şi probe inflamatorii modificate (CRP, Fb, hemoleucograma). S-a făcut o singură determinare datorită cantității mici de teste şi prețul crescut al testului.

REZULTATE

Pentru a determina acuratețea PCT în diagnosticul sepsisului neonatal precoce, utilizând valori cut-off standard, s-au evaluat pe baza unei abordări probabilistice complexe corelațiile PCT cu factorii de risc materni, neonatali, semnele clinice şi semnele paraclinice ale nou-născuților din cele două loturi. A fost evaluată prevalența factorilor de risc privind cele două loturi analizate: lot I reprezentând nou-născuți cu infecție dovedită ce prezentau semne şi simptome clinice plus culturi pozitive şi lot II ce a inclus nou-născuți cu infecție suspectată ce prezentau semne şi simptome clinice, culturi negative, dar cel puțin două teste screening pozitive (CRP, HLG sau Rx).

Dintre factorii de risc implicați în infecția nou-născutului, cei ce prezintă o frecvență semnificativ mai mare la nou-născutul cu infecție dovedită sunt: MR >18h (p=0,043), colonizare cu streptococ de grup B (p=0,003), toți factorii de risc neonatali (VG, greutate mică la naştere), detresa respiratorie (p=0,0008), distensia abdominală (p=0,0042), edemul şi scleredemul (p<<0,05) şi EUN (p<<0,05).

În acest studiu un nivel seric al PCT≥0,5ng/mL a fost acceptat ca fiind patologic.

În loturile de studiu PCT pozitivă (>0,5 ng/mL) s-a asociat semnificativ (r=0,85, p=0,01) cu prezența infecției dovedite (hemocultură pozitivă). La nou-născuții ce prezentau hemoculturi pozitive s-au regăsit 87,5% valori ale PCT>0,5ng/mL, în timp ce nou-născuții la care culturile au fost negative doar 11.02% prezentau valori ale PCT>0,5ng/mL (tab. II, III).

Suspiciunea prematurului pentru infecție a fost demonstrată de faptul că frecvența nou-născuților prematuri cu infecție dovedită şi procalcitonină pozitivă (81,25%) este semnificativ mai mare (r=0,88, p=0,0012, χ^2=10,15, 95%CI) comparativ cu frecvența nou-născuților la termen cu infecție suspectată şi procalcitonină pozitivă (0,79%) (tab. IV). Valorile mari ale procalcitoninei la nou-născutul prematur demonstrează riscul prematurului pentru infecție datorită imaturității imunologice şi factorilor de risc.

TABELUL I

Prevalența factorilor de risc implicați în infecția neonatală

FACTORI DE RISC		Nou-născuți cu infecție dovedită	Nou-născuți cu infecție suspectată	r	p	
				95% Interval de încredere		
Factori de risc Materni	MRS>18h	50%	30,71%	0,578	0,0403	ss
	Coriamniotita	6,25%	4,72%	0,207	0,7242	ns
	Bacili Gram-negativi	59,38%	40,63%	0,591	0,0038	ss
	Infecție de tract urinar	25%	32,28%	0,153	0,4251	ns
Factori de risc Neonatali	Vârsta de gestație (săpt.)	87,5%	52,76%	0,826	0,0003	ss
	Greutatea la naştere (gr.)	1448,4±707,2	2635,8±913,8		0,00047	ss
Semne clinice	Detresă respiratorie	87,5%	21,26%	0,954	0,0008	ss
	Distensie abdominală	31,25%	11,02%	0,681	0,0042	ss
	Drenaj gastric bilios	6,25%	9,45%	0,126	0,5681	ns
	Edem de perete abdominal	37,5%	17,32%	0,594	0,0128	ss
	Scleredem	59,38%	33,86	0,628	0,0037	ss
	Icter	59,38%	81,89%	0,636	-0,0065	ss
	EUN clinic +radiologic	71,8%	14,17%	0,895	0,000	ss
Semne paraclinice	PCT>2ng/mL	87,5%	11,02%	0,902	0,000	ss
	CRP >8mg%	84,38%	12,02%	0,871	0,000	ss
	Leucopenie <5000 mm^3	6,25%	5,51%	0,501	0,009	ss
	Leucocitoză >30000 mm^3	24,41%	9,38%	0,734	0,006	ss
	Fb (peste 3.4g‰)	59,06%	28,13%	0,491	0,001	ss
	Bilirubina directă	3,46±7,8	1,09±0,57	0,503	0,002	ss
	Acidoză metabolică	31,25%	25,98%	0,166	0,548	ns

TABELUL II

Procalcitonina pozitivă în loturile studiate

	PCT pozitiv (>0,5ng/mL)		PCT negativ (<0,5ng/mL)		Total
	Nr, cazuri	%	Nr ,cazuri	%	
Lot I	28	87,50%	4	12,50%	32
Lot II	14	11,02%	113	88,98%	127
Total			**159**		

TABELUL III

Parametrii estimați în testarea asocierii PCT pozitiv vs. lotul de studiu

95% interval de încredere	**Chi-pătrat χ^2**	**p**
Pearson Chi-pătrat - χ^2	76,42	0,037035
M-L Chi-square	73,02	0,026856
Coeficient de corelație (Spearman Rank R)	0,85370	0,016179

TABELUL IV

Parametrii estimați în testarea asocierii PCT pozitive vs. prematuritate

95% interval de încredere	**Chi-pătrat χ^2**	**p**
Pearson Chi-pătrat - χ^2	10,15197	0,00144
M-L Chi-square	12,03849	0,00052
Coeficient de corelație (Spearman Rank R)	0,8827309	0,00128

Pentru a evidenția utilitatea procalcitoninei şi corelarea acesteia cu factorii de risc implicați în apariția infecției neonatale, s-au evaluat rezultatele corelației multiple, avându-se în vedere modificările valorilor procalcitoninei în funcție de factorii de risc.

Tabelul conține factorii de risc predictivi pentru valorile procalcitoninei în ordinea importanței lor. Pe baza rezultatelor se poate aprecia faptul că EUN (r=0,74, p=0,0001), prezența edemelor (r=0,79, p=0,0004) şi scleredemelor (r=0,69, p=0,0025), vârsta de gestație (r=0,66, p=0,0018), valorile crescute ale CRP (r=0,67, p=0,0144), MR>18h (r=0,65, p=0,028), prezența leucocitozei (r=0,62, p=0,032) şi greutatea mică la naştere (r=0,61, p=0,035) sunt factori de risc semnificativi predictivi pentru valorile pozitive ale procalcitoninei (tab. V). Rezultatele analizei multivariate sunt prezentate sugestiv în graficul următor (fig. 1).

TABELUL V

Corelația multiplă a valorilor procalcitoninei cu factorii de risc implicați în infecția neonatală

Corelația multiplă	**Valoare estimată**
Coeficient de corelație multiplă	0,852344
Multiple R^2	0,726490
F	7,802520
p (95%CI)	0,000000
Std.Err. of Estimate	2,254536

Corelație parțială PCT>2ng/ml	Coeficient de corelație (Beta)	Std.Err. (Beta)	B	Std.Err. B	t	p *95% interval de confidență*
Intercept			2.95090	4.789845	0.61607	0.540818
EUN	0,741629	0,106018	3,40026	0,816269	4,16561	0,000132
edeme	0,799004	0,131509	3,80784	1,003532	3,79444	0,000423
scleredeme	0,690642	0,098764	3,63871	1,62441	3,20781	0,002571
VG	0,661120	0,109611	2,87819	0,873620	3,29455	0,001879
CRP	0,674226	0,147314	0,02981	0,011733	2,54032	0,014439
MR>18H	0,655055	0,099850	1,66284	0,737747	2,25394	0,028903
leucocitoza	0,623525	0,121286	1,88147	0,900096	2,09030	0,032026
GN	0,611373	0,164910	0,00170	0,000899	1,88814	0,035189
detresa	0,637662	0,109152	0,56898	0,379232	1,50034	0,140215
Inf.urinara	0,885864	0,093454	0,77256	0,815011	0,94791	0,348022
Distensie abd.	0,122716	0,177449	0,13140	0,190001	0,69156	0,492618
acidoza	0,544855	0,111232	0,44055	0,899410	0,48983	0,626536
fibrinogen	0,370657	0,097807	0,00064	0,001692	0,37896	0,706425
leucopenie	0,472282	0,127563	0,00002	0,000057	0,37023	0,712872
corioamniotita	0,070331	0,087537	0,10733	1,335970	0,08034	0,936308
icter	0,075457	0,128719	0,04873	0,831378	0,05862	0,953506
Drenaj gastr.bil.	0,023744	0,102247	0,03269	1,407500	0,02322	0,981571

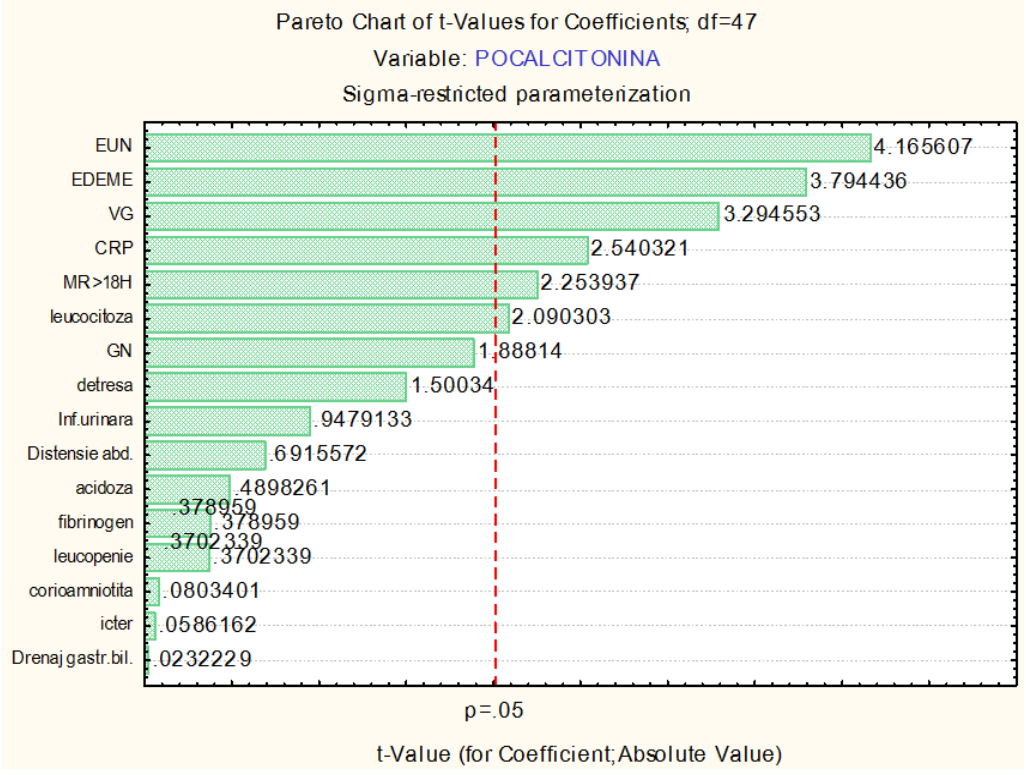

Fig. 1. *Valoare absolută a statisticii "t" în evaluarea analizei multivariate*

Corelarea multiplă a demonstrat că valori ale procalcitoninei peste 2 ng/ml s-au asociat cu infecția cu punct de plecare digestiv (EUN), cu semnele cutanate de suspiciune de infecție (edeme, scleredeme) cu vârsta de gestație mică, cu CRP, MR>18h, leucocitoză, ceea ce demonstrează utilitatea determinării procalcitoninei ca test rapid pentru evaluarea riscului de infecției şi ulterior urmărirea tratamentului.

TABELUL VI

Asocierea PCT pozitive vs. valori CRP

	CRP pozitiv (>8mg/L)		CRP negativ (<8mg/L)		Total
	Nr. cazuri	%	Nr. cazuri	%	
PCT pozitiv (>0.5ng/ml)	35	85,37%	19	16,10%	**54**
PCT negativ (<0.5ng/ml)	6	14,63%	99	83,90%	**105**
Total			**159**		

Nivelul seric al procalcitoninei la nou-născuții cu CRP pozitiv a fost peste 0.5 ng/ml în 85,37% din cazuri, iar la nou-născuții cu CRP negativ a fost de 16,1% (tab. VI). Asocierea între valorile pozitive ale CRP şi valorile pozitive al PCT este semnificativă (r=0,87, p<<0,05, 95%CI). De asemenea, se remarcă faptul că nivelul PCT creşte mai rapid în infecția neonatală decât nivelul CRP (r=0,56, p=0,0067, 95% CI) (fig. 2).

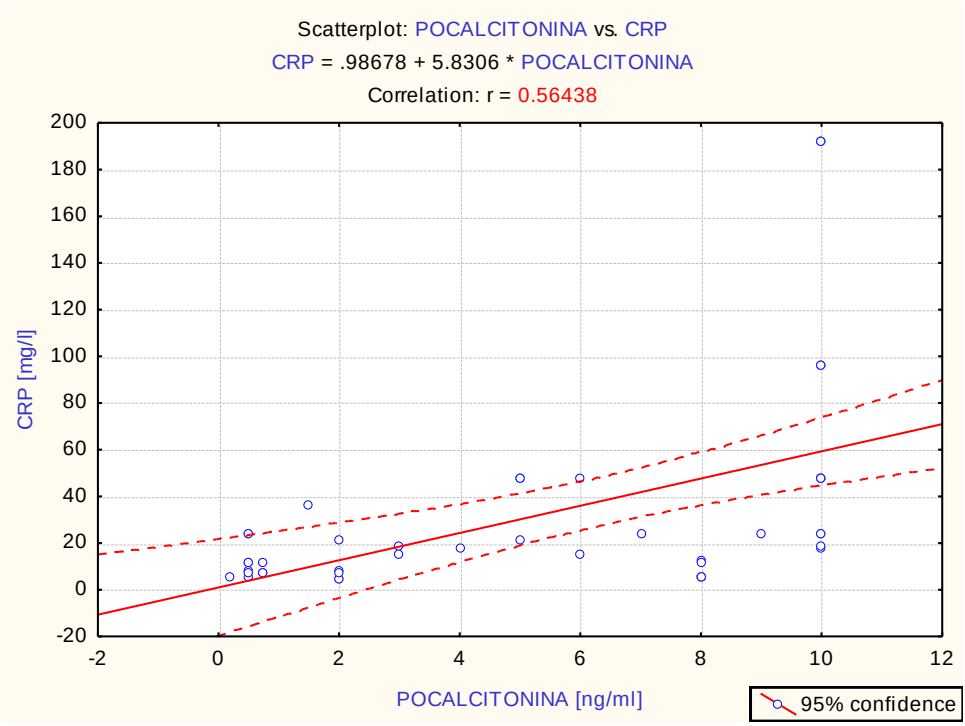

Fig. 2. *Dreapta de regresie PCT vs. CRP*

Utilizând hemocultura ca metodă gold standard, s-au calculat sensibilitatea, specificitatea, valorile predictiv pozitive şi predictiv negative şi acurateţea nivelului PCT pentru infecţia neonatală. Astfel, sensibilitatea şi specificitatea mare a procalcitoninei (specificitate=88,98%, sensibilitate=87,5%) demonstrează că acurateţea determinării procalcitoninei în loturile de studiu a fost de 88,68% în intervalul 24-72 ore de viaţă.

CONCLUZII

Nivelurile serice crescute ale PCT în loturile studiate pledează pentru utilizarea PCT în stabilirea rapidă şi precoce a diagnosticului de suspiciune şi sepsis neonatal.

În cazurile cu PCT pozitive şi hemoculturi (-) sau CRP (+) şi semne clinice de infecţie PCT poate fi un test util în iniţierea sau întreruperea antibioterapiei, ceea ce ar duce la scăderea costurilor.

Utilizarea PCT ca şi test screening la cazurile cu risc de infecţie, datorită costului ridicat al investigaţiei necesită studii privind raportul cost/beneficiu şi implicaţiile pe termen lung ale sepsisului nou-născuţilor şi al antibioterapiei.

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OBSERVAŢII EPIDEMIOLOGICE LEGATE DE SUPRAVIEŢUIREA ÎN PRIMUL AN DUPĂ INFARCT MIOCARDIC ACUT CU SUPRADENIVELARE DE SEGMENT ST (STEMI) TRATAT PRIN ANGIOPLASTIE PRIMARĂ

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EPIDEMIOLOGICAL OBSERVATIONS ON SURVIVAL IN THE FIRST YEAR AFTER ACUTE ST-ELEVATION MYOCARDIAL INFARCTION (STEMI) TREATED BY PRIMARY ANGIOPLASTY (Abstract): Conditional analysis of survival factors after acute myocardial infarction is important in care practice. We analyzed the survival and occurrence of coronary ischemic events during the first year after the episode of acute myocardial infarction with ST segment elevation (STEMI). We prospectively evaluated at one month and one year the patients with STEMI assisted in November 2008 - February 2010 at Heart Institute "Niculae Stăncioiu" with primary coronary angioplasty (ACP) performed during the first 12 hours of the onset of pain. We analyzed statistically survival and occurrence of ischemic events. Survival rate was 95.6% (CI95%: 92.7 to 97.3) at one month and 92.1% (CI95%: 87.8 to 94.2) ate one year after STEMI. Survival was significantly reduced in anterior STEMI (83.4%), history of ischemic heart disease (83.4%), stroke (81.2%), females (81.8%) and ischemia exceeding > 6 hours (83.6%). The rate of ischemic events avoidance was 96.5% (CI95%: 0.938 to 0.980) at one month and 81.7% (CI95%: 76.7 to 85.6) at one year after STEMI. The ischemic events, as evaluated through avoidance rate of phenomena, were significantly more frequent in females (69.9%), dyslipidemic (72.9%), hypertensive (73.7%) patients, having history of ischemic heart disease (72.4%) and total ischemia time longer than 6 hours (70%). Evolution of patients with STEMI after ACP is significantly better in patients with non-anterior location, males, without history of coronary disease, stroke, hypertension, dyslipidemia, ischemic time > 6 hours. **Keywords**: STEMI, PRIMARY CORONARY ANGIOPLASTY, SURVIVAL.

Bolile cardiovasculare cauzează peste 12 milioane de decese anual în lume (raportul OMS 1991-93), iar boala cardiacă ischemică reprezintă principala cauză de deces la adult. Peste 25% din decesele de cauză cardiovasculară se datorează infarctului miocardic acut (IMA), dar cifrele pot subestima realitatea (1). Supravieţuirea după un episod de IMA şi evenimentele apărute post-STEMI reprezintă o preocupare permanentă a cardiologilor. Cunoscând factorii care pot influenţa evoluţia acestor pacienţi şi acţionând în consecinţă, se poate îmbunătăţi prognosticul.

Fatalitatea în prima lună după un infarct miocardic acut este de aproximativ 50%,

dintre care aproximativ 50% se produc în primele 2 ore (1). Mortalitatea înaltă prespitalicească din primele ore nu a suferit modificări importante în ultimii ani. Mortalitatea intraspitalicească avea incidenţă de aproximativ 16% în era pre-reperfuzie, dar mortalitatea la o lună s-a redus la 4-6% după introducerea terapiei cu fibrinolitice şi a intervenţiilor coronariene percutane (2).

Trialurile randomizate efectuate în ultimii 20 de ani pe pacienţi cu STEMI, mortalitate pe termen scurt (la 30 de zile de la debut) cu o tendinţă de evoluţie încurajatoare: aproximativ 6-7% (trialul GUSTO-I raportează mortalitate la 30 de zile post-STEMI de 6,9%) (3). În schimb, mortalitatea raportată de registrele STEMI variază între 15-20%. Rezultatele trialurilor nu pot fi aplicate în practica clinică la aproximativ 50% din pacienţi, deoarece pacienţii incluşi în studiile randomizate sunt selectaţi, fiind la risc mai mic decât cei din practica de zi cu zi (4, 5).

Puţinele date existente referitoare la mortalitatea prin IMA în România arată că ţara noastră se situează printre ţările cu cea mai ridicată fatalitate prin IMA din Europa, STEMI fiind o problemă majoră de sănătate comunitară.(4, 6, 7, 8, 9)

Tratamentul STEMI urmăreşte restabilirea promptă şi cât mai completă a fluxului coronar prin una din cele două metode de reperfuzie disponibile: angioplastia primară, respectiv trombofibrinoliza (10). Succesul terapeutic depinde de timpul total de ischemie (timpul de la debutul durerii toracice până la momentul revascularizaţiei, TTI), deoarece unica variabilă asupra căreia se mai poate acţiona în evoluţia pacienţilor cu STEMI (IMA cu supradenivelare de segment ST) este timpul (11, 12, 13, 14). Factorii de risc ai cardiopatiei ischemice şi unele boli coexistente pot influenţa supravieţuirea acestor pacienţi şi frecvenţa evenimentelor post-STEMI.

Scopul lucrării a fost analizarea supravieţuirii şi a evenimentelor medicale coronariene apărute în primul an după episodul acut de STEMI la pacienţii trataţi de urgenţă prin angioplastie coronariana primară percutanată.

MATERIAL ŞI METODĂ

Am analizat supravieţuirea şi evenimentele coronariene care apar în primul an după angioplastia primară transcutanată la pacienţii cu IMA cu supradenivelare de segment ST (STEMI) prin supravegherea prospectivă a cazurilor.

Criteriile de includere a cazurilor au fost: asistarea la camera de urgenţă a Institutului Inimii din Cluj-Napoca, însoţită de terapia de reperfuzie miocardică prin angioplastie primară în primele 12 ore de la debutul durerii precordiale, capacitatea pacientului sau a aparţinătorilor de a da relaţii despre episodul actual de STEMI, acceptul de participare la studiu. Nu au fost incluşi: pacienţii de la care nu s-au putut culege informaţii despre evenimentele petrecute între debutul durerii şi internarea în secţia specializată, cei care au refuzat participarea la studiu şi pacienţii cu infarct miocardic sechelar.

Eşantionul a fost format din cazurile care au corespuns criteriilor de includere stabilite, care s-au prezentat în serviciul de urgenţă al Institutului Inimii „Niculaie Stăncioiu” în perioada noiembrie 2008 – februarie 2010. Fiecărui caz selectat i s-a completat o fişă de culegere de date, de concepţie originală, în care s-au înregistrat: datele personale, antecedente personale patologice (prezenţa unor factori de risc coronarian: statusul de fumător, prezenţa în antecedente a diagnosticelor de hipertensi-

une arterială, diabet zaharat, dislipidemie, accident vascular cerebral sau cardiopatie ischemică), date privind internarea, date diagnostice, durata asistenţei în alte servicii medicale anterior internării, date privind intervenţia de reperfuzie.

Pentru realizarea supravegherii prospective, tuturor pacienţilor li s-a solicitat prezentarea în serviciul de cardiologie pentru reevaluare la 30 de zile şi la 12 luni după episodul acut. Datele culese la reevaluare au fost înregistrate în acelaşi formular de supraveghere, incluzând relaţii despre angorul recurent, reinternările pentru angor, reinfarctizarea, insuficienţa cardiacă gravă sau decesul. Culegerea tuturor datelor necesare s-a încheiat în februarie 2011.

Baza de date pentru analizarea evoluţiei în primul an după STEMI, a fost realizată în programul informatic Excel, iar prelucrarea statistică în *Epi Info*.

Analiza statistică a urmărit rata de supravieţuire a pacienţilor, rata de apariţie a evenimentelor ischemice cardiace şi relaţia acestora cu factorii de risc urmăriţi, precum şi timpul total de ischemie. În prelucrarea statistică a parametrilor de studiu s-a folosit metoda Kaplan Meier, metoda actuarială şi metoda Log Rank pentru analizele comparative.

REZULTATE

În perioada noiembrie 2008 – februarie 2010, pe baza criteriilor de selectare stabilite, au fost incluşi în studiu 321 de pacienţi cu STEMI. Pacienţii au avut vârsta medie de 59,9 ani (între 24 şi 88 ani), 67,6% (IC95%: 62,1-72,6; p<0,001) au provenit din mediul urban, 73,8% din cazuri (IC95%: 68.6-78.5; p<0,001) au fost de gen masculin; sunt caracteristici semnificative statistic ţinând cont de structura populaţiei din regiune (14).

În prima lună după STEMI, în eşantionul studiat au apărut 14 decese, corespunzător unei rate de supravieţuire de 95,6% (IC95%: 92,7-97,3) (tab. I). La un an de la STEMI, rata de supravieţuire a fost de 92,1% (IC95%: 87,8-94,2), în total înregistrându-se un număr de 24 de decese. Pe parcursul studiului 58 de pacienţi (17,5%) au fost pierduţi din supraveghere.

TABELUL I

Ratele de supravieţuire la o lună şi la un an după infarctul miocardic acut.

Interval de timp (zile)	Vi	Di	Li	Ni	S(ti+1/ti)	S(ti+1)	IC
[0, 30]	321	14	0	321	0,956	0,956	0,927-0,973
[31, 365]	307	10	58	278	0,964	0,921	0,878-0,942

Vi :numărul de subiecţi vii la începutul intervalului; Di: numărul de subiecţi decedaţi în intervalul de timp; Li: numărul de subiecţi vi la ultimele noutăţi, la care timpul de participare se opreşte în intervalul de timp stabilit; Ni: numărul de subiecţi la risc; S(ti+1/ti): rata supravieţuire la finele intervalului; S(ti+1): rata cumulată de supravieţuire; IC: intervale de încredere.

Evenimentele ischemice sub formă de angor recurent şi reinfarctizare au fost evitate cu o rată cumulativă de 96,5% (IC95%: 93,8-98) în prima lună de evoluţie după STEMI şi de 81,9% (IC95%: 76,7-85,6) în primul an după STEMI (tab. II).

TABELUL II

Ratele de apariţie a evenimentelor ischemice coronariene la o lună şi la un an după infarctul miocardic acut.

Interval de timp (zile)	FIi	ISCHi	Li	Ni	S(ti+1/ti)	S(ti+1)	IC
[0, 30]	321	11	0	321	0,965	0,965	0,938-0,980
[31, 365]	310	43	58	281	0,846	0,817	0,767-0,856

FIi: numărul de subiecţi la care s-a montat stent şi sunt fără fenomen ischemic la începutul intervalului; ISCHi: numărul de subiecţi care au prezentat un fenomen ischemic în intervalul de timp; Li: numărul de subiecţi fără fenomen ischemic la ultimul control, la care timpul de participare se opreşte în intervalul de timp stabilit; Ni: numărul de subiecţi la risc; S(ti+1/ti): rata de evitare a fenomenelor ischemice la finele intervalului; S(ti+1): rata cumulată de evitare a fenomenelor ischemice; IC: intervale de încredere.

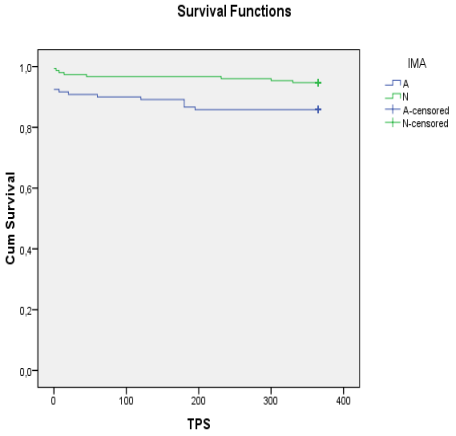
Supravieţuirea la un an a fost semnificativ mai redusă la pacienţii cu infarct miocardic localizat anterior (rata de supravieţuire de 83,4% [75,6-89,1]; p: 0,011 < 0,05), la cei cu antecedente de cardiopatie ischemică (rata de supravieţuire 83,4% [75,4-89,2]; p: 0,003 < 0,05) sau accident vascular cerebral (rata de supravieţuire 81,2% [64,6-91,1]; p: 0,04 < 0,05), la pacienţii de gen feminin (rata de supravieţuire 81,8% [70,8-89,2]; p: 0,016 < 0,05) şi la cei la care timpul total de ischemie (TTI) a fost mai mare de 6 ore (rata de supravieţuire 83,6% [75,6-89,3] ; p: 0,009 < 0,05) (tab. III, fig. 1). Nu s-au identificat diferenţe semnificative ale supravieţuirii pacienţilor care prezentau diabet zaharat, hipertensiune arterială, dislipidemie, erau fumători şi cei care au provenit din mediul rural.

TABELUL III

Ratele de supravieţuire la un an după STEMI în funcţie de caracteristicile pacienţilor, de localizarea infarctului şi de durata de ischemie până la reperfuzare.

Caracteristici	Rata de supravieţuire la un an (%)	Intervale de încredere (95%)	Semnificaţia statistică (p)
IMA anterior	83,4	75,6-89,1	0,011 < 0,05
IMA nonanterior	94	88,9-96,8	
CI în antecedente	83,4	75,4-89,2	0,003 < 0,05
Fără CI în antecedente	93,7	88,8-96,5	
AVC în antecedente	81,2	64,6-91,1	0,04 < 0,05
Fără AVC în antecedente	90,6	86,2-93,7	
Gen feminin	81,8	70,8-89,2	0,016 < 0,05
Gen masculin	92	87,4-94,9	
TTI sub 6 ore	93,6	88,7-96,5	0,009 < 0,05
TTI peste 6 ore	83,6	75,6-89,3	

IMA: infarct miocardic acut; CI: cardiopatia ischemică; TTI: timp total de ischemie.



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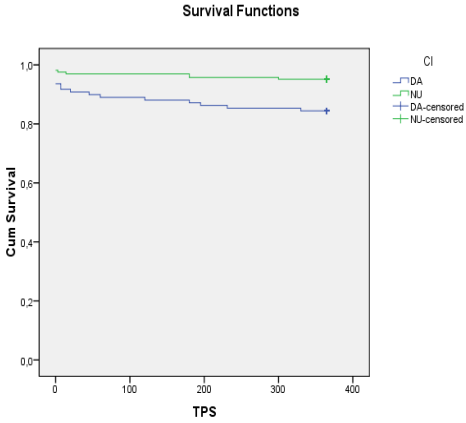

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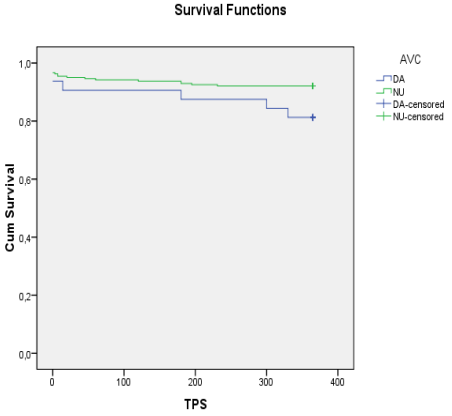

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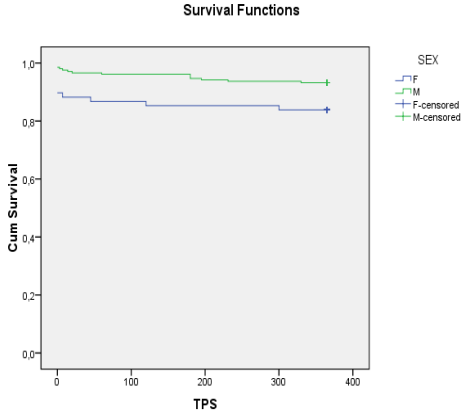

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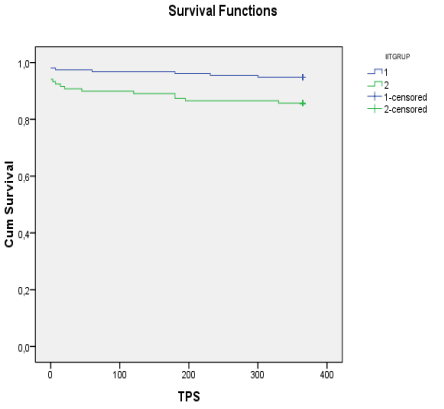

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Fig. 1. Curbele de supravieţuire funcţie de localizarea infarctului (a), prezenţa cardiopatiei ischemice în antecedentele pacienţilor (b), prezenţa accidentului vascular cerebral în antecedentele pacienţilor (c), gen (d) şi timpul total de ischemie mai mare de 6 ore (e).

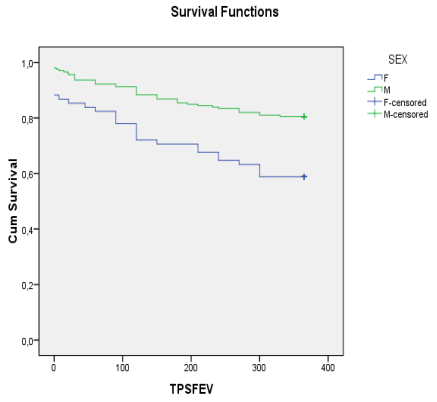
Evenimentele ischemice coronariene în primul an după infarctul miocardic au fost mai frecvente în mod semnificativ la pacienţii de gen feminin (rata de evitare a ischemiei la 69,6% [57,1-79,4].; p: 0,001< 0,05), la cei cu dislipidemie (rata de evitare a ischemiei la 72,9% [65,2-79,2]; p: 0,008 < 0,05), cu hipertensiune arterială (rata de evitare a ischemiei la 73,7% [66,7-79,6]; p: 0,018 < 0,05), cu antecedente de cardiopatie ischemică (rata de evitare a ischemiei la 72,4% [63,1-79,9]; p: 0,03 < 0,05) şi la cei la care TTI a fost mai mare de 6 ore (rata de evitare a ischemiei la 70% [60,5-77,7]; p: 0,003 < 0,05) (tab. IV, fig. 2). Frecvenţa evenimentelor ischemice coronariene nu a fost diferită la pacienţii cu infarct miocardic localizat anterior, la cei care prezentau diabet zaharat, cei care aveau accident vascular cerebral în antecedente, erau fumători şi cei care au provenit din mediul rural.

TABELUL IV

Ratele de evitare a evenimentelor ischemice în primul an după STEMI în funcţie de caracteristicile pacienţilor şi de durata de ischemie până la reperfuzare.

Caracteristici	Rata de evitare a evenimentelor ischemice la un an (%)	Intervale de încredere (95%)	Semnificaţia statistică (p)
Gen feminin	69,6	57,1-79,4	0,001
Gen masculin	82,1	76,2-86,8	(< 0,05)
Dislipidemie în antecedente	72,9	65,2-79,2	0,008
Fără dislipidemie	87,6	80-92,4	(< 0,05)
HTA în antecedente	73,7	66,7-79,6	0,018
Fără HTA în antecedente	89,8	81,6-94,5	(< 0,05)
CI în antecedente	72,4	63,1-79,9	0,03
Fără CI în antecedente	83,6	76,9-88,5	(< 0,05)
TTI sub 6 ore	85,4	78,9-90,1	0,003
TTI peste 6 ore	70	60,5-77,7	(< 0,05)

HTA: hipertensiune arterială; CI: cardiopatia ischemică; TTI: timp total de ischemie.



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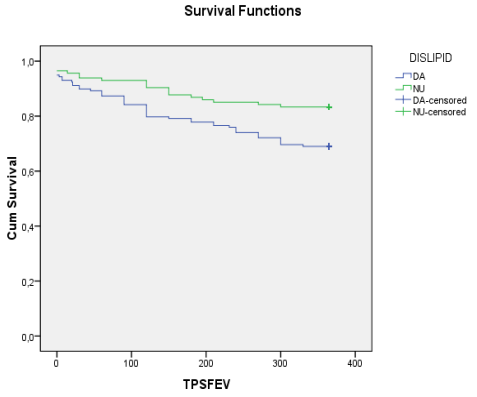

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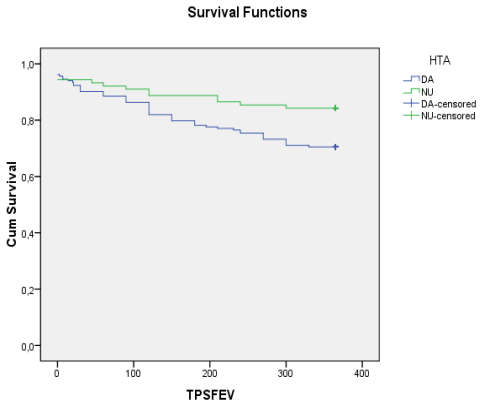

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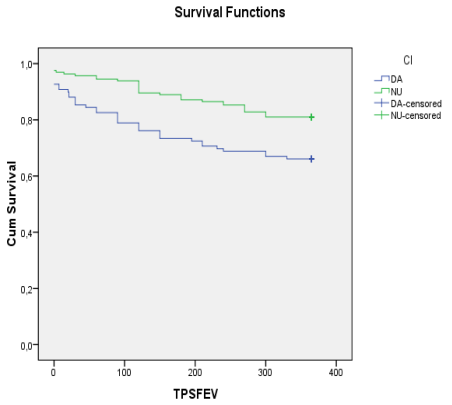

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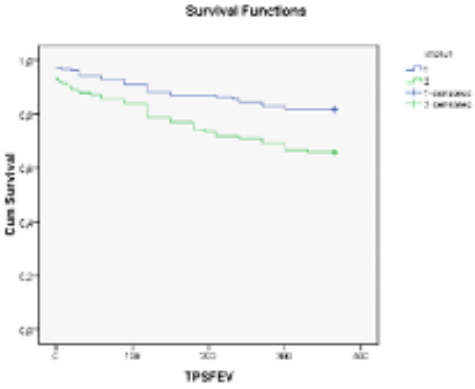

e

Fig. 2. Curbele de supravieţuire privind apariţia unui eveniment ischemic coronarian funcţie de gen (a), dislipidemie (b), hipertensiune arterială (c), cardiopatia ischemică în antecedentele pacienţilor (d) şi timpul total de ischemie (e).

DISCUŢII

Mortalitatea în prima lună post-STEMI la lotul studiat este de 4,4%, ceea ce corespunde cu datele statistice publicate in literatura de specialitate: trialul GUSTO-I raportează mortalitate la 30 de zile post-STEMI de 6,9%. Mortalitatea la un an post-STEMI al eşantionului studiat este de 7,9%, cifră care de asemenea se plasează în marja de 5-10% publicată în literatură (3).

Trei din cele zece variabile urmărite au influenţat statistic semnificativ atât supravieţuirea până la deces, cât şi până la evenimentele urmărite în primul an post-STEMI: genul feminin, TTI peste 6 ore, respectiv prezenţa diagnosticului de CI în antecedentele pacientului. TTI mai lung presupune o pierdere mai mare de miocard viabil (13, 14, 16). Diagnosticul de CI în antecedente presupune o evoluţie mai prelungită a bolii cardiace ischemice, cu afectare coronariană mai extinsă.

IMA de perete anterior ventricular stâng (VS) este considerat mai grav ca evoluţie deoarece scoate din funcţie o cantitate mai mare de miocard şi predispune mai des la

dezvoltarea de anevrism VS (5, 11, 16).

Genul feminin influenţează negativ evoluţia pacienţilor post-STEMI, cunoscându-se faptul ca femeile dezvoltă mai rar IMA, dar prezintă forme mai grave (17, 18).

AVC antecedent la un pacient care suferă un STEMI presupune existenţa unei afectări vasculare cu localizări multiple, fiind deci un bolnav cu ateroscleroză difuză, ceea ce explică evoluţia lor grevată de complicaţii.

Contrar aşteptărilor, DZ şi fumatul nu au influenţat semnificativ rata apariţiei evenimentelor ischemice cardiace la pacienţii din prezentul studiu. Această constatare se poate datora faptului că s-au luat în studiu doar pacienţii cu STEMI care au fost asistaţi în timp util de la debutul simptomelor (sub 12 ore). Se cunoaşte că bolnavii cu DZ prezintă mai des forme oligo- sau chiar asimptomatice de IMA, ceea ce contribuie la întârzierea apelării la un serviciu specializat (19, 20) Tot aceasta ar explica şi numărul mai mic de diabetici cu STEMI incluşi în studiu.

Evenimentele ischemice în primul an post-STEMI, înregistrate în special la persoane de gen masculin, cu dislipidemii şi hipertensiune arterială, sunt factori de risc independenţi pentru cardiopatia ischemică. Prezenţa lor determină evoluţia bolii de bază (ateroscleroza coronariană), explicând totodată apariţia evenimentelor coronariene post-STEMI urmărite in studiu şi prognosticul mai rezervat al acestor pacienţi.

Limitele studiului realizat rezultă din culegerea anamnestică a datelor legate de timpul de ischemie şi de componentele acestuia, de la pacienţi sau aparţinători ai acestora, deci pot conţine inexactităţi asociate impactului psihologic aferent evenimentului acut de STEMI. De asemenea, în cadrul reevaluărilor, datele culese (adeseori telefonic) despre eventuala prezenţă a insuficienţei cardiace grave, aprecierea dispneei (subiectivă pe baza relatărilor pacientului), pot fi surse de bias.

CONCLUZII

Supravieţuirea în primul an post-STEMI tratat prin angioplastie primară este influenţată semnificativ de localizarea STEMI pe peretele anterior al VS, apartenenţa la genul feminin, TTI mai mare de 6 ore, prezenţa diagnosticului de cardiopatie ischemică sau accident vascular cerebral în antecedente. Implicit aceste caracteristici sunt factori de risc pentru decesul prin STEMI.

Evenimentele ischemice post-STEMI sunt influenţate negativ semnificativ de genul feminin, TTI peste 6 ore, prezenţa hipertensiunii arteriale, a dislipidemiei şi cardiopatiei ischemice în antecedentele pacienţilor. Aceşti factori pot fi consideraţi factori de prognostic negativ în evoluţia pacienţilor cu STEMI.

Educarea pacienţilor coronarieni şi informarea explicită a populaţiei generale privind importanţa vitală a recunoaşterii simptomelor precoce ale IMA şi a solicitării consultului medical precoce sunt esenţiale pentru ameliorarea prognosticului imediat şi de durată în STEMI.

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LAMBOUL MUSCULOCUTANAT PLATISMA ÎN RECONSTRUCȚIA DUPĂ PAROTIDECTOMIA EXTINSĂ

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THE PLATYSMA MYOCUTANEOUS FLAP (PMF) FOR RECONSTRUCTION OF DEFECTS AFTER EXTENDED PAROTIDECTOMY (Abstract). In order to reestablish the facial symmetry after the tumor removal, we can use sternocleidomastoid muscle transposition, dermis-fat graft, superficial musculoaponeurotic system (SMAS) plication, temporoparietal fascia flaps, microvascular free flaps and the platysma muscle flap. The technic depends on the size of the defect. MATERIAL AND METHODS. In this study we present our results of using a superiorly based platysma myocutaneous flap for reconstruction of defects after extended parotidectomy. The patients were treated for malign tumor of parotid gland which invaded the skin. RESULTS. All flaps were readily harvested at the time of tumor resection. Only two patients presented venous congestion. No flap was lost. There was no morbidity of donor site. The cosmetic outcome was satisfactory. DISCUSSION. The platysma myocutaneous flap is an alternative to more complex reconstructive technics, such as free flap transfer, and gives a satisfactory cosmetic result. CONCLUSIONS. The platysma myocutaneous flap is easy to harvest and has low donor site morbidity. The main disadvantage is represented by the vascularity problems. KEYWORDS. PAROTIDECTOMY, PLATYSMA, FLAP, RECONSTRUCTION.

Prima parotidectomie a fost raportată de către Bernard în 1823. De atunci au apărut numeroase modificări ale tehnicilor de îndepărtare a tumorilor parotidiene, de la simpla enucleere până la parotidectomia totală, cu sacrificarea sau prezervarea nervului facial (1). Parotidectomia este realizată pentru afecțiuni benigne, maligne primare sau secundare ale glandei parotide. Variantele de tratament chirurgical includ parotidectomia superficială, totală sau radicală, în funcție de tipul histopatologic şi extensia tumorii (2). Complicațiile postoperatorii cele mai frecvente sunt: pareza de facial, modificarea conturului feței, Sindromul Frey, tulburări de sensibilitate ale lobului urechii, hematoamele, sialocelele şi infecția plăgii (3, 4). Îndepărtarea unei tumori maligne de glandă parotidă implică uneori rezecții ample cu pierderi importante de substanță, însoțite de sacrificarea nervului facial, care determină tulburări funcționale şi cosmetice semnificative (5). Nitzan (6) a propus un chestionar adresat pacienților cu tumori benigne şi maligne în care erau evaluate postoperator durerea,

modificarea fizionomiei şi sindromul Frey. Dintre aceştia 58% s-au plâns asupra anomaliilor de contur facial.

Parotidectomia superficială sau totală, care nu este urmată de reconstrucție, determină o înfundare a regiunii preauriculare şi retromandibulare. De aceea, pentru a reface simetria facială sunt folosite mai multe tehnici de plastie: lamboul muscular din sternocleidomastoidian (7), din muşchiul digastric (8), sistemul musculoaponevrotic superficial (SMAS) (3), grefa de grăsime autologă (9), grefă din *fascia lata* (10), lamboul fascial temporoparietal (11), lambou liber parascapular deepitelizat (12), lambou musculocutanat paltisma (13) şi plasarea de implante sintetice (14). Pentru defectele mari, consecință a parotidectomiilor extinse, pierderea conturului facial necesită tehnici reconstructive complexe care să refacă estetica pacienților şi care, tot odată, să reducă la maximum complicațiile radioterapiei.

Transferul liber de țesuturi în reconstrucția defectelor extinse parotidiene, mastoidiene şi a tegumentelor adiacente (15) este din ce în ce mai folosit. Unii autori recomandă utilizarea lambourilor microvasculare şi în plastia defectelor parotidiene mai puțin extinse (16, 17).

Un interes deosebit îl poate prezenta şi folosirea lamboului musculocutanat platisma în reconstrucția defectelor postparotidectomie extinsă.

MATERIAL ŞI METODĂ

În prezentul studiu am inclus un număr de 6 pacienți care au fost operați în perioada 2006 – 2010 în Clinica de Chirurgie Orală şi Maxilofacială Iaşi pentru tumori maligne parotidiene şi la care reconstrucția s-a efectuat cu lambou musculocutanat platisma. Raportul bărbați/femei a fost de 5/1, cu vârste cuprinse între 59 şi 70 ani. În 4 cazuri examenul anatomopatologic a evidențiat carcinom scuamocelular, iar în două situații a fost carcinom adenoid chistic. La toți bolnavii s-a practicat parotidectomie totală extinsă cu îndepărtarea tegumentului invadat de tumoră, iar în 4 cazuri şi a unei părți din urechea externă (fig. 1), cu sacrificarea nervului facial infiltrat integral sau parțial.

Fig. 1. Pacient cu carcinom scuamocelular parotida stângă cu paralizie facial şi invazia lobului urechii şi a regiunii parotidiene

La fiecare pacient a fost efectuată şi evidarea ganglionară laterocervicală ipsilaterală modificată. Nici un bolnav nu a urmat radioterapie pre sau postoperatorie. Pentru reconstrucție s-a utilizat lamboul musulocutanat platisma pediculat cu pedicul superior.

REZULTATE

Toate lambourile au fost recoltate în momentul curei chirurgicale, iar evidarea ganglionară efectuată a fost uşurată deoarece, după ridicarea lambourilor regiunii cervicale grupurile ganglionare au fost

expuse larg, nemaifiind nevoie de incizii suplimentare.

Imediat postoperator 2 lambouri au prezentat congestie venoasă care s-a remis în 72 de ore. Un singur caz a avut necroză marginală de sub un cm pentru care s-a efectuat debridare şi cicatrizare secundară, cu efecte cosmetice satisfăcătoare. Nici un pacient nu a avut necroză totală a lamboului, iar rezultatul estetic a fost mulţumitor. Nu a existat morbiditatea zonei donatoare la nici un bolnav (fig. 2,3).

Fig. 2. Aspect postoperator cu paralizia nervului facial.

Fig. 3. Absența morbidității zonei donatoare şi a necrozei marginale.

Doi pacienţi au prezentat lagoftalmie paralitică şi nu au urmat tratament chirugical pentru corectarea acesteia prin implantarea unei plăcuțe din aur în pleoapa superioară ori prin blefarorafie definitivă datorită refuzului şi slabei cooperări ale acestora. Au preferat metodele conservative de tratament simptomatic, protector, care a constat în umezirea corneei şi protejarea acesteia de traumatismele locale. Bolnavii au folosit în acest scop lacrimi artificiale şi unguente oftalmice.

DISCUȚII

Parotidectomia extinsă determină alterări semnificative ale esteticii faciale. Calea de abord şi planul de tratament vor ține cont următorii factori: de localizarea, extensia şi tipul histopatologic al tumorii (18). În trecut, când o leziune malignă invada zone greu accesibile, precum fosa infratemporală sau baza craniului, acestea erau declarate inoperabile din cauza abordului dificil, a riscului hemoragic crescut şi a rezultatelor estetice şi funcționale slabe (18). În aceste cazuri, analiza preoperatorie a datelor obținute prin CT şi RMN creează o imagine tridimensională a poziției, volumului şi gradului de extensie al tumorii.

În cazul tumorilor extinse poate fi necesară excizia conductului auditiv, rezecție de os temporal, ram ascendent al mandibulei şi îndepărtarea tegumentelor infiltrate tumoral. Dacă se ia decizia sacrificării nervului facial, iar reconstrucția cu grefon nervos nu este fezabilă, trebuiesc luate măsuri de reabilitare a paraliziei de facial cât mai repede posibil.

În cazul parotidectomiei totale sau radicale, tehnicile locoregionale de reconstrucție şi refacere a simetriei faciale au anumite limitări. Plastia cu mușchi sternocleidomastoidian sau platisma creşte incidența leziu-

nilor nervoase, mai ales a marelui nerv auricular, şi determină înfundarea regiunii de unde a fost recoltat (3). Lamboul fascial temporoparietal şi transpoziția SMAS-ului sunt utile pentru plastiile după parotidectomii superficiale, dar nu conferă volum suficient necesar umplerii defectelor consecutive parotidectomiilor totale (19). Grefele grăsoase şi dermogrăsoase autologe pot restabili conturul facial (20), dar au dezavantajul resorbției variabile în timp, cu asimetrie consecutivă.

O altă tehnică pentru a restabili atât conturul facial cât şi funcția, este reprezentat de transferul liber de muşchi gracilis sau de lambouri libere microneurovasculare.

O alternativă viabilă şi relativ uşor de folosit o reprezintă lamboul musculocutanat paltisma. A fost descris pentru prima dată în 1887 de către Robert Gersuny pentru plastia unui defect al obrazului posttraumatic. Totuşi, abia în 1978 a fost descris de Futrell (21) pentru reconstrucția după carcinoame intraorale. Ulterior, în 1999, Kim (13) a folosit acest lambou pentru repararea defectelor postparotidectomie. Lamboul platisma mai poate fi utilizat pentru plastia defectelor cutanate, sau musculocutanate, din jumătatea inferioară a feței, de la nivelul gâtului şi cavității orale. Versatilitatea acestuia l-au mai recomandat în repararea stricturilor benigne de hipofaringe, refacerea bărbiei şi a buzelor, reconstrucția traheei la pacienții cu neoplasm esofagian, replantări de ureche, plastia defectelor cutanate din regiunile parotidiană, mastoidiană şi geniană (22).

Vascularizația lamboului provine din ramul submental din artera facială, din artera tiroidiană superioară, artera occipitală şi auriculară posterioară (23). Vena jugulară externă şi vena submentală asigură drenajul venos, care are o orientare predominat pe verticală spre deosebire de vascularizația arterială multiaxială (24).

Lamboul musculocutanat paltisma poate fi cu pedicul superior, posterior sau inferior. Primele două oferă suficient țesut pentru repararea defectelor mici şi medii cu localizare cutanată sau intraorală. Ultimul pedicul are vascularizația din artera tiroidiană superioară, astfel că nu are aplicație în plastia orală sau facială (25).

Lambou musculocutanat platisma cu pedicul superior are avantajul că este uşor de recoltat, fără a necesita incizii suplimentare dacă s-a efectuat şi evidare ganglionară, are textură similară cu tegumentul regiunii primitoare, iar zona donatoare are morbiditate redusă (22, 26). Un alt avantaj este că asigură a protecție adecvată în cazul refacerii continuității nervului facial cu un grefon nervos.

Principalul dezavantaj este dat de problemele de vascularizație. Atât aportul arterial, cât şi drenajul venos pot fi compromise în momentul recoltării lamboului, în urma radioterapiei preoperatorii, sau în cazul evidărilor ganglionare sau a inciziilor cervicale preexistente (27). Unii autori au raportat tulburări ale vascularizației şi în cazul chimioterapiei neoadjuvante (28). Lamboul cu pedicul superior are o vascularizație foarte bună asigurată de arterele facială şi submentală, în schimb drenajul venos este mai puțin eficient şi se realizează prin venele comunicante anterioare în vena jugulară anterioară, tiroidiană superioară sau în vena facială (29). Lamboul cu pedicul posterior are un drenaj venos bun prin vena jugulară externă, dar vascularizația arterială este la întâmplare din artera occipitală (29).

Nu sunt de neglijat nici problemele oncologice şi limitările evidării ganglionare

date de necesitatea păstrării unei vascularizații decente a lamboului. În general, adesea, jugulara externă nu poate fi păstrată, de unde şi cea mai importantă complicație reprezentată de congestia venoasă a lamboului. Alte complicații sunt necroza parțială sau totală şi dehiscența plăgii.

CONCLUZII

Lamboul musculocutanat platisma este o metodă viabilă şi uşor de folosit pentru reconstrucția defectelor după parotidectomia extinsă cu pierdere cutanată. Pentru defectele voluminoase, care includ şi alte structuri în afara glandei şi tegumentelor, sunt recomandate lambourile liber transferate.

Lamboul musculocutanat platisma are textură asemănătoare cu a zonei acceptoare.

Morbiditatea zonei donatoare este minimă.

Prin facilitarea evidării ganglionare, timpul necesar recoltării lamboului este compensat. Durata redusă şi simplitatea tehnicii reprezintă argumente importante pentru utilizarea ei în cazul unor pacienți cu stare generală afectată de vârstă, afecțiuni concomitente ori stadiul bolii.

Principalul dezavantaj îl reprezintă problemele de vascularizație, dar şi posibilitatea limitată de refacere a conturului şi simetriei faciale.

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TOXICOLOGIC SCREENING OF SOME SURFACTANTS USING MODERN *IN VIVO* BIOASSAYS

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TOXICOLOGIC SCREENING OF SOME SURFACTANTS USING MODERN *IN VIVO* BIOASSAYS (Abstract): This paper aims to evaluate the degree of skin irritation using specific *in vivo* tests. The completion of the study is to develop models with wide applicability in toxicological area. HET-CAM or chorioallantoic membrane assay is a new method accepted as an INVITTOX protocol that is a substitute of Draize test. The methods applied in present study were CAM assay on embryonated egg and CD1 Nu/Nu experimental model. The evaluation of erythema that is an important toxic effect of surfactants was done using a Mexameter MX18 (Courage Khazaka research line). The main observations were that sodium lauryl sulphate is the most toxic compound on our series but the non-ionic surfactants are not completely non-noxious. Non-invasive methods can be associated with other test such as CAM assay to evaluate irritant compounds. **Key words:** HET-CAM BIOASSAY, IRRITATION SCORE, DERMAL IRRITATION, SURFACTANT

The main goal of toxicological scientific endeavours is to safeguard human beings against the possible adverse effects of diverse types of chemicals, including pharmaceuticals, cosmetics and, of course, tensioactive compounds with a known noxiousness. The exposure can be incidental, accidental or intentional, even when the noxious tested formulation is a cosmetic or a drug. One of the most common effects of the exposition and accidental contact with a new chemical is eye and skin irritation evaluated by Draize test. Generally, the physiological response to a chemical stimulus is irritation, which involves objective changes (e.g., local redness and oedema) and subjective sensations (e.g., pruritus and pain) and these aspects could characterize noxious ingredients also and could be evaluated by scores (1). Even today, the final preclinical safety assessment of chemicals is largely based on animal experiments. However, ethical concerns involving the use of laboratory animals, the validity of animal eye and skin as human eye and skin models and the need for more efficient and cost-effective preclinical validation methods have promoted the development of alternative methods to assess irritation that will substitute the classical ones (1).

Surfactants, due to their surface or interface activities and physico-chemical properties, are among the most used excipients in cosmetic and pharmaceutical products for cleaning or other properties or for realising the forms. The application of the preparations containing these compounds may result in eye and skin irritation or other side reactions and important noxious consequences (2). As a consequence, it is of great interest to identify surfactants with low irritant

properties and, during this screening process, it is also necessary to have rapid assays to assess noxiousness and deep damaging effects. The evaluation of the irritant potential of chemicals *in vivo* is traditionally conducted in animals, particularly in rabbits (the Draize test method), rodents or other tests (3, 4, 5, 6). However, due to increasing concern over animal use and obvious ethical implications of using directly human subjects, *in vitro* alternative methods should now be encouraged and they are enough developed (5). Different *in vitro* approaches have been proposed for assessing eye irritation as an alternative to the Draize eye irritation test, including the use of cultured cells, isolated organs or fertilized hen eggs (6).

The mechanism by which surfactants cause skin irritation is not fully understood. Series of factors contributing to their irritation potential include : (a) direct action on the *stratum corneum* components (lipids and proteins), (b) interaction with living epidermal cells altering their proliferative capacity and (c) action on dermal components inducing mediator release and inflammation. No single *in vitro* model and no single assay will be able to mimic all the events that occur *in vivo* in the human skin, thus, a complex of methods are required to conclude about the surfactants toxicity and to offer a detailed evaluation (7).

The chorioallantoic membrane (CAM) is the vascularised respiratory membrane that surrounds a chick developing inside an egg. There are several variations in CAM assay protocols. One of the scores determines the irritation potential from a formula which includes the time in seconds at which haemorrhage, vasoconstriction and coagulation appear and it is useful to prove also the antiangiogenic effect of antitumoral compounds. The irritation potential ranges from 0 to 21 and the analysed substances will be included in this interval. This method allows different types of substances such as surfactants (8), cosmetics, ingredients (9), different chemical products (10,11,12) or dental adhesives (13) to be evaluated as irritant and noxious ingredients.

The Cosmetic Toiletry and Fragrance Association performed an evaluation of alternatives to the Draize primary eye irritation test because of a lot of things (ethical, details of mechanism, etc). They concluded that none of the assays exhibited 100% sensitivity and 100% specificity. However, the CAM method achieved 94% sensitivity and 100% specificity (14). The combination of histological and visual HETCAM tests is of interest, as it may give a more sensitive evaluation of the innocuousness of cosmetic active ingredients, easy to develop (15). The amount of stain fixed on the damaged membrane is quantified and evaluated (16,17). We have introduced a modification to the method, whereby the membrane weight is measured after dissection and the results are expressed as a function of the membrane weight (18).

Excessive exposure of skin to physical and environmental agents implicitly favours the appearance of photoaging. Interventions on the body skin through aggressive environmental factors such as formulations with surfactants are matters of concern to current research and to offer details about the application of alternative methods. These represent the arguments for our study.

MATERIALS AND METHODS

Sodium lauryl sulphate (SDS or SLS), Tween 20 and Tween 60 solutions were in concentration 0.5%. They were offered by the UMFT Pharmaceutical Technology department. A vehicle control solution was prepared for the application on the CAM ; the pure reagent was diluted with distilled water (1 : 10, v/v).

SDS. SDS was administered onto the CAM as positive control using a solution 1mg/ml in distilled water.

HET CAM Assay

The bioassay is used to evaluate the potential ocular irritancy of any test substance as measured by its ability to induce toxicity at the vascular level of the chorioallantoic membrane of the chicken (19). The HET-CAM bioassay was performed following ICCVAM recommendations published in November 2006 in Appendix G and adapted to our laboratory conditions (20,21). In brief, fertilized eggs were horizontally incubated 7 day prior to use, at 37^0C, in controlled wet atmosphere. On the 3rd day of incubation, in order to detach the chorioallantoic membrane, 3 ml of albumen were aspired through a perforation at the more pointed end of the eggs. The hole was resealed and returned to the incubator. The next day, a big window was cut and resealed on the superior side of the shell. The eggs were returned to incubate until the 8th day. 5 eggs were used for each test substance. After inspecting and recording the surface of the CAM by means of a stereomicroscope, 0.3 ml of the control and test solution diluted in NaCl 0.9% were applied to the CAM. The reactions produced were observed over a period of 300 seconds. The time for the appearance of each selected endpoint was registered in seconds. The endpoints observed were : haemorrhage, H (bleeding from the vessels), vascular lysis, L (blood vessel disintegration), coagulation, C (intra- or extra- vascular protein denaturising). A variety of analysis methods may be used to assess irritancy potential of test substances. One analysis method that has been used extensively is an irritation score (IS). The formula used to generate an IS value is (22,23) :

$$IS = 5 \times \frac{301 - SecH}{300} + 7 \times \frac{301 - SecL}{300} + 9 \times \frac{301 - SecC}{300}$$

where : H = haemorrhage ; L = vessel lysis ; C = coagulation ; Sec = start second

Haemorrhage time (Sec H) = observed start (in seconds) of haemorrhage reactions on CAM, Lysis time (Sec L)= observed start (in seconds) of vessel lysis on CAM, Coagulation time (Sec C) = observed start (in seconds) of coagulation formation on CAM After the treatment time of 5 min, the main reaction was scored (either haemorrhage or lysis, or coagulation) according to the following scheme : 0 = no reaction ; 1 = slight reaction ; 2 = moderate reaction ; 3 = severe reaction. Mean scores were determined.

Animals and treatment

CD1 Nu/Nu mice were purchased from Charles River (Sulzfeld, Germany), 4 mice /group. From the week 8 the tested solution was applied on the dorsal area : group A. whitness-just solvent (water), group B SLS 5% solution, group C Tween 20 5% and group D Tween 60 5%, 2 times/day each. The final results were carted after 30 days. The work protocol followed all NIAH-National Institute of Animal Health rules : animals were maintained during the experiment in standard conditions : 12h light-dark cycle, food and water *ad libidum*, temperature 24°C, humidity above 55%.

The mexametric measurements were performed using a research device from Courage Khazaka, with a Mexameter MX18 (Courage &Khazaka Electronics, Cologne, Germany). The maximum units for Mexameter are 999 (interval 0-999) and the measurement is based on the absorption/reflexion. For the measurements, the mice were anesthetised with xylazine and ketamine. The time of measuring was continuous for 20 seconds. The protocol followed the observations on haemoglobin status (erythema). The device was applied on most obvious affected areas and maintained on the skin for 20 seconds. The data were registered by the specific soft from the Mexameter MX18 device and then expressed as units. All data were processed as initial and final measurements values on the same area.

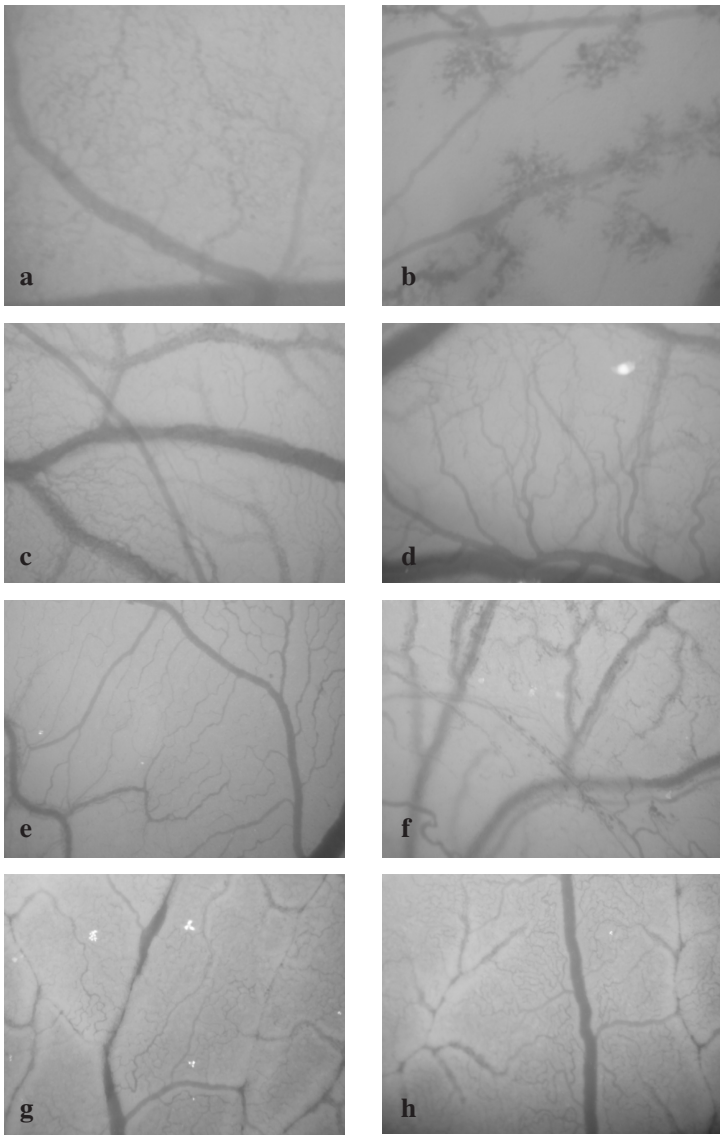

Fig. 1. HET CAM bioassay, en face images of chorioallantoic membrane before (left) and after 5 min of contact with the tested compounds (right), Stereomicroscope mycrographs x20 : a) SDS -before, b) SDS-after, c) Na Cl 0.9% -before, d) NaCl 0.9% - after, e) Tween 20 - berfore, f) Tween 20 - after, g) Tween 60 -before, h) Tween 60 - after

RESULTS AND DISCUSSIONS

The effects induced by the tested compounds as well as the selected controls were registered as microphotographs representing the en face surface of the chorioallantoic membranes before and after 5 min of substance contact (fig. 1).

Prior to the determination of irritation score, the results obtained for irritation thre-

TABLE I
Irritation score, severity and effect classification

Test compound and controls	Irritation score (mean)	Irritation severity (mean)	Classification of the effect
SDS (1 mg/mL) Positive control	17.99	3	Severe reaction
Na Cl 0.9% Negative, vehicle control	0	0	No reaction
Tween 20 (1 mg/mL)	5.43	1	Slight to moderate reaction
Tween 60 (1 mg/mL)	8.45	0.75	Slight reaction

shold was considered. The test chemical concentrations were selected based on the irritation threshold results. All the three end points, hemorrhage, coagulation and lysis were observed for SDS, Tween 20 and Tween 60 respectively. The mean irritation score for SDS is 17.99. The test showed 0 irritation score for solution of Na Cl 0.9% (tab. I; fig. 2).

The results show a great difference between the positive control, SDS and the two test surfactants Tween 20 and Tween 60. SDS induced major dammage at the vascular level of the chorioallantoic membrane; after the application of 0.3 mL of solution (1 mg/mL), a large area was affected by high number of mycrohemorrhages, the death of the specimen was registered after 20 min of contact with the solution. The other specimens had somehow a better viability, until the next day.

The two evaluated surfactants did not produce severe irritative reaction on the capillary plexus of the treated CAM. Both of them showed mycrocoagulation signs. Tween 20 expressed a more generalised and severe reaction , which emerged later, explaining in this way the lower value of the irritation score. Also there was no sign of lysis and the vasodilation and slight haemorragy came out only at the end of the observing time. In what concerns Tween 60, there were a few

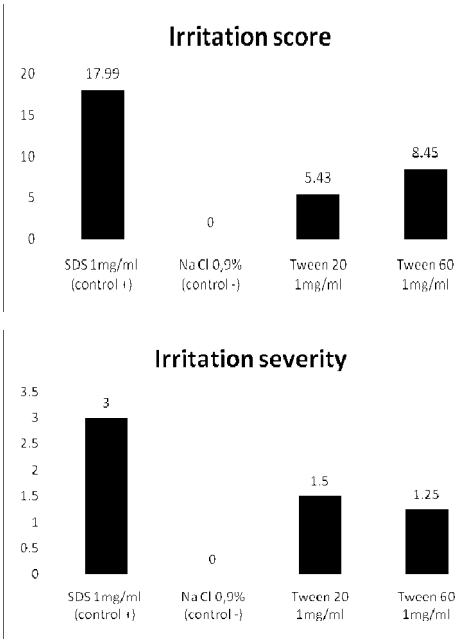

Fig. 2. Diagrams of the irritation score and its grade of severity for the tested surfactants and the control solutions

areas of mycrocoagulation and of vascular lysis, but which have been registered earlier, causing a higher irritation score, even if the severity of the reaction was classified as slight.

In vivo experiments sustain the theory of irritancy for tested compounds aspects ob-

A. Whitness B.Tween20 C.Tween60 D. SLS

Fig. 3. *In vivo* evaluation of surfactants solution observed on macroscopic images on CD1 Nu/Nu mice after 30 days of continuous application

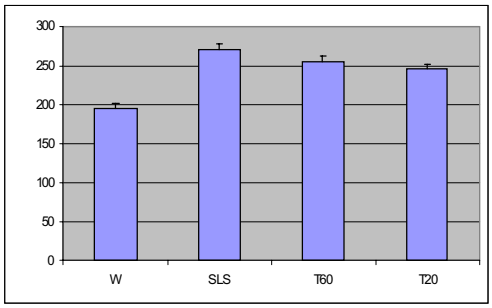

Fig. 4. Erythema units measured as comparative values at the end of experiment (day 30)

served on macroscopic images (fig. 3). The most irritative compound is the reference one SLS but also the nonionics (Tweens) developed dry skin and irritant reactions (fig. 3 and 4). The mexametric values of the erythema induced after the application of surfactants are in correlation with the irritation score obtained after the HET-CAM evaluation. Considering these results, Tween 20 showed the lowest irritative potential comparing to Tween 60, which induced a more severe reaction and to SDS, which is the positive control for toxic effects. The irritation severity can be considered a more subjective parameter, while the irritation score has a better prediction capacity.

Surfactants, due to their surface and interface activities, are among the most common and versatile components of pharmaceutical and cosmetic preparations, cleansers, soaps and shampoos with an intense usage. Thus, the concentrations used in commercial formulations should avoid adverse reactions such as skin irritation and other damages. For this reason, the search for new non-irritative surfactants has generated considerable interest, although pre formulation developmental trials are needed (24). Furthermore, there is a pressing need to develop biodegradable and biocompatible surfactants with low toxicity and excellent emulsifying properties, particularly in light of increasing concerns about energy consumption and environmental and toxicological dangers (25, 26). Our study underline the noxious potential of tensioactive compounds using as reference SLS, a well known irritant. Neither the Tweens are without signs of noxious effects if they are tested as single compounds. Future studies will evaluate the formulations with combinations of surfactants or with compounds that can lead to macromiceles formation to evaluate the differences.

HET-CAM was proposed to mimic the effects that may occur in the conjunctiva following exposure to a test substance. Chicken embryo models have long been used by embryo toxicologists (27,28,29) and our evaluations proved its applicability. Extending the use of chicken embryos, the HET-CAM test was proposed by Luepke as an alternative in vitro method.

Studies also have suggested that the extra embryonal vascular systems (yolk sac, CAM)

are not sensitive to pain. These studies suggest that on day 9 of incubation, there is no pain perceived by the developing embryo during the HET-CAM test (20).

In the present study, a good correlation was found between the HET-CAM test results and reported data from others irritation tests. Non-invasive methods such as mexametric measurements can be applicable to evaluate irritation potential and to confirm noxiousness evaluated by other methods.

CONCLUSIONS

In summary, according to the presented *in vivo* tests, surfactants non-ionic compounds developed a lower irritation potential than the well known SLS even on CAM assay and in *vivo*-erythema measurement tests.

A large number of studies have been undertaken to find tests that replace the need for animals in eye and skin safety testing and it seems that it is adequate. The tests on mouse skin that is a fidel test on a total body can be correlated with CAM assay. A battery of tests should be established as no single assay can fulfill the requirements for risk assessment using an *in vitro* method and part of these tests could be the simple tests on embryonated eggs or measurements on animals skin with non-invasive techniques.

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INFLUENŢA TEHNICILOR DE FINISARE ASUPRA RUGOZITĂŢII SUPRAFEŢELOR COMPONENTELOR METALICE ALE RESTAURĂRILOR PROTETICE

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INFLUENCE OF FINISHING TECHNIQUES ON THE SURFACE ROUGHNESS OF METALLIC COMPONENTS OF PROSTHETIC RESTORATIONS (Abstract): The paper proposes to examine the influence of mechanical and electrolytic finishing techniques on metal surface roughness. **Material and method**: Six non-noble dental alloys and three versions of finishing have been taken into consideration: mechanical polishing with rubber polisher, mechanical polishing with cotton polisher and abrasive paste and electrolytic polishing. Examinations on roughness have made reference to R_z, R_a and R_q. **Results**: The experimental results obtained have shown that mechanical polishing with rubber polisher is recommended for Co-Cr-Mo alloys, while mechanical polishing with cotton polisher and abrasive paste or electrolytic polishing are suited for Ni-Cr alloys. **Conclusion**: Analysis of values obtained led to formulation of recommendations concerning the best polishing method for each non-noble alloy considered. **Key words**: NON-NOBLE DENTAL ALLOYS, POLISHING TECHNIQUES, ROUGHNESS R_Z, R_A, R_Q

Componentele metalice ale restaurărilor protetice sunt supuse operaţiei de lustruire care are ca scop îmbunătăţirea calităţii de suprafaţă, prin micşorarea considerabilă a rugozităţii stratului superficial (1).

Rugozitatea unei suprafeţe este o abatere a profilului real de la forma profilului ideal şi ea este reprezentată prin: striaţii şi rizuri periodice sau pseudoperiodice, smulgeri de material, urme lăsate de scule şi goluri aperiodice.

Descrierea matematică a profilului microneregularităţilor se poate face cu ajutorul unor mărimi simplificatoare: R_z – rugozitatea medie a suprafeţei; R_a – abaterea medie aritmetică a profilului; R_q – abaterea medie pătratică a profilului (2).

SCOPUL STUDIULUI

Studiul şi-a propus efectuarea unei analize comparative privind influenţa tehnicilor de finisare asupra rugozităţii suprafeţei componentelor metalice ale restaurărilor protetice realizate din diferite aliaje nenobile.

Presupunând existenţa unei dependenţe între rugozitatea suprafeţelor metalice şi procesul de dezvoltare a plăcii bacteriene, această analiză va permite identificarea ulterioară a corelaţiei dintre tehnicile de finisare, rugozitatea suprafeţei şi integrarea biologică (3).

MATERIALE ŞI METODĂ

Cercetările experimentale au fost efectuate pe şase aliaje nenobile (4) cu utilizare frecventă în protetica dentară (tab. I).

Pe epruvete cilindrice (φ7 x 10 mm) realizate din aceste aliaje, a fost executată o faţetă plană (l=3 mm) la nivelul căreia s-au efectuat, în trei zone ale aceleiaşi direcţii longitudinale, măsurători ale rugozităţii.

Tehnicile de finisare aplicate la nivelul

TABELUL I
Aliaje nenobile dentare supuse cercetărilor

Aliaje nenobile	Marca aliajului	Compoziţia chimică, [%]						Destinaţia aliajului
		Co	Ni	Ti	Cr	Mo	Nb	
Aliaje Co-Cr-Mo	Sismo	63,3	-	-	30,0	5,0	-	Componente metalice ale protezelor dentare: şei metalice, conectori principali şi secundari, croşete, culise
	Robur 400	62,0	-	-	29,1	5,8	-	
Aliaje Ni-Cr	Kera N	-	62,0	-	26,7	10,2	-	
	Ugirex III	-	62,0	-	26,0	9,4	-	
Aliaje pe bază de titan	Biotan	-	-	99,5	-	-	-	
	Biotan-Nb	-	-	87,0	-	-	7	

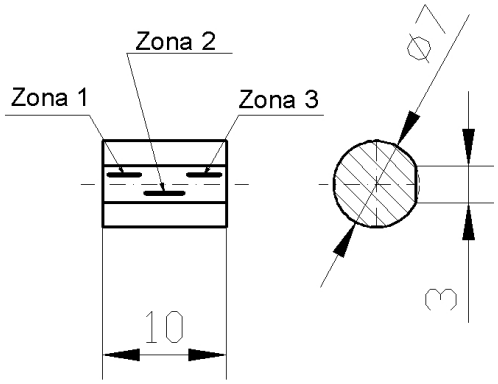

Fig. 1. Epruvete destinate măsurătorilor de rugozitate

faţetei au fost : lustruirea mecanică - pentru toate aliajele şi lustruirea electrolitică - specifică numai aliajelor Co-Cr-Mo şi Ni-Cr.

Lustruirea mecanică a fost realizată cu un micromotor Schick C3 Master, utilizând discuri gumate abrazive sau pufuri din bumbac şi paste abrazive speciale.

Pentru lustruirea electrolitică, probele au fost imersate într-o soluţie acidă, tensiunea aplicată între electrozi fiind U = 12 Vcc, iar durata procesului t = 15 min.

REZULTATE

Măsurătorile de rugozitate au fost realizate pe un echipament special Mitutoyo SJ-301 (Japonia), pe fiecare epruvetă fiind efectuate trei determinări la nivelul aceleiaşi direcţii, după care s-a calculat valoarea medie a fiecărui parametru.

Soft-ul rugozimetrului (5) a permis trasarea profilului microneregularităţilor (fig. 2, 3 şi 4) şi determinarea tuturor mărimilor simplificatoare (tab. II, III şi IV).

DISCUŢII

Valorile medii ale parametrilor de rugozitate (R_z, R_a şi R_q) determinate prin măsurătorile efectuate pe suprafaţa plană a epruvetelor din aliajele nenobile dentare studiate sunt reprezentate grafic în fig. 5, 6 şi 7.

Analizând comparativ rezultatele prezentate în tab. II, III, IV, se pot face următoarele discuţii referitoare la valorile celor trei mărimi simplificatoare specifice rugozităţii.

- Rugozitatea medie (R_z) a suprafeţelor plane lustruite mecanic şi electrolitic
  - după lustruirea mecanică cu disc gumat abraziv cele mai mici valori s-au obţinut la aliajele Co-Cr-Mo, valori medii pentru aliajele Ni-Cr şi cele mai mari pentru aliajele de bază de titan (tab. II) ; dispersia valorilor este largă, deoarece : R_z (Robur 400) = 57,57% · R_z (Biotan) ;
  - valorile cele mai mici pentru (R_z) după lustruirea mecanică cu puf şi pastă abrazivă, sunt obţinute la aliajele Ni-Cr, valori medii pentru aliajele Co-Cr-Mo şi cele mai mari pentru aliajele pe bază de titan ; dispersia valorilor este mai restrânsă, deoarece : R_z (Kera N) = 44,57% · R_z (Biotan-Nb) ;
  - lustruirea electrolitică conduce la obţinerea unor valori cu dispersie mare atât între aliajele din aceeaşi grupă, cât şi

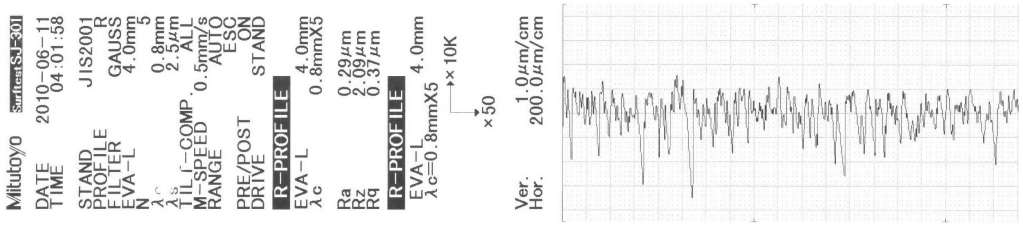

Fig. 2. Profilul microneregularităţilor pentru epruvete din aliaje nenobile lustruite mecanic cu disc gumat abraziv

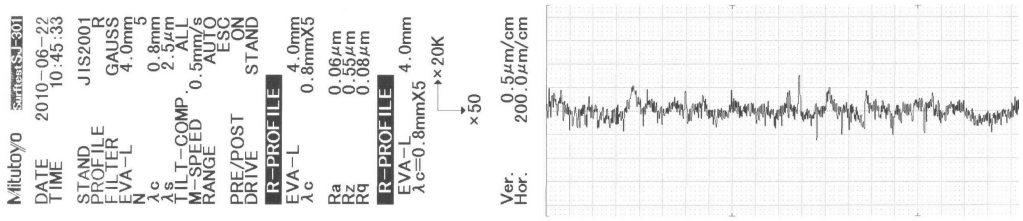

Fig. 3. Profilul microneregularităţilor pentru epruvete din aliaje nenobile lustruite mecanic cu puf şi pastă abrazivă

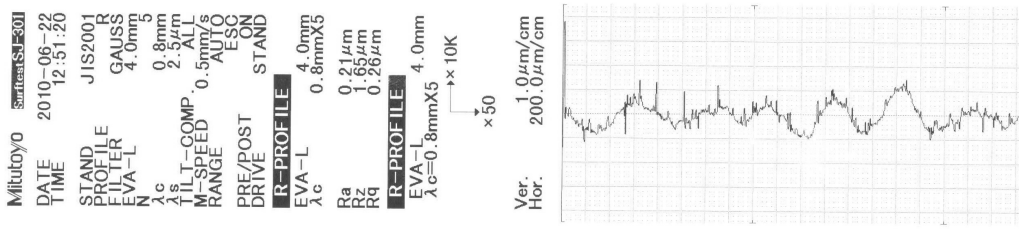

Fig. 4. Profilul microneregularităţilor pentru epruvete din aliaje nenobile lustruite electrolitic

TABELUL II

Valorile medii ale parametrilor de rugozitate (R_z, R_a şi R_q), pentru suprafeţele metalice lustruite mecanic cu disc gumat abraziv şi cu puf şi pastă abrazivă

Nr. crt.	Aliaje nenobile	Marca aliajului	Valori medii după lustruirea cu disc gumat abraziv, [μm]			Valori medii după lustruirea cu puf şi pastă abrazivă, [μm]		
			R_z	R_a	R_q	R_z	R_a	R_q
1	Aliaje Co-Cr-Mo	Sismo	1.817	0.246	0.330	0.617	0.067	0.093
2		Robur 400	1.686	0.220	0.287	0.603	0.067	0.090
3	Aliaje Ni-Cr	Kera N	2.097	0.293	0.377	0.536	0.057	0.073
4		Ugirex III	2.526	0.334	0.487	0.580	0.060	0.083
5	Aliaje pe bază de titan	Biotan	3.973	0.580	0.737	0.843	0.113	0.143
6		Biotan-Nb	2.833	0.480	0.590	0.967	0.120	0.160

între aliajele din grupe diferite. Astfel : R_z (Sismo) = 53,62% · R_z (Robur 400), iar R_z (Kera N) = 34,82% · R_z (Ugirex III), în timp ce R_z (Sismo) = 39,51% · R_z (Ugirex III).

- Abaterea medie aritmetică (R_a) a profilului microneregularităţilor suprafeţelor plane lustruite mecanic şi electrolitic

TABELUL III

Valorile medii ale parametrilor de rugozitate (R_z, R_a şi R_q), pentru suprafeţele metalice lustruite electrolitic

Nr. crt.	Aliaje nenobile	Marca aliajului	Valori medii după lustruirea electrolitică, [μm]		
			R_z	R_a	R_q
1	Aliaje Co-Cr-Mo	Sismo	2.506	0.203	0.310
2		Robur 400	3.247	0.304	0.453
3	Aliaje Ni-Cr	Kera N	2.700	0.210	0.325
4		Ugirex III	4.143	0.326	0.503

TABELUL IV

Dispersia valorilor parametrilor de rugozitate R_z, R_a şi R_q

Aliaje nenobile	Marca aliajului	Lustruire mecanică cu disc gumat abraziv			Lustruire mecanică cu puf şi pastă abrazivă			Lustruire electrolitică		
		R_z	R_a	R_q	R_z	R_a	R_q	R_z	R_a	R_q
Aliaje Co-Cr-Mo	Sismo							39.51	37.73	38.36
	Robur 400	57.57	62.07	61.05						
Aliaje Ni-Cr	Kera N				44.57	49.56	43.75			
	Ugirex III							100	100	100
Aliaje pe bază de Ti	Biotan	100	100	100	100	100	100	-	-	-
	Biotan-Nb							-	-	-

Notă: 100% - valoarea maximă a parametrului de rugozitate; x%: - valoarea minimă a parametrului de rugozitate.

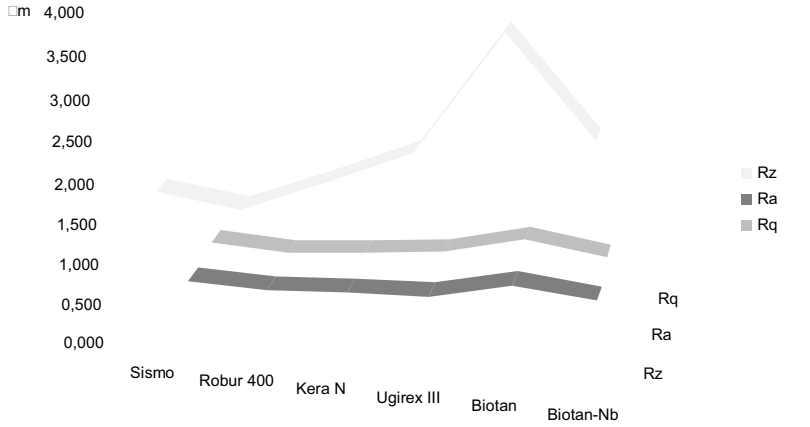

Fig. 5. Variaţia valorilor medii ale parametrilor de rugozitate (R_z, R_a şi R_q) pentru epruvetele lustruite mecanic cu disc gumat abraziv

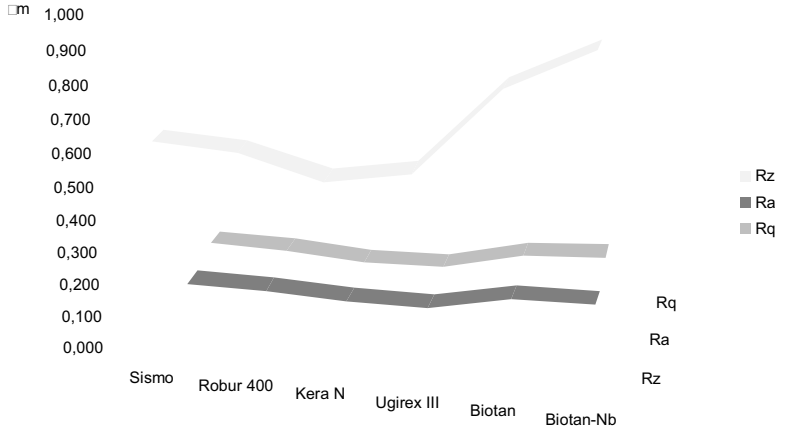

Fig. 6. Variaţia valorilor medii ale parametrilor de rugozitate (R_z, R_a şi R_q) pentru epruvetele lustruite mecanic cu cu puf şi pastă abrazivă

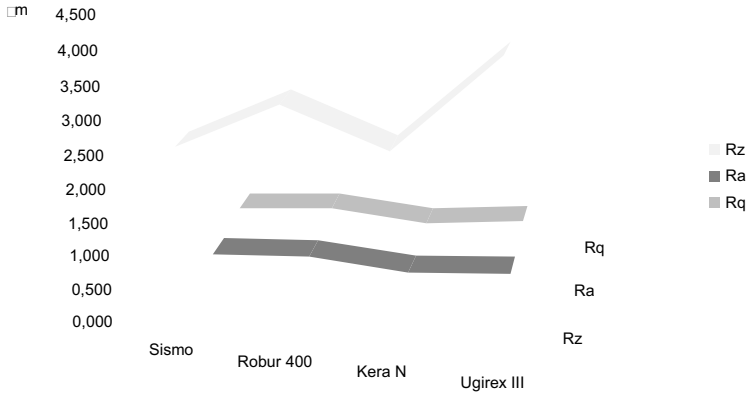

Fig. 7. Variaţia valorilor medii ale parametrilor de rugozitate (R_z, R_a şi R_q) pentru epruvetele lustruite electrolitic

– după lustruirea mecanică cu disc gumat abraziv, cele mai mici valori s-au obţinut la aliajele Co-Cr-Mo, valori medii pentru aliajele Ni-Cr şi cele mai mari pentru aliajele pe bază de titan; dispersia rezultatelor este largă, deoarece: R_a (Kera N) = 62,07% · R_a (Biotan);

– valorile cele mai mici pentru (R_a) după lustruirea mecanică cu puf şi pastă abrazivă, sunt obţinute la aliajele Ni-Cr, valori medii pentru aliajele Co-Cr-Mo şi cele mai mari pentru aliajele pe bază de titan; dispersia rezultatelor este mai restrânsă, deoarece: R_a (Kera N) = 49,56%

$\cdot R_a$ (Biotan);
- după lustruirea electrolitică, se obţine o dispersie mică a valorilor între aliajele Co-Cr-Mo şi Ni-Cr, iar R_a (Sismo) = 37,73% $\cdot R_a$ (Ugirex III).

• Abaterea medie pătratică (R_q) a profilului microneregularităţilor suprafeţelor plane lustruite mecanic şi electrolitic
- după lustruirea mecanică cu disc gumat abraziv, cele mai mici valori s-au obţinut la aliajele Co-Cr-Mo, valori medii pentru aliajele Ni-Cr şi cele mai mari pentru aliajele pe bază de titan; dispersia rezultatelor este largă, deoarece: R_q (Robur 400) = 61,05% $\cdot R_q$ (Biotan);
- valorile cele mai mici pentru (R_q) după lustruirea mecanică cu puf şi pastă abrazivă, sunt obţinute la aliajele Ni-Cr, valori medii pentru aliajele Co-Cr-Mo şi cele mai mari pentru aliajele pe bază de titan; dispersia rezultatelor este mai restrânsă deoarece: R_q (Kera N) = 43,75% $\cdot R_q$ (Biotan);
- după lustruirea electrolitică se menţine o dispersie considerabilă a valorilor specifice între aliajele Co-Cr-Mo şi Ni-Cr, când R_q (Sismo) = 38,36% $\cdot R_q$ (Ugirex III).

CONCLUZII

Pe baza rezultatelor obţinute prin măsurători ale rugozităţii efectuate pe aceeaşi direcţie longitudinală a unei suprafeţe plane, se pot formula unele concluzii generale:
- au fost studiate şase mărci de aliaje aparţinând la trei clase de aliaje nenobile, cu utilizare frecventă în protetica dentară;
- probele prelevate din fiecare aliaj au fost lustruite prin trei tehnici: lustruire mecanică cu disc gumat abraziv, lustruire mecanică cu puf şi pastă abrazivă şi lustruire electrolitică;
- rugozitatea suprafeţei lustruite a fost evaluată prin trei mărimi simplificatoare specifice: R_z, R_a şi R_q;
- analiza valorilor medii determinate evidenţiază aspecte tehnice importante:

• pentru toate aliajele, cea mai mică rugozitate se obţine după lustruire mecanică cu puf şi pastă abrazivă, iar cea mai mare rezultă după lustruirea electrolitică;
• lustruirea mecanică cu disc gumat abraziv şi cea electrolitică sunt recomandate aliajelor Co-Cr-Mo;
• lustruire mecanică cu puf şi pastă abrazivă este destinată aliajelor Ni-Cr şi celor pe bază de titan.

- diferenţele remarcabile dintre valorile rugozităţii obţinute prin lustruirea mecanică şi cea electrolitică pot fi explicate prin natura mecanismelor specifice celor două procese: deformarea plastică prin fluaj a microneregularităţilor profilului – pentru lustruirea mecanică cu puf şi pastă abrazivă, respectiv descompunerea anodică a vârfurilor microneregularităţilor – pentru lustruirea electrolitică;
- se constată că alunecarea datorată fluajului va asigura un grad de netezire a suprafeţei superior, comparativ cu cel rezultat prin descompunerea anodică.

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PREVALENŢA TUBERCULOZEI ÎN JUDEŢUL MUREŞ ŞI ÎN ŢARĂ ÎN PERIOADA 2006-2008

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TUBERCULOSIS PREVALENCE IN MURES COUNTY IN 2006-2008 (Abstract): The aim of this study is analysis of tuberculosis prevalence in Mures county for a period of three years (2006-2008). **Material and method**: We monitored the prevalence of tuberculosis cases in the Mureş area recorded at Tg. Mureş TB Dispensary, the new cases and readmitted cases, chronic or abandonment, separately for children and adults, evolving for 2006-2008, data compared with national registration. **Results**: From 2006 to 2008, frequency of TB illness in our area decreased instead of the share of patients with positive results (BK+) wich have increased, associated with an amplified risk. In our study the assessment during the three years showed a decrease of the total number of TB cases, instead of increased frequency of new cases of acute than those of chronic, increased cases of adults against children and cases of failed healing. Number of new cases of TB in Mureş urban areas, by years, showed the same decreasing trend for cities monitored, except Tg. Mureş where the number of new cases recorded in 2008 was higher than in 2006. **Conclusion**: TB control programs in order to be effective, must be conducted throughout the all country, to be permanent, to the satisfaction of the population and to integrated structure of community health. **Key words**: TUBERCULOSIS, RISK FACTORS, MANAGEMENT

În România, în ultima perioadă au fost raportate aproximativ 28.000 de cazuri noi de tuberculoză (TBC) şi recidive anual. Incidenţa globală a bolii a avut după anul 1985 o evoluţie constant ascendentă, ajungând în anul 2002 la valoarea maximă de 142,2%ooo. În anii următori s-a înregistrat o tendinţă de stabilizare şi chiar de uşoară scădere a valorilor acestui indicator. Cu toate acestea, în anul 2004 România se situa, pe locul 3 din 52 de ţări ale Regiunii Europa a OMS - după Federaţia Rusă (cu 121.426 de cazuri) şi Ukraina (cu 38.403 de cazuri) - iar ca incidenţă pe locul 42 din 211 ţări din întreaga lume şi pe locul 2 (după Kazakhstan) în Regiunea Europa a OMS (1,2). În perspectiva integrării în Uniunea Europeană, România se află cu siguranţă pe primul loc printre ţările UE atât ca număr de cazuri, cât şi ca incidenţă.

Analiza endemiei TBC din România arată că, spre deosebire de celelalte ţări europene, amploarea fenomenului nu se datorează nici migraţiei (în fiecare an sunt raportate doar câteva cazuri la persoane născute în afara ţării) şi nici infecţiei HIV (procentul infectaţilor HIV din numărul cazurilor de tuberculoză raportate a fost în ultimii ani sub

0,5%). Foarte probabil că un factor important al severităţii endemiei îl reprezintă problemele socio-economice grave cu care se confruntă ţara noastră. Scăderea nivelului de trai a influenţat cu certitudine evoluţia incidenţei, dovadă fiind şi faptul că incidenţa cea mai mare se înregistrează în judeţele din sudul ţării şi din Moldova, cele care au şi cele mai importante probleme socio-economice (3,4).

Factorii de risc de infecţie sunt determinaţi de cei de îmbolnăvire, astfel cel mai important determinant al instalării infecţiei la un contact cu un caz de tuberculoză activă este severitatea cazului din punct de vedere bacteriologic (infecţiozitatea sa). Urmează apoi condiţiile de expunere (volumul de aer respirat împreună cu sursa) : aglomerarea şi intimitatea surselor, timpul petrecut împreună. La acelaşi grad de infecţiozitate, aglomerare de persoane şi intimitate a contactului, stilul de viaţă al persoanelor devine un factor de risc de infecţie : igiena locuinţei şi igiena personală. (2)

Caracteristicile ultimei perioade de timp pe plan mondial dar şi naţional evidenţiază o modificare în distribuţia cazurilor, tuberculoza fiind net mai frecventă la grupele populaţionale ce prezintă anumite particularităţi. Aceste subgrupuri pot fi identificabile şi un sistem de supraveghere a tuberculozei trebuie să fie orientat în primul rând către cartografierea grupelor cu risc crescut, pentru orientarea adecvată a acţiunilor antituberculoase. Abilitatea de a identifica aceste grupuri şi locuri geografice particulare în care ele apar este însă afectată de acurateţea diagnosticului, de corectitudinea declarării cazurilor şi de colectarea corectă a datelor.

Obiectivul acestui studiu este de a analiza datele de prevalenţă ale tuberculozei la nivelul judeţului Mureş şi în ţară, pe o perioadă de 3 ani (2006-2008). Am comparat suplimentar aceste date judeţene cu cele naţionale, prin constituirea profilului naţional demografic de bază al pacienţilor cu tuberculoză.

MATERIAL ŞI METODĂ

Am monitorizat prevalenţa şi trendul cazurilor de tuberculoză din arealul judeţului Mureş înregistrate la Dispensarul TBC din Tg. Mureş, pe cazuri noi şi cazuri readmise, cele cronice sau de abandon, separat pentru copii şi adulţi, evolutiv pentru anii 2006-2008. Datele naţionale sunt înregistrate la Institutul Naţional de Pneumologie Marius Nasta din Bucureşti, prin raportări judeţene anuale.

REZULTATE

Prima parte a acestui studiu s-a concentrat pe detaliile privind incidenţa TBC în mediul urban mureşan completată de caracteristicile de monitorizare, în dinamică pe 2006-2008. Am urmărit comparativ, datele corespunzătoare principalelor oraşe din judeţ : Tg. Mureş, Reghin, Luduş, Sighişoara şi Târnăveni.

Per total, cele mai multe cazuri de TBC înregistrate, indiferent de ani sau categorie, au fost în Tg. Mureş (municipiul de judeţ), iar cele mai puţine în Luduş.

Din 2006 până în 2008, frecvenţa îmbolnăvirilor a scăzut de la 359 la 336 pentru Tg. Mureş, iar în total per judeţ de la 725 la 661. Deşi numărul de cazuri de TBC înregistrate în perioada 2006-2008, a scăzut, ponderea pacienţilor cu rezultate pozitive (BK+) a crescut de la 70,2% la 71,55%, cu un risc de gravitate amplificat (tab. I).

Cazurile noi de TBC au arătat aceeaşi scădere uşoară din 2006 faţă de 2008 (de la 534 de cazuri la 530), în schimb am observat o creştere a frecvenţei cazurilor acute din totalul celor monitorizate de la un an la altul (reprezentând 73,65% din pacienţii pe 2006, 78,92% din pacienţii pe 2007, respectiv 80,18% din pacienţii din 2008). Au fost asociate şi cu o creştere a frecvenţei cazurilor pozitive de la 63,1% la 66,22% (BK+) (tab. I).

Cazurile readmise de TBC au arătat o scădere din 2006 faţă de 2008 (de la 103 de cazuri la 93), asociate însă cu o creştere

TABELUL I

Situaţia statistică a cazurilor de tuberculoză înregistrate în judeţul Mureş (perioada 2006-2008), corespunzătoare pentru adulţi

Dispensar	Total judeţ						Caz nou						Caz readmis						Copii					
	2006		2007		2008		2006		2007		2008		2006		2007		2008		2006		2007		2008	
		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %		BK+ %
Tg. Mureş	359 49,51	250	307 51,77	205	336 50,83	230	40 38,83	38	34 50,0	38	34 50,0	38	40 38,83	38	34 50,0	29	33 35,48	30	31 54,38	3	22 57,89	4	24 63,15	3
Reghin	127 17,51	86	103 17,36	75	120 18,15	90	18 17,47	16	15 22,0	18 17,47	15 22,0	16	18 17,47	16	15 22,0	14	28 30,1	25	2 3,5	0	2	1	1 2,63	-
Luduş	46 6,34	31	36 60,7	30	30 4,53	21	8 7,76	8	5 7,35	8 7,76	5 7,35	8	8 7,76	8	5 7,35	5	1 1,0	1	4 7,0	0	-	-	3 7,89	1
Sighişoara	121 16,68	97	72 12,14	56	105 15,88	85	25 24,27	22	8 11,76	25 24,27	8 11,76	22	25 24,27	22	8 11,76	8	21 22,58	20	4 7,0	0	1 2,63	-	2 5,26	1
Târnăveni	68 9,37	45	75 12,64	46	70 10,59	47	12 11,65	9	6 8,82	12 11,65	6 8,82	9	12 11,65	9	6 8,82	6	10 10,75	9	-	-	13 34,21	1	8 21,05	-
TOTAL	**725**	**509 70,2**	**593**	**412 69,47**	**661**	**473 71,55**	**103**	**93 90,29**	**68**	**103**	**68**	**93 90,29**	**103**	**93 90,29**	**68**	**62 91,17**	**93**	**85 91,39**	**57**	**8 14,03**	**38**	**6 15,78**	**38**	**5 13,15**

mai uşoară a frecvenţei cazurilor pozitive de la 90% la 91,39% (BK+) (tab .I).

Pacienţii copii au avut o frecvenţă mai mică decât a adulţilor, de asemenea cu un trend în scădere din 2006 către 2008 (de la 57 la 38 de cazuri) şi frecvenţe apropiate de la un an la altul (cu o medie de 14%) (tab. I).

Cazurile de abandon sunt destul de puţine dar de interes curativ şi preventiv, de la 23 de abandonuri în monitorizare în 2006 la 15 cazuri în 2008, cu o medie anuală de 2,5% din pacienţii înregistraţi (tab. II).

Cazurile de eşec curativ sunt apropiate ca număr şi frecvenţă cu cele de abandon, 2,75% din totalul pacienţilor monitorizaţi, în scădere ca evoluţie pe parcursul perioadei studiate (de la 20 în 2006 la 14 în 2008) (tab. II).

La capitolul de cronici, ponderea este mai scăzută (5,65% din totalul pacienţilor), cu o scădere de la 41 de cazuri în 2006 la 7 în 2008, reducere semnificativă ($p < 0,004$) (tab. II).

Per total, în lotul nostru de evaluare, pe parcursul celor 3 ani de urmarire, a scăzut numărul de cazuri de TBC, în schimb a crescut frecvenţa cazurilor noi acute, faţă de cele cronice, au crescut cazurile de adulţi faţă de copii, precum şi situaţiile de eşec curativ.

Numărul cazurilor noi de TBC din judeţul Mureş, comparativ pe ani şi mediul urban au arătat acelaşi trend descrescător pentru toare oraşele monitorizate, cu excepţia municipiului Tg. Mureş unde numărul cazurilor noi înregistrate în 2008 a fost mai mare decât cel din 2006 (284 faţă de 271). Astfel, pe parcursul a 3 ani, aici a scăzut frecvenţa cazurilor totale de pacienţi cu TBC, pe fondul cazurilor de cronici, în schimb au crescut cazurile noi înregistrate (tab. I şi fig. 1).

Numărul cazurilor de TBC la copiii din judeţul nostru, comparativ pe ani şi mediu urban a demonstrat o evoluţie în scădere de la un an la altul, fără diferenţe semnificative de la un oraş la altul, excepţie făcând oraşul Târnăveni singurul la care a crescut simţitor numărul şi frecvenţa pacienţilor copii cu TBC din 2006 către 2008, cu un vârf de

TABELUL II

Situaţia statistică a cazurilor de tuberculoză din judeţul Mureş (2006-2008), corespunzătoare cazurilor de cronici, abandon şi eşec

Dispensar	Abandon						Cronici						Eşec					
	2006		2007		2008		2006		2007		2008		2006		2007		2008	
		+		+		+		+		+		+		+		+		+
Tg. Mureş	18 78,26	18	11 73,33	11	10	9	20 48,78	19	7 46,66	7	2 28,57	2	10 50,0	10	13 48,14	13	6 42,85	6
Reghin	2 8,69	2	2 13,33	2	2 13,33	2	6 14,63	6	3 20,0	3	1 14,28	1	5 25,0	3	5 18,51	5	-	-
Luduş	1 4,34	1	1 4,54	1	1 4,54	1	2 4,87	2	1 6,66	1	-		3 15,0	2	1 3,7	1	4 28,57	4
Sighişoara	0	0	1 4,54	1	-	-	12 29,26	12	4 26,66	4	3 42,85	3	2 10,0	2	3 11,11	3	-	-
Târnăveni	2 8,69	1	-	-	2 13,33	2	1 2,43	1	-	-	1 14,28	1	0	0	5 18,51	2	4 28,57	4
TOTAL	**23**	**22 95,65**	**15**	**15 100**	**15**	**14 93,33**	**41**	**40 97,56**	**15**	**15 100**	**7**	**7 100**	**20**	**17 85**	**27**	**24 88,88**	**14**	**14 100**

frecvenţă de 13 cazuri corespunzător anului 2007, urmat de o scădere la 8 cazuri.

Numărul cazurilor de TBC la cronicii din judeţul Mureş, comparativ pe ani şi mediu urban a arătat de asemenea o scădere de la un an la altul, fără diferenţe semnificative de la un oraş la altul, cele mai multe cazuri fiind în Tg. Mureş iar cele mai puţine în Târnăveni aspect pozitiv ţinând cont că aceşti pacienţi au o colaborare mai dificilă şi o toleranţă mai scăzută la tratamentul complex care adesea durează 12-18 luni.

Evoluţia în dinamică a cazurilor de TBC din judeţul Mureş (2006-2008), arată o grafică similară pentru categoriile importante în evaluare (adulţii, copii, cronicii şi cazurile acute), cu menţiunea că cea mai mare frecvenţă o prezintă cazurile noi de TBC, iar cele mai mici sunt cazurile cronice şi de copii, din lotul întreg (fig. 2).

DISCUŢII

Am dorit compararea datelor judeţene mureşene cu cele naţionale, pentru aceeaşi perioadă de timp, prin constituirea profilului naţional demografic de bază al pacienţilor cu tuberculoză, analiza migraţiei acestora şi spitalizării pe anumite spitale sau zone geografice, precum şi respectarea recomandărilor Programului Naţional de Control al tuberculozei (4). Astfel, la nivel de ţară şi în Mureş, 85% dintre bolnavii de tuberculoză se internează în spitale din judeţul de domiciliu, această pondere fiind aproximativ aceeaşi cu cea pentru restul pacienţilor de pe alte secţii. Absenţa migraţiei inter-judeţene sugerează o acoperire suficientă cu servicii spitaliceşti specifice la nivelul fiecărui judeţ (5,6).

În această perioadă anuară, cea mai mare pondere a cazurilor de tuberculoză din totalul cazurilor spitalizate în ţară se constată în judeţul Giurgiu (3%), în restul judeţelor ponderea fiind sub 2%, respectiv judeţul Mureş fiind pe locul zece ca frecvenţă.

Proporţia mare a cazurilor de tubercu-

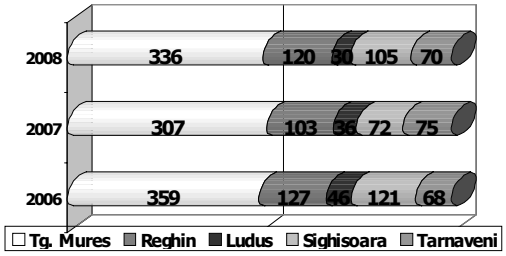

Fig. 1. Numărul total al cazurilor de TBC din judeţul Mureş, comparativ pe ani şi mediul urban

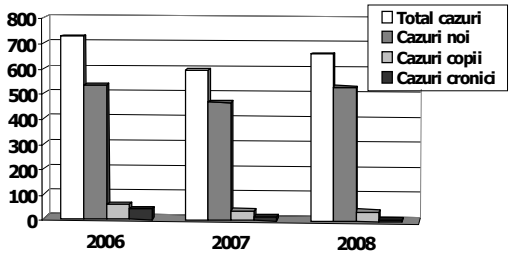

Fig. 2. Evoluţia în dinamică a cazurilor de TBC din judeţul Mureş (2006-2008)

loză este desigur specifică sanatoriilor TBC şi a spitalelor de pneumoftiziologie; proporţiile cele mai ridicate sunt deţinute de Spitalul de pneumoftiziologie Bixad şi Drajna.

În România, ca de altfel în întreaga lume, cazurile de îmbolnăvire la bărbaţi sunt de 2,1 ori mai frecvente decât cele la femei. Repartiţia pe grupe de vârste indică diferenţe între cele două sexe: cele mai multe îmbolnăviri apar la bărbaţi la vârste adulte, între 40 şi 54 de ani, iar la femei la vârste mai tinere, între 20 şi 34 de ani. Această observaţie conduce la concluzia că transmiterea infecţiei în populaţie nu este deocamdată pe deplin controlată (7,8). Aproape trei sferturi dintre cazurile de tuberculoză spitalizate în ţară sunt bărbaţi (72,6%), medie uşor mai crescută decât cea din Mureş (70,4%). Concentrarea cazurilor de TBC spitalizate se face la vârsta mijlocie (jumătate au vârsta între 40-60 ani).

Cea mai des întâlnită formă de tuberculoză este cea pulmonară (aproape 95%), cele mai frecvente cazuri (70%) fiind cele cu confirmare a diagnosticului prin examen microscopic al sputei şi cele cu investigaţii bacteriologice sau histologice negative.

Diagnosticul de tuberculoză este însoţit în jumătate de cazuri şi de comorbidităţi, cea mai frecventă fiind insuficienţa respiratorie cronică (3,89%), urmată de HTA esenţială (2,87%) şi BPOC (2,36%), asemănătoare cu datele mureşene (3,5).

Procedurile efectuate se rezumă, în general, la explorări funcţionale şi radiologice; radiografiile reprezintă mai mult de 10% din procedurile efectuate acestor pacienţi iar intervenţiile chirurgicale la bolnavii de TBC sunt rare (sub 1%).

Ponderea cazurilor fără asigurare la pacienţii cu tuberculoză este semnificativ mai mare comparativ cu ceilalţi pacienţi (13,83% faţă de 2,72%), în Mureş fiind de 10,7%.

Una din caracteristicile afecţiunii este perioada mare de spitalizare (38,59 zile), cu o medie de aproape 6 săptămâni în cazul tuberculozei faţă de o săptămână în cazul altor afecţiuni.

Tuberculoza nu este o boală înalt contagioasă. Dacă se ia în considerare infecţiozitatea pe unităţi de durată a expunerii, tuberculoza apare net mai puţin infecţioasă ca celelalte boli comunicabile ale copilului. Numai în circumstanţele ce permit un contact prelungit cu sursa eliminatorie de bacili (nedescoperirea la timp a cazurilor), în condiţiile unei aerisiri incorecte a încăperilor (condiţii de habitat deficitare) şi a unor concentraţii foarte mari în atmosfera respirată a particulelor infectante se poate ajunge la o rată mare de infecţie (9,10).

Factorii de risc de dezvoltare a unei boli active după o infecţie ţin în mare măsură de caracteristicile intrinseci ale indivizilor: vârstă, sex, conformaţie corporală (tip constituţional "ectomorfic" sau "liniar"), susceptibilitate genetică. În general, toate cauzele ce pot altera imunitatea mediată celular duc la un risc crescut de îmbolnăvire (11,12,13).

Totuşi, trebuie subliniat că vârsta este

un factor important pentru apariţia bolii, tuberculoza având o mare predilecţie pentru persoanele tinere. Faptul că tuberculoza boală apare astăzi îndeosebi la adulţi se datorează doar scăderii ratei infecţiei printre copii.

CONCLUZII

Din 2006 până în 2008, frecvenţa îmbolnăvirilor în arealul mureşan a scăzut, însă ponderea pacienţilor cu rezultate pozitive (BK+) a crescut, cu un risc de gravitate amplificat.

În lotul nostru de evaluare, pe parcursul celor 3 ani evolutiv, a scăzut numărul total de cazuri de TBC, în schimb a crescut frecvenţa cazurilor noi acute, faţă de cele cronice, au crescut cazurile de adulţi faţă de copii, precum şi situaţiile de eşec curativ.

Numărul cazurilor noi de TBC din judeţul Mureş, comparativ pe ani şi mediul urban au arătat acelaşi trend descrescător pentru toare oraşele monitorizate, cu excepţia municipiului Tg. Mureş unde numărul cazurilor noi înregistrate în 2008 a fost mai mare decât cel din 2006.

Aproape jumătate dintre cazurile de tuberculoză spitalizate au vârsta între 40 şi 60 de ani, respectiv trei sferturi dintre cazurile de tuberculoză spitalizate sunt bărbaţi

Diagnosticul de tuberculoză este însoţit în jumătate de cazuri şi de comorbidităţi, cea mai frecventă fiind insuficienta respiratorie cronică.

La peste jumătate dintre cazuri internarea se face prin trimitere de la medicul din ambulatoriu, iar durata medie de spitalizare este de 39 de zile.

Boală cu răspândire în masă, tuberculoza fiind o boală cu răspândire în masă, nu trebuie considerată numai ca boală a individului ci şi a comunităţii în care trăieşte acesta. Programele de combatere a tuberculozei, pentru a fi eficiente, trebuie să se realizeze pe întreg teritoriul ţării, să fie permanente, să răspundă cerinţelor populaţiei şi să fie integrate în structura sanitară a colectivităţii.

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FACTORI DE RISC ASOCIAŢI ÎN APARIŢIA MANIFESTĂRILOR ORALE LA PACIENŢII CU DISCRAZII SANGUINE

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ASSOCIATED RISK FACTORS IN DEVELOPING ORAL MANIFESTATIONS IN PATIENTS WITH BLOOD DYSCRASIA (Abstract) : The oral and dental complications arising in cancer patients can be attributable to the malignant disease itself and to the various modalities of cancer therapy. Up to 37.2% of all patients receiving cancer chemotherapy develop acute oral complications and up to 31.1% of all patients receiving cancer radiotherapy develop acute oral manifestations. Oral complications may result in significant morbidity, impaired nutrition, treatment delays, and dose reductions which are affecting the prognosis of the primary disease. **Material and method** : We investigated 312 cases of blood dyscrasia which 296 cases had oral manifestations (86.2%). They had a mean age of 40,6 years (range from 7 to 86 years). The male to female ratio was 1.0 : 0.75 (178/134). Data were statistically analyzed by t test, chi-square, Wilcoxon, Kruskal-Wallis and two-tailed exact Fischer, using the *SPSS* program, at a significance level of 5%. **Results** : Oral complications are frequently encountered in patients receiving anticancer therapy and these complications may result in significant morbidity, treatment delays, dose reductions, and nutritional deficiencies. The acute effects of anticancer chemotherapy upon the oral cavity include mucositis, infection, hemorrhage, xerostomia and nutritional deficiencies. Patients which undergo radiotherapy, specific in head and neck carcinomas, display a 30-60% incidence of the oral manifestations. The antineoplastic therapy will interfere with the turnover of the epithelial cells, followed by mucosal injury, later by infections, due to indirect invasion of Gram-negative bacteria and fungal species, as most of the anti-cancer drugs cause immunological changes. **Conclusions** : The mucosal surface that is the oral cavity may provide insight into the immune function of the patient. Differential diagnosis is important as many disorders may manifest themselves similarly in the buccal area. **Key words** : ORAL MANIFESTATION, CANCER THERAPY, CHEMO-THERAPY, RADIATION, BLOOD DYSCRASIA

ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM *CARUM CARVI* L. CULTIVATED IN NORTH-EASTERN ROMANIA

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ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM *CARUM CARVI* L. CULTIVATED IN NORTH-EASTERN ROMANIA (Abstract): Essential oils have significant antioxidant activity, being used frequently as preservatives in the food and cosmetic industries. **Aim**: To assess the *in vitro* antioxidant activity of essential oil from *Carum carvi* L. (caraway) cultivated in northeastern Romania. **Materials and Methods**: The essential oil was isolated by hydrodistillation from dried caraway fruits. The chemical composition was investigated by gas chromatography and gas chromatography–mass spectrometry analyses. Antioxidant activity was evaluated by three different *in vitro* antioxidant assays (DPPH· and $ABTS^{\bullet+}$ scavenging and reducing power assays). Butylhydroxyanisole was used as reference standard. **Results and Discussion**: Carvone was the major compound of essential oil of caraway fruits (48.53%), followed by limonene (44.42%). Evaluation of the antioxidant activity (DPPH·and $ABTS^{\bullet+}$ scavenging activity and reducing power) revealed significant effects, with IC_{50} values of 46.51 ± 1.61 µg/mL, 5.34 ± 0.07 µg/mL and 7.64 ± 0.22 µg/mL, respectively, as compared to those of the reference standard, butylhydroxyanisole (6.09 ± 0.27 µg/mL, 1.49 ± 0.00 µg/mL and 3.39 ± 0.07 µg/mL, respectively). **Conclusions**: Essential oil of *Carum carvi* cultivated in northeastern Romania belongs to carvone chemotype. Due to its high antioxidant activity it might be a potential alternative to conventional preservatives in the food, cosmetic and pharmaceutical industries. **Keywords:** *CARUM CARVI* L., CARVONE, ESSENTIAL OIL, ANTIOXIDANT ACTIVITY.

Medicinal plants are used for thousands of years due to their content in bioactive compounds, endowed with therapeutic and nutritional effects. Among these compounds, essential oils have been targeted for investigation with regard to their biological properties, especially concerning their antioxidant activity and their potential use as preservatives in the food and cosmetic industries (1,2). Essential oils have a complex chemical composition, therefore their antioxidant properties are related to interactions among constituents, which may act synergistically or exhibit antagonistic effects (3).

The antioxidant activity of essential oils is well documented (3,4); however, their uses can be hampered due to the chemical variability related to several factors includ-

ing climatic and agronomic conditions, soil, harvesting time, plant vegetative development and type of isolation (3,5).

Carum carvi L. (Apiaceae) has a long history of use in phytotherapy due to its pharmacological effects (spasmolytic, antifoaming, galactagogue and antimicrobial), but also as spice and flavoring agent in food products (6). The dried fruits contain essential oil (3-7%), with carvone (40-65%) and limonene (30-45%) as main constituents (7).

Continuing our previous researches focused on the influence of agricultural techniques (plant spacing) on the yield and chemical composition of essential oils of several spices (8,9), in this study we aimed to assess the antioxidant activity of essential oil isolated from *Carum carvi* L. cultivated in northeastern Romania.

MATERIAL AND METHODS

Chemicals. All solvents and chemicals were of analytical grade and were purchased from Sigma–Aldrich Chemie (Steinheim, Germany), Merck (Darmstadt, Germany) and Riedel-de Haën (Seelze, Germany).

Plant material. Caraway fruits were collected from biennial plants cultivated in the field of Agricultural Research and Development Center, Secuieni, Neamt county (Romania). A voucher specimen was deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy *Grigore T. Popa* - Iasi.

Isolation of the essential oil. The essential oil was isolated by hydrodistillation of powdered dried fruits in a Clevenger-type apparatus (3 h) (10), dried over anhydrous sodium sulphate and stored in a sealed glass at -4°C until analysis.

Chemical analysis of the essential oil. Gas chromatography–mass spectrometry (GC–MS) analysis of *C. carvi* essential oil was performed on an Agilent type 7890A gas chromatograph equipped with an Agilent 5975C mass spectrometer and a DB-5MS capillary column (30 m×0.25 mm, 0.25 μm film thickness). The oven temperature was increased with a rate of 10°C/min from 35°C to 280°C (kept isothermally for 7.5 min). The other characteristics of the analysis program were: inlet temperature: 250°C; split ratio: 50:1; carrier gas: helium (flow rate: 1 mL/min); injected volume: 0.2 μL of essential oil. *Gas chromatography (GC) analysis* of *C. carvi* essential oil was carried out using an Agilent 6890 gas chromatograph equipped with a flame ionization detector. The column and GC conditions were the same as the ones described above. *Identification of volatile components.* Individual essential oil components were identified by comparison of their retention indices (RI) relative to (C8–C20) *n*-alkanes with those reported in the literature, and by matching their recorded mass spectra with with those stored in the Wiley 275 mass spectral library from GC-MS database. Quantitative data were obtained from the electronic integration of the FID peak areas.

In vitro antioxidant activity analysis. The antioxidant activity of the essential oil from *C. carvi* fruits was assessed by three methods previously reported (11,12), namely DPPH radical and ABTS radical cation scavenging assays and reducing power test.

In all antioxidant assays butylhydroxyanisole (BHA) was used as positive control. Results were expressed as IC_{50} values (μg/mL), which was calculated through linear interpolation between values above and below 50% activity, except for the

reducing power assay, where IC_{50} value represents the concentration of essential oil/positive control that leads to an absorbance of 0.5. A lower IC_{50} value corresponds to a higher antioxidant activity.

RESULTS AND DISCUSSION

Chemical composition of the essential oil. GC analysis of the essential oil from caraway fruits cultivated in northeastern Romania revealed the presence of twenty compounds, accounting for 98.82% of the total essential oil composition (tab. I).

Carvone (48.53%) was the major compound of caraway fruit essential oil, followed by limonene (44.42%). The chemical class characterization showed that *Carvi aetheroleum* is mainly composed of monoterpene ketones (48.89%), represented by carvone, *cis*-dihydrocarvone and *trans*-dihydrocarvone, and monoterpene hydrocarbons (44.84%) with limonene as main constituent, followed by monoterpene aldehydes (3.09%) and low levels of monoterpene alcohols, oxides and esters, sesquiterpenes and aliphatic compounds (tab. I).

Several factors, including location, genotype, nutrient supply and growing conditions are known to influence carvone and limonene content in caraway fruits (13). Our results are in accordance with those of Laribi *et al.* (14), who reported the predominance of carvone and limonene in essential oils of Tunisian, German and Egyptian caraway ecotypes. Our study revealed that the Romanian caraway essential oil belongs to the carvone chemotype, similar to the caraway essential oils from Tunisia (76.78–80.53% carvone) (15), Serbia (32.3% carvone) (16) and Czech Republic (72.55–81.76%) (17). As carvone is an important flavouring agent in the food industry and has important therapeutic properties (18), Romanian caraway fruits are an important source for the isolation of this monoterpene ketone.

TABLE I

Essential oil composition of caraway fruits from northeastern Romania

RI	Compound	%
929	α-thujene	tr
938	α-pinene	0.03
978	sabinene	0.06
983	β-pinene	0.01
991	myrcene	0.32
1004	octanal	0.07
1036	limonene	44.42
1049	*trans*-β-ocimene	tr
1063	γ-terpinene	tr
1102	L-linalool	0.49
1138	*cis*-limonene oxide	0.12
1142	*trans*-limonene oxide	0.25
1196	*cis*-dihydrocarvone	0.23
1203	*trans*-dihydrocarvone	0.13
1219	*trans*-carveol	0.25
1238	L-carvone	48.53
1288	perilla-aldehyde	3.09
1323	methyl geranate	0.59
1419	β-caryophyllene	0.19
1479	germacrene D	0.03
	Chemical classes	
	Monoterpene hydrocar-	44.84
	Monoterpene ketones	48.89
	Monoterpene aldehydes	3.09
	Monoterpene alcohols	0.74
	Monoterpene oxides	0.37
	Monoterpene esters	0.59
	Sesquiterpenes	0.22
	Aliphatic compounds	0.08
	Total identified	**98.82**

RI - Retention index relative to C8–C20 *n*-alkanes on a DB-5 column; tr=traces (< 0.01%)

In vitro antioxidant activity. Antioxidant activity is very complex, involving a sum of different mechanisms, such as free radical scavenging, prevention of hydrogen abstraction, reducing capacity and binding of transition metal ions (3). Essential oils are complex mixtures of bioactive compounds endowed with multifunctional properties as they have different functional groups and chemical behavior (1). Therefore, the antioxidant activity of *Carvi aetheroleum* was assessed by three different *in vitro* antioxidant assays (DPPH· and $ABTS^{\bullet+}$ scavenging assays and reducing power test) and compared with the activity of a commonly used synthetic antioxidant, BHA (tab. II).

The radical scavenging capacity of the essential oil increased in a concentration-dependent manner (data not shown). Although the DPPH and ABTS methods are based on the same principle, *Carvi aetheroleum* was more potent in reducing the preformed $ABTS^{\bullet+}$ (IC_{50} = 5.34 ± 0.07 µg/mL) than reducing DPPH· to $DPPH_2$ (IC_{50} = 46.51 ± 1.61 µg/mL). In the reducing power assay, caraway essential oil was able to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in a dose-dependent manner (data not shown) and its IC_{50} value (7.64 ± 0.22 µg/mL) was only 2.2 fold lower than that of the positive control, BHA (3.39 ± 0.07 µg/mL) (tab. II).

However, compared with the standard BHA, in all antioxidant assays caraway essential oil showed significant antioxidant activities (tab. II). A survey of the literature showed that there are several reports on the antioxidant activity of *C. carvi* essential oil (4,19,20), but a direct comparison of our results with those reported in other studies was not possible due to the different experimental protocols and different ways of expressing antioxidant activity.

TABLE II

Antioxidant activities of essential oil of *Carum carvi* fruits cultivated in North-Eastern Romania

Essential oil/positive control	IC_{50} (µg/mL)		
	DPPH· scavenging assay	$ABTS^{\bullet+}$ scavenging assay	Reducing power assay
C. carvi essential oil	46.51 ± 1.61	5.34 ± 0.07	7.64 ± 0.22
BHA	6.09 ± 0.27	1.49 ± 0.00	3.39 ± 0.07

CONCLUSIONS

Our study revealed that the essential oil of *Carum carvi* L. cultivated in northeastern Romania belongs to carvone chemotype. Due to its high antioxidant activity it might be an alternative to conventional preservatives in the food, cosmetic and pharmaceutical industries.

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CATECHINS PROFILE, CAFFEINE CONTENT AND ANTIOXIDANT ACTIVITY OF *CAMELLIA SINENSIS* TEAS COMMERCIALIZED IN ROMANIA

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CATECHINS PROFILE, CAFFEINE CONTENT AND ANTIOXIDANT ACTIVITY OF *CAMELLIA SINENSIS* TEAS COMMERCIALIZED IN ROMANIA (Abstract). **Aim**: Catechins profile, caffeine content and antioxidant activity of different green tea and white tea samples commercialized on the Romanian market were investigated. **Material and methods**: Five green tea samples and five white tea samples commonly available on the Romanian market were processed by infusion and the lyophilisates of infusions were analyzed. Total phenolic content was determined using the Folin-Ciocalteu method. Catechins and caffeine profile was analyzed by RP-HPLC-DAD (Agilent Eclipse XDB-C18 column, binary mobile phase (A) 3% acetic acid and (B) methanol). *In vitro* antioxidant activity was assessed by free radical scavenging and ferrous ion chelating assays. **Results and discussions**: Total phenolic content ranged between 44.73±0.63 and 63.57±0.45 GAE% in green tea samples and between 9.69±0.90 and 52.99±0.45 GAE% in white tea samples. RP-HPLC-DAD analysis allowed the identification of epigallocatechin gallate (45.18-118.58 mg/g lyophilisate) and caffeine (47.79-108.07 mg/g lyophilisate) in all tea samples; epicatechin was detected in all samples (5.04-31.04 mg/g lyophilisate) except for two white teas infusions. Green tea samples scavenged DPPH radical and chelated ferrous ion with EC_{50}=9.68±0.02-16.11±0.02 µg/mL and 10.91±0.04-18.65±0.03 µg/mL, respectively. For white teas, EC_{50} values varied between 9.50±0.02-20.95±0.02 µg/mL in DPPH assay and 12.49±0.03-20.32±0.07 µg/mL in ferrous ion chelating assay. **Conclusions**: This study showed a large variability in the content of catechins and caffeine and in the antioxidant capacity of both green and white tea samples. **Keywords**: CATECHINS, CAFFEINE, GREEN TEA, WHITE TEA, ANTIOXIDANT.

Tea is the second most consumed beverage in the world. All types of tea come from the leaves of *Camellia sinensis* (L.) Kuntze (Theaceae), a plant originally native to China, Thailand, Laos and Vietnam (1). Based upon the degree of fermentation, tea can be classified into unfermented tea (white tea and green tea), semi fermented tea (oolong tea) and fully fermented tea (black tea and Pu-erh tea) (2). Tea provides complex chemical substances, like: flavanols (catechins), flavonols and flavones glycosides, proantocyanidols, bisflavanols, theaflavins, thearubigins, theasinensins, caffeine, amino acids and minerals. Catechins are the representative class of compounds from green and white tea, with epigallocatechin-3-O-gallate (EGCG) as the most abundant constituent

(50-80% of the total catechins). Among polyphenols, catechins are primarily responsible for almost all the beneficial effects of tea: antioxidant, chemopreventive, neuroprotective, radioprotective, antimicrobial, anti-aging, hypoglycemiant, and hypolipemiant. Caffeine is the main purine alkaloid of tea with CNS stimulant effects (3).The chemistry and quality of tea are significantly influenced by the environmental conditions of the regions where the plants grow, sampling time, cultivation and processing practices. Therefore, numerous studies have reported a considerable variability among different tea types and brands (2).

The aim of this study was to analyze the catechins profile, caffeine content, and antioxidant activity of different green and white tea samples commercialized on the Romanian market.

MATERIAL AND METHOD

Chemicals

Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid, and methanol were purchased from Merck (Darmstadt, Germany); Folin-Ciocalteu's phenol reagent, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (ferrozine), and epicatechin (EC) were purchased from Fluka (Steinheim, Germany); iron (II) sulfate heptahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, acetic acid, gallic acid, and epigallocatechin gallate (EGCG) were obtained from Sigma-Aldrich (Steinheim, Germany); caffeine was supplied by Carl Roth (Karlsruhe, Germany).

Plant material

Five green tea samples and five white tea samples of different origins (China, India, Vietnam), of different varieties (Bi Luo Chan, Darjeeling, Fanning, Pai Mu Tan), and in different packages were purchased from a local market in Iasi, Romania (tab. I). Voucher samples are deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa" – Iaşi, Romania.

TABLE I

Sample numbers, varieties, origins and types of package

Sample		Variety	Origin	Type of package
Green teas	GT1	BI LUO CHAN	China	Bulk
	GT2	DARJEELING	India (packaged in Austria)	Bulk
	GT3	FANNING	Vietnam	Sachet
	GT4	Unknown	China	Bulk
	GT5	BI LUO CHAN	China	Bulk
White teas	WT1	PAI MU TAN	China (packaged in Austria)	Bulk
	WT2	PAI MU TAN	China (packaged in Czech Republic)	Bulk
	WT3	Unknown	China (packaged in Canada)	Sachet
	WT4	PAI MU TAN	China (packaged in Austria)	Sachet
	WT5	PAI MU TAN	China	Bulk

Bi Luo Chun is one of the most famous ten Chinese teas originated in Dong Ting Mountain (Jiangsu Province). Its specific flavor is determined among other things by various fruit trees (apricot, peach, plum) which are found on tea plantations. After

harvest, the leaves are manually sorted, treated with heat (190-200 °C) for 3-5 min, and then rolled. Darjeeling tea is obtained from plantations located in the region with the same name in West Bengal, India, and it is distinguished by its unique flavor. Plant material consists only of the first two leaves from the top or from the apical buds. The processing of leaves is achieved by exposing them to a hot air flow and subsequent mechanical twisting. In the case of Pai Mu Tan tea ("white peony"), after harvest, tea leaves are sun-dried or dried in closed rooms, without any further technological intervention (4).

Extraction

2 g of tea leaves were placed into boiled water (200 mL) and incubated at 80-90 °C for 3 min. This condition was similar to an actual brewing condition for a cup of tea. After cooling, each sample solution was filtered through Whatman filter papers. Each extract was concentrated under reduced pressure at < 40 °C (Büchi R-210 rotary evaporator, Büchi V-850 vacuum controller, Büchi V-700 vacuum pump, Büchi B-490 heating bath, Büchi, Switzerland) and then freeze-dried (Alpha 1-2 LD Plus freeze-dryer, Christ, Germany).

Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu assay. The results were expressed as g gallic acid equivalents (GAE)/100 g extract (5).

RP-HPLC-DAD analysis

Reversed-phase high performance liquid chromatography (RP-HPLC) analysis was conducted on an Agilent 1200 HPLC system (Agilent Technologies, PaloAlto, CA, USA) equipped with a DAD detector. The chromatographic separations were performed on an Agilent Eclipse XDB C-18 (150 mm x 4.6 mm, i.d. 5 µm) column, with column temperature set at 20 °C. The binary mobile phase consisted of (A) 3% acetic acid and (B) methanol; the flow rate was 1 mL/min; the detection wavelength was set at 280 nm. The gradient elution profile started with 0% B; then B was gradually increased to 5% at 10 min, to 10% at 35 min, to 50% at 45 min, maintained at 50% at 50 min, and decreased back to 0% at 55 min [6]. Catechins and caffeine were identified by comparing their UV spectra and retention times with those of commercial standards. EGCG, EC and caffeine were quantified on the basis of their calibration curves (0.125-1 mg/mL), and the results were expressed as mg compound/g lyophilisate. The calibration curves were: for EGCG: A=1227.3c-69.206, R^2=0.9997; for EC: A=504.72 c+19.928, R^2=0.9998; for caffeine: A=1790.4c+9.114, R^2=1.

DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was performed according to Malterud et al (7, 8, 9). Epicatechin was used as positive control. The radical-scavenging activity (%) was calculated as follows: %=[(absorbance of DPPH solution - absorbance of DPPH solution with sample after 5 min)/absorbance of the DPPH solution]×100.

Ferrous ion chelating ability assay

This assay was done according to Dinis et al (10). Epicatechin was the positive control. The percent inhibition of Fe^{2+}-ferrozine complex formation was calculated according to the following equation: % inhibition=[(absorbance of control - absorbance of sample)/absorbance of control]×100.

Statistical analysis

All experiments were conducted in triplicate. The results were expressed as mean

± standard deviation (SD). The EC_{50} values were calculated by linear interpolation between values above and below 50% activity.

RESULTS AND DISCUSSION

Phenolic content and profile

The total phenolic content ranged between 44.73±0.63 and 63.57±0.45 g GAE% in green tea samples and between 9.69±0.90 and 52.99±0.45 g GAE% in white tea samples, respectively (fig. 1). Green tea samples contained higher amounts of polyphenols compared to white tea samples. RP-HPLC-DAD analysis revealed the presence of EGCG in all infusions; EC was detected in all samples except for two white tea samples (WT1 and WT5) (fig. 2). Also, caffeine was identified in all tea samples. EGCG was the major catechin in tea samples; it has been found in concentrations between 49.85 and 118.58 mg/g lyophilisate in green teas. In white tea samples, EGCG concentrations were lower (45.18-85.01 mg/g lyophilisate), and four samples had a concentration of less than 65 mg EGCG/g lyophilisate (fig. 3). Although green tea contained higher amounts of EGCG, the variability of its content was much higher than in white tea samples. This may be due to the fact that commercial samples of green tea were more heterogeneous (Darjeeling, Fanning, Bi Luo Chun) while white tea samples were predominantly Pai Mu Tan. Compared to EGCG, the EC content values were much lower (5.04-31.04 mg/g lyophilisate) (fig. 4); GT4 green tea sample contained the highest concentration of EC. The content of EC can vary up to 6 times between the two types of tea.

Except WT2 sample that contained 27.79 mg EC/g lyophilisate, all other white tea samples showed lower concentrations of EC (5.04-8.76 mg/g lyophilisate) compared to green teas; therefore, white tea was characterized by a more pronounced heterogeneity. Regarding caffeine content, all samples of green tea had at most 65.19 mg/g lyophilisate (GT3); in white tea, the amounts of caffeine were found to be higher (67.44-108.07 mg/g lyophilisate) (fig. 5).

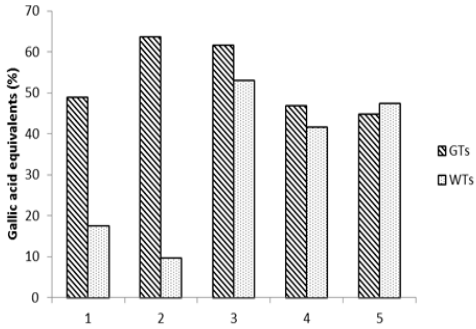

Fig. 1. Total phenolic content of tea samples

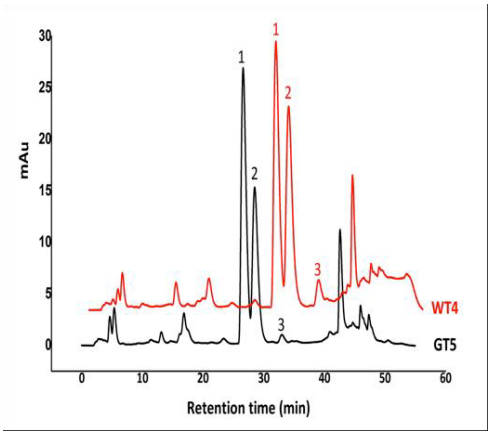

Fig. 2. RP-HPLC-DAD of tea samples (1-caffeine, R_t=29.49 min; 2-EGCG, R_t=32.69 min; 3-EC, R_t=37.36 min; GT-green tea, WT-white tea)

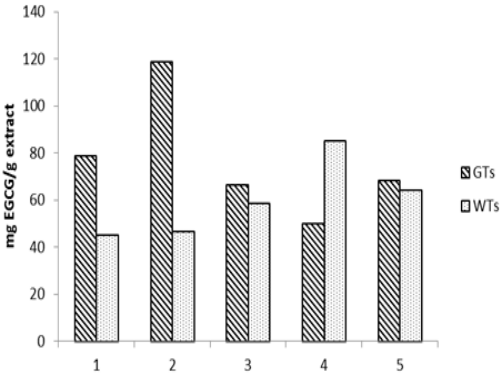

Fig. 3. EGCG concentration of tea samples

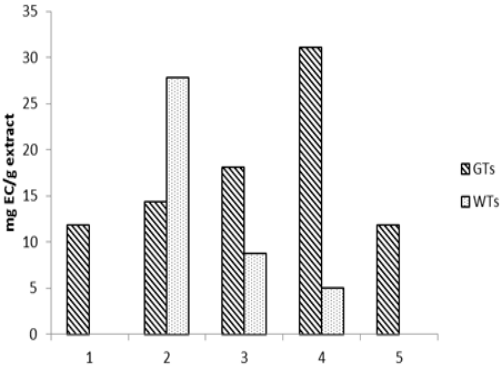

Fig. 4. EC concentration of tea samples

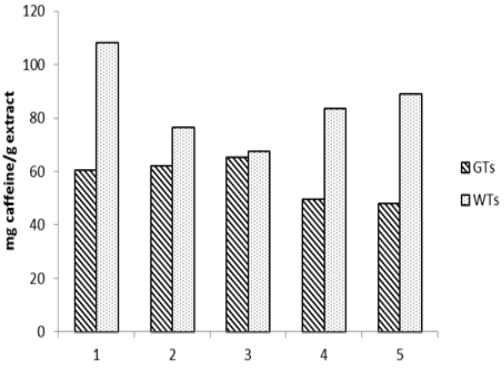

Fig. 5. Caffeine concentration of tea samples

DPPH radical scavenging assay

Both types of tea scavenged DPPH radical in a concentration dependent manner; at the concentration of 41.66 μg/mL, all samples with the exception of WT2 showed a DPPH radical scavenging activity higher than 90% which was similar to that of the positive control (EC) (tab. II).

At the concentration of 10.41 μg/mL, DPPH radical scavenging activity of samples decreased reaching values lower than 50%. Therefore, at low concentrations we noticed a greater heterogeneity of DPPH radical inhibitory activity: between 30.26% and 64.15% for green tea samples, and between 23.28% and 53.55% for white tea samples, respectively. DPPH scavenging activity of white tea samples was lower than that of green teas. The samples of white tea showed a greater variability of free radical scavenging activity (EC_{50}=9.50±0.02-20.95±0.02 μg/mL) than that of green tea (EC_{50}=9.68±0.02-16.11±0.02 μg/mL) (tab. II).

Ferrous ion chelating ability assay

Ferrous ion chelating abilities of both types of tea samples increased with concentration. At the concentration of 41.66 μg/mL, all tea samples showed the highest values of ferrous ion chelating activity (77.79±0.20%-92.26±0.19% for white teas and 88.35±0.21-99.31±0.16% for green teas, respectively) (tab. III). Overall, green tea samples were more active as ferrous ion chelating agents (EC_{50}=10.91±0.04-18.65±0.03 μg/mL) than white teas (EC_{50}=12.49±0.03-20.32±0.07 μg/mL). Also, some of the samples, either green tea or white tea, were even more potent than the positive control, EC (EC_{50}=15.44±0.01 μg/mL) (tab. III).

TABLE II
DPPH scavenging activity of tea samples

Sample/ Positive control	DPPH scavenging activity (%)±SD			EC_{50}±SD (µg/mL)
	41.66 µg/mL	20.83 µg/mL	10.41 µg/mL	
GT1	94.59±0.06	73.52±0.04	47.7±0.11	11.14±0.01
GT2	94.82±0.11	90.92±0.08	64.15±0.08	9.68±0.02
GT3	94.29±0.03	77.33±0.08	47.19±0.12	10.92±0.01
GT4	92.87±0.04	78.40±0.14	30.26±0.16	16.11±0.02
GT5	91.65±0.08	69.95±0.11	33.63±0.11	15.39±0.01
WT1	93.42±0.08	57.89±0.09	34.97±0.11	14.87±0.01
WT2	78.98±0.10	42.67±0.05	23.28±0.04	20.95±0.02
WT3	93.25±0.04	80.02±0.06	41.99±0.13	12.92±0.02
WT4	92.65±0.18	68.89±0.08	53.55±0.15	9.50±0.02
WT5	93.97±0.12	81.83±0.05	49.30±0.21	10.93±0.02
EC	95.64±0.08	94.93±0.08	60.24±0.03	8.33±0.01

TABLE III
Ferrous ion chelating activity of tea samples

Sample/ Positive control	Ferrous ion chelating activity (%)±SD			EC_{50}±SD (µg/mL)
	41.66 µg/mL	20.83 µg/mL	10.41 µg/mL	
GT1	98.12±0.16	63.34±0.21	40.91±0.10	12.97±0.03
GT2	96.49±0.14	80.17±0.12	40.17±0.20	13.28±0.05
GT3	99.31±0.16	69.80±0.16	48.28±0.16	10.91±0.04
GT4	88.35±0.21	49.14±0.04	22.15±0.01	18.65±0.03
GT5	88.67±0.20	58.89±0.02	32.63±0.11	16.12±0.05
WT1	78.39±0.04	52.46±0.08	28.52±0.06	19.77±0.04
WT2	77.79±0.20	45.64±0.08	24.17±0.19	20.32±0.07
WT3	88.92±0.13	58.50±0.20	41.70±0.04	13.21±0.02
WT4	84.81±0.10	67.69±0.09	38.91±0.11	13.59±0.02
WT5	92.26±0.19	55.15±0.09	43.59±0.08	12.49±0.03
EC	94.56±0.08	71.73±0.02	21.93±0.06	15.44±0.01

CONCLUSIONS

In our study, samples of green tea were characterized by a richer content in total polyphenols (that can be almost 5 times higher) compared to white teas. EGCG and EC were found in higher concentrations in green teas. EGCG content showed a higher degree of variability in green tea samples

while EC content exhibited a great heterogeneity in white teas. The highest concentrations of caffeine, exceeding 65 mg/g lyophilisate, were identified in white teas samples. Overall, green tea samples were more active as free radical scavengers and ferrous ion chelating agents than white tea samples.

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PATIENTS' PERCEPTION TOWARDS INFECTION TRANSMISSION IN DENTAL PRACTICES IN IASI, ROMANIA

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PATIENTS' PERCEPTION TOWARDS INFECTION TRANSMISSION IN DENTAL PRACTICES IN IASI, ROMANIA (Abstract): **Aim**: To assess patients' perception and attitudes concerning the risk of infection transmission in dental practices in Iasi, Romania. **Material and methods:** A questionnaire-based study was conducted amongst 280 patients aged between 21 and 62 in 32 private dental offices in Iasi, Romania. The 16 questions were related to patients' attitude towards the risk of infection and their involvement in assessing the safety protocols. The data were analyzed by gender, age and education level using the *SPSS 15.0* statistical package ($p<0.05$) and Chi square test. **Results:** Most patients (82.6%) manifest their trust in the medical staff to protect them against the spread of infection. 10,9% of the subjects avoid dental care as a result of the risk of getting infected. 46,5% of the patients are concerned about the safety measures used during their dental treatment. Women desire to involve themselves in assessing the implementation of infection control measures more than men (63.6%, 50.0%, respectively). HIV infection, viral hepatitis B and C and influenza are considered by the patients to be the most frequently transmitted during dental treatment. Older subjects (81.4%) and those with secondary educational level (67.3%) do not consider that their implication in infection control is necessary. The dental assistant is associated with infection transmission by 62.7% of patients. **Conclusions**: The assessment of patients' specific requirements and needs is essential in order to increase the medical team knowledge and awareness regarding the level of patient safety. **Keywords:** PATIENT PERCEPTION, DENTAL PRACTICE, INFECTION PREVENTION.

The high quality standards of modern dentistry imply major demands regarding patient safety and infection risk management. The patient safety is a complex concept involving social, economical, financial, cultural, and management issues with major benefits regarding the quality of the dental treatment (1). In the dental office the germs can be transmitted by direct contact with blood and contaminated saliva, indirectly through contaminated objects (surfaces, instruments or equipments) but also from one patient to another by medical personnel (cross-infection) (2).

The advanced specific dental procedures, the unique relationship between the patient and the dentist, as the medical technological progress require not only a continuous adaptation of infection control protocols but also an increased concern of the medical staff and public in this domain (3).

The actual public high level of health

education influences the patient requirements regarding the protection from infectious diseases like HIV infection, viral hepatitis B and C, tuberculosis and respiratory infections. The educated patient's participation in his own treatment must increase the responsibility of the dentist and his team for the safety of the dental procedures (4).

MATERIAL AND METHODS

The study based on an anonymous questionnaire included 280 patients in 32 private dental practices in Iasi, Romania.

The self-administrated questionnaire comprised 16 questions regarding the patient's trust in the medical team, the desire to assess medical procedures safety, dentist's protective equipment, attitudes towards infection control, knowledge concerning diseases which can be transmitted during dental treatments and procedures with high risk of infection (tab. I). Patients were asked to answer the survey questions in the waiting room of the dental offices and their participation was voluntary.

TABLE I.

Questionnaire for patients' opinion assessment

No.	*Question*
1.	Do you trust the medical staff to protect you against the transmission of infectious diseases during the dental treatment?
2.	Do you think you could get sick during dental treatment?
3.	Do you avoid the dental treatment because of the fear of getting infected?
4.	Do you consider you have to get involved in checking the measures to prevent infection transmission in the dental office?
5.	Did you discuss with your dentist about the risk of contracting infectious diseases throughout the dental treatment?
6.	Did you watch the application of the protocols for preventing the transmission of infectious diseases during dental treatments?
7.	Which are the diseases that pose a threat during dental treatment : hepatitis A, hepatitis B, hepatitis C, HIV infection, tuberculosis, influenza?
8.	Do you accept to be treated in the same dental office where an HIV infected patient was treated?
9.	Do you consider that the dentist appearance increases the confidence in the safety of the dental treatments?
10.	Do you want the dentist to wear protective equipment (gown, mask, gloves)?
11.	Do you consider that these equipments protect you against the infection transmission?
12.	Which are the dental instruments associated with a high risk of disease transmission during dental treatment: dental drills, endodontic needles, scaling instruments, dental extraction instruments, scalpels?
13.	Which are the factors incriminated in infection transmission (instrument sterilization failure, surfaces and equipments disinfection deficiencies, poor compliance of dental personnel to protective equipment)
14.	Which of the following maneuvers are important in preventing infection transmission during dental treatment: medical staff hand washing, use of an individual kit of instruments for each patient, dental unit disinfection, changing the glass for mouth rinses for each patient, changing the mask and gloves after each patient?
15	Do you think dental assistant may be involved in the transmission of the infectious diseases during dental treatment?
16.	Do you consider the dental assistant should wear protective equipment (gown, mask, gloves)?

A cross-sectional epidemiological study was initiated, the data being analyzed by age, gender and educational level. The data was processed using the *SPSS 15.0* statistical package (statistical significance $p<0.05$) and *Chi square test.*

RESULTS

The survey included 37% men and 63% women aged between 21 and 62. Depending on age, the structure of the study group was as follows: 41, 7% in the 20-30 years old group; 28.4%, 31-40; 20.7%, 41-50 and 9.2% in the over 51 years old group (tab. II).

TABLE II

Structure of the patients study group by age

No	*Age group (years)*	*Percentage of patients (%)*
1.	20-30	41.7
2.	31-40	28.4
3.	41-50	20.7
4.	over 51	09.2
	TOTAL	100.0

Regarding the educational level, 26.5% of the subjects were highly educated, 45.2% had a secondary level education and 28.3% a primary level of education.

The data from the questionnaires revealed that most of the subjects (87.3%) trust the dental medical professionals to protect them against infectious diseases transmission. 10.9% of the respondents avoid dental treatment because they feel exposed to infections. 1.8% of the patients had no opinion on this matter.

Men showed a higher level of confidence in the medical staff than women (94.5%, 75.8% respectively) (fig.1).

About half of subjects (46.5%) expressed their interest in infection control protocols applied after each patient (environmental surface disinfection, changing the protective equipment, hands hygiene). Among those, the majority are young active people included in the groups of 20-30 years of age (44.8%) and 31-40 years of age (37.3%). Regarding the gender, women desire to involve themselves in assessing the implementation of infection control measures more than men (63.6%, 50.0%, respectively). A significant proportion of older subjects (81,4%) and of those with secondary educational level (67.3%) do not consider that their implication in infection control is necessary. 1.9% of the subjects are not interested in those aspects and 12.7% admit that they do not have the necessary knowledge in this domain (fig. 2).

In terms of patient–doctor communication, 27.5% of the patients have discussed with their dentist about the risk of contracting infectious diseases throughout the dental treatment, women being more interested in this topic than men (43.4% and 28.3%, respectively). Patients in the 31-40 age groups are the most interested to receive information about the risk of diseases transmission and the methods to decrease this risk (58.5%).

The severe diseases as HIV infection, viral hepatitis B and C, but also influenza and tuberculosis are considered by the patients to be frequently transmitted during dental treatment (100%, 76.3%, 82.5%, 96.5% and 23.7%, respectively) (fig.3). The HIV infection is a major concern for subjects with a secondary level of education (82.7%) while the highly educated patients give a greater importance to the risk to get infected with hepatitis B and C viruses (75.4%).

Regarding the attitude towards HIV infection, 86.5% of patients do not accept to get theirs treatment in a dental office in which an infected patient was previously attended to.

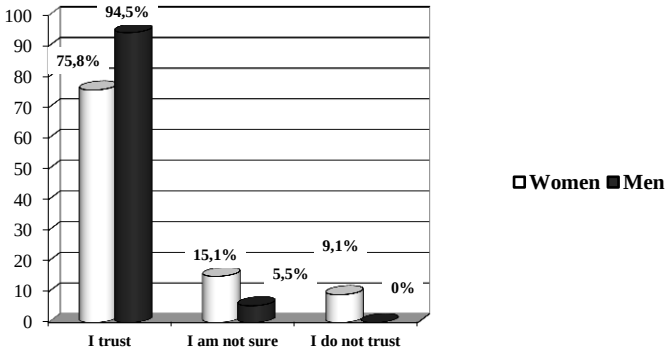

Fig.1. Dental patients' confidence in dentist by gender

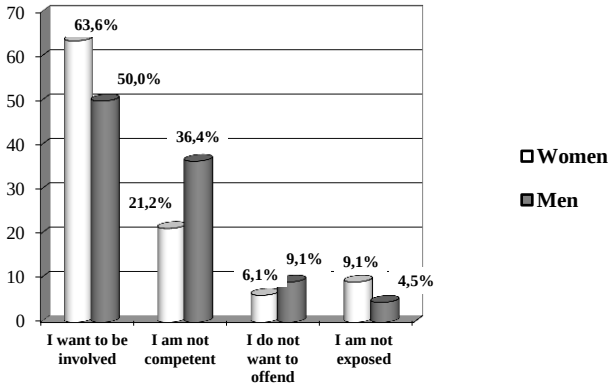

Fig.2. Patients' involvement desire in infection control by gender

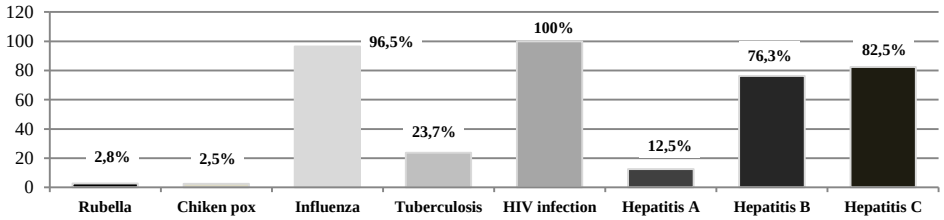

Fig. 3. Infectious diseases considered as having a high risk of transmission

The subjects' answers demonstrate that most of them desire the medical team to always use the protective equipment: rubber gloves (88.0%), face mask (63.6%) and protective glasses (47.2%); doctor's hair protection is a concern for only 27.7% of the patients. 97.3% of the patients feel protected from infectious diseases by the use of those barriers, with no significant differences by gender, age or education level.

Most of patients (67.8% women and 76.8% men) believe that dental assistant is involved in the transmission of infection and should use the same equipment as the dentist.

A significantly high percentage (65.5%),

especially highly educated individuals and women, appreciate that the patient's satisfaction over the medical care quality and safety is increased by the dentist's professional attire. 23.6% would like the traditional white gown and over half of the patients would like the doctor to wear an ID badge.

The failure of instruments' sterilization as well as the deficiencies in surfaces and equipments disinfection in the dental practice are regarded by most patients as the main cause of infection transmission (80.0% and 54.5%, respectively).

The most efficient infection control methods considered to ensure the safety in the dental practice are: dental personnel hands washing (78.2%), environmental surfaces disinfection (56.4%) and the safe manipulation of the instruments between patients (45.5%).

DISCUSSION

Given the complexity of clinical procedures in the dental office, the patient safety and protection represent an important challenge during oral health care. The dental medical personnel must take the proper measures in order to avoid the risk of infection transmission by applying the needed methods and protocols in the spirit of the Standard Precautions concept. In this context the patient must be considered as a partner in his own oral health care whose needs and opinions should be respected. The results of our study highlight that majority of patients trust the medical staff to protect them by applying the needed infection control procedures. However, a high percentage of the patients, especially men, expressed their concern about catching an infection during dental treatment. Our results are similar to those reported by Smith et al. regarding the patients' perceptions of risk, the major concerns of patients revolving around hand hygiene practices rather than risks from bloodborne viruses and Creutzfeldt-Jacob Disease (5).

The young subjects and women mainly expressed their concerns regarding the taken measures to reduce the risk of infection transmission, whereas the older patients claim their lack of knowledge in this domain or the desire to avoid offending the medical staff.

The dentist's protective equipment is perceived to have major benefits in patient's safety by acting as a barrier against contaminated fluids, especially blood and saliva. Rubber gloves are considered the most efficient barrier but the facial mask and the protective glasses are also required, the results of our study being similar to those reported in the literature. In his study Barghout reported that 83.5% of the respondents are of the opinion that the dentist must wear gloves and 74.8% want him to wear facial masks (6). These results demonstrate a positive, encouraging evolution of the public knowledge regarding the safety of the dental treatment compared, for example, with the data obtained by Samaranayake who reported in 1990 for the Glasgow region that the majority of the patients were not interested at all in the protective equipment (gloves or masks) and one third of them lacked knowledge about dental instruments sterilization methods (7).

The patients expressed their desire to get involved in the dental care in a low percentage as a consequence of their lack of knowledge in the domain of infection transmission risk, but also due to the confidence in the professionalism and responsibility of the medical team. The highly educated patients wish to be informed and to have the opportunity to evaluate the infection control protocols used in the dental office.

Regarding the dentist's professional at-

tire our findings confirm the results of other investigations revealing the patients' preferences for traditional medical appearance (8,9). Highly educated subjects consider that the doctor's appearance reflects the level of his professional training and is related to treatment quality and safety. The majority of patients want the medical team to wear an ID badge which is seen as a sign of assuming the medical procedure responsibility, a proportion similar to that reported by Shulman in his survey involving 1500 subjects (10). An effective communication with the dentist and a patient-dentist relationship based on trust and respect is considered, especially by the patients with a high level of education, to ensure the increased quality level of the dental treatment (11,12).

CONCLUSIONS

The results of our study demonstrate that majority of patients trust the dentist to use the proper safety measures and this fact should motivate the medical team in complying with the safety standards. The beneficiaries of oral health care have a realistic perception regarding the importance of the protective equipment and should be encouraged to request, accept and supervise the safety procedures in the dental practice.

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FIXED DOSE COMBINATIONS WITH SELECTIVE BETA-BLOCKERS: QUANTITATIVE DETERMINATION IN BIOLOGICAL FLUIDS

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FIXED DOSE COMBINATIONS WITH SELECTIVE BETA-BLOCKERS: QUANTITATIVE DETERMINATION IN BIOLOGICAL FLUIDS (Abstract): Hypertension is one of the most common causes of death, a complex and incompletely controlled disease for millions of patients. Metoprolol, bisoprolol, nebivolol and atenolol are selective beta-blockers frequently used in the management of arterial hypertension, alone or in fixed combination with other substances**.** This study presents the most used analytical methods for simultaneous determination in biological fluids of fixed combinations containing selective beta-blockers. Articles in PubMed, Science Direct and Wiley Journals databases published between years 2004-2014 were reviewed. Methods such as liquid chromatography–mass spectrometry–mass spectrometry (LC-MS/MS), high performance liquid chromatography (HPLC) or high performance liquid chromatography–mass spectrometry (HPLC-MS) were used for determination of fixed combination with beta-blockers in human plasma, rat plasma and human breast milk. LC-MS/MS method was used for simultaneous determination of fixed combinations of metoprolol with simvastatin, hydrochlorothiazide or ramipril, combinations of nebivolol and valsartan, or atenolol and amlodipine. Biological samples were processed by protein precipitation techniques or by liquid-liquid extraction. For the determination of fixed dose combinations of felodipine and metoprolol in rat plasma liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS/MS) was applied, using phenacetin as internal standard. HPLC-MS method was applied for the determination of bisoprolol and hydrochlorothiazide in human plasma. For the determination of atenolol and chlorthalidone from human breast milk and human plasma the HPLC method was used. The analytical methods were validated according to the specialized guidelines, and were applied to biological samples, thing that confirms the permanent concern of researchers in this field. **Keywords:** HYPERTENSION, SELECTIVE BETA-BLOCKERS, FIXED DOSE COMBINATION, HPLC, LC-MS/MS.

Arterial hypertension is one of the most common cardiovascular diseases with serious chronic complications such as heart failure, hypertensive cardiopathy, severe renal failure, hypertensive encephalopathy or lower limb peripheral arterial disease (1). According to statistics from the World Health Organization (WHO) in 2012 approximately 33.3% of the population had cardiac problems, data obtained from 194

countries (2). The prevalence of this disease in Romania is between 40% and 44.92%. A retrospective study including 1064 patients, conducted between January 2011 and December 2012 in Iasi, showed a prevalence of hypertension of 38.72% men and 61.28% women with ages between 40-80 years (3). The occurrence of hypertension is favored by behavioral type (41.2%), high fat diet (38.2%), sedentary life (25.8%), excess salt (9.0%), and also passive smoking (4.7%) (4).

Beta-blockers have been described for the first time in 1964, being initially administered for angina and arrhythmias. Subsequently, their spectrum of indications was extended and was approved by the Food and Drug Administration (USFDA) as antihypertensive agents in 1967. They are classified according to their selectivity for ß1 or ß2 receptors in non-selective and ß1 selective beta-blockers. Distribution of beta-adrenergic receptors varies depending on the species and organ system. ß1-adrenergic receptors are mainly present in cardiac and adipose tissues, and ß2 receptors in vascular tissue.

The action mechanism of selective beta-blockers is multifactorial supposing decreased cardiac output, central nervous system effects, resetting of baroreceptor levels, and reduction in vasomotor tone and plasma volume. Competitive ß-adrenergic receptor antagonism is responsible for blood pressure lowering.

Selective beta-blockers such as metoprolol, bisoprolol, nebivolol and atenolol are recommended for hypertension control alone or as fixed combinations with substances from other drug classes such us diuretics, lipid-lowering agents, calcium channel blockers and angiotensin-converting enzyme inhibitors (5).

Both the Brithish Hypertension Society guidelines and the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure recommend fixed combinations containing selective beta-blockers and thiazide diuretics as first choise medication. Thiazide diuretics are designed to minimize sodium retention and increase antihypertensive efficacy when fixed dose combinationsare used.

Non-dihydropyridine calcium channel blockers, such as verapamil and diltiazem should be avoided in fixed combination with selective beta-blockers because of the risk of symptomatic bradycardia and atrioventricular block. Instead, a proven efficacy have shown the combinations with selective beta-blockers and dihydropyridine calcium channel blockers, such as nifedipine, amlodipine, felodipine. Following the administration of metoprolol and felodipine it has been noticed that selective beta-blockers suppress renin secretion which potentiates the vasodilatory properties of calcium channel blockers. In the case of fixed combination with atenolol, a selective long-acting beta-blocker, and nifedipine, with short duration of action, the effect is not so obvious, but it is a combination fairly used to treat hypertension.

Studies have shown that the blood pressure lowering effect after the administration of a selective beta-blocker with an angiotensin-converting enzyme inhibitor is less obvious than after the administration of a fixed combination with a beta-blocker and a thiazide diuretic (6).

The potential disadvantages of fixed combinations with selective beta-blockers include the increased risk of adverse effects and drug interactions. The initial doses are lower than those administrated alone, so it

is harder to achieve the desired plasma concentration, fact that increases the risk of orthostatic hypotension in elderly patients and in those with diabetic autonomic neuropathy (7).

Due to the complex composition of biological fluids, before applying quantitative analysis method a pre-treatment of the biological samples required in order to remove proteins and potential interferences. Currently known and widely used techniques for the preparation of the biological samples are protein precipitation, liquid-liquid extraction and solid phase extraction. Protein precipitation technique has the advantage of simplicity and universality for drug molecules in plasma, but the interferences are high and the amount of substance recovered is reduced. Instead, the liquid-liquid extraction not only purifies, but also concentrates the sample, and is a commonly used technique (8).

The most used analytical methods for quantitative determination in biological fluids of the fixed dose combinations which contain selective beta-blockers were identified by consulting the publications in PubMed, ScienceDirect, and Willey Journals databases, from years 2004-2014. Were used methods such as: liquid chromatography–mass spectrometry–mass spectrometry (LC-MS/MS), liquid chromatography–atmospheric pressure ionization–mass spectrometry (LC-API-MS/MS), liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS/MS), high performance liquid chromatography (HPLC) or high performance liquid chromatography–mass spectrometry (HPLC-MS) (tab. I).

TABLE I

Methods for quantitative determination of fixed combinations with selective beta-blockers

Fixed-dose combination	Biological fluid	Method of analisys	References
Metoprolol succinate / simvastatin	Human plasma	LC-MS/MS	Selvan, 2009 (9)
Metoprolol succinate / amlodipine besylate	Human plasma	LC-MS/MS	Sarkar, 2008 (10)
Metoprolol / hydrochlorothiazide	Human plasma	LC-MS/MS	Gao, 2010 (11)
Metoprolol tartrate / ramipril	Human plasma	LC-MS/MS	Gowda, 2007 (12)
Metoprolol / felodipine	Rat plasma	LC-ESI-MS/MS	Kallem, 2012 (13)
Bisoprolol / amlodipine	Rat plasma	LC-MS/MS	Chang, 2012 (8)
Bisoprolol / hydrochlorothiazide	Human plasma	HPLC-MS	Tutunji, 2009 (14)
Nebivolol / valsartan	Human plasma	LC-API-MS/MS	Selvan, 2007 (15)
Atenolol / chlorthalidone	Human plasma	HPLC	Elgawish, 2011 (16)
Atenolol / nifedipine	Human plasma	LC-ESI-MS/MS	Kallem, 2013 (17)
Atenolol / amlodipine	Human plasma	LC-MS/MS	Kallem, 2013 (18)
Atenolol / chlorthalidone	Human breast milk	HPLC	El-Gindy, 2008 (19)

TABLE II

Techniques and solvents used for biological sample processing

Biological fluid	Extraction method	Solvent	References
Human plasma	Protein precipitation technique	Methanol	Selvan, 2009 (9)
Human plasma	Liquid-liquid extraction	Chloroform	Sarkar, 2008 (10)
Human plasma	Liquid-liquid extraction	Diethyl ether-dichloromethane (60:40, v/v)	Gao, 2010 (11)
Human plasma	Liquid-liquid extraction	Diethyl ether-dichloromethane (70:30, v/v)	Gowda, 2007 (12)
Rat plasma	Liquid-liquid extraction	Tert-butyl-methyl ether	Kallem, 2012 (13)
Rat plasma	Liquid-liquid extraction	Tert-butyl-methyl ether	Chang, 2012 (8)
Human plasma	Protein precipitation technique	Acetonitrile	Tutunji, 2009 (14)
Human plasma	Protein precipitation technique	Acetonitrile	Selvan, 2007 (15)
Human plasma	Protein precipitation technique	Acetonitrile	Elgawish, 2011 (16)
Human plasma	Liquid-liquid extraction	*	Kallem, 2013 (17)
Human plasma	Liquid-liquid extraction	*	Kallem, 2013 (18)
Human breast milk	Protein precipitation technique	Acetonitrile and phosphoric acid	El-Gindy, 2008 (19)

* Not specified

In this study are presented the techniques for processing biological samples, methods for extracting drug substances from biological fluids, respectively the solvents used for extraction (tab. II). It is noticed that the most widely used sample preparation technique is liquid-liquid extraction.

The most prescribed combinations in the treatment of hypertension are those that contain selective beta-blockers and thiazide diuretics. As methods for quantitative determination were described LC-MS/MS for analysis of fixed combination with metoprolol and hydrochlorothiazide in human plasma (11), HPLC-MS for bisoprolol and hydrochlorothiazide (14), also HPLC for atenolol and chlorthalidone in human plasma and human milk breast (16, 19). For fixed combinations with selective beta-blockers and calcium channel blockers, LC-ESI-MS/MS is the method used to determine a combination of metoprolol and felodipine in rat plasma (13), and also of atenolol and nifedipine in human plasma. For the analysis of the combination containing a selective beta-blocker and amlodipine in human plasma LC-MS/MS was used (12, 18). LC-MS/MS has been applied also for the simultaneous determination in human plasma of metoprolol and ramipril, using atenolol as internal standard (12). Various separation columns, mobile phases and internal standards are used in each case (tab. III).

TABLE III

Particularities of analytical methods used for simultaneous determination of fixed combinations with selective beta-blockers

Method of analysis	Separation column	Mobile phase	Flow rate	Internal standard	Lower limit of quantification	References
LC-MS/MS	Gemini-C18 (50 mm x 2.0mm i.d., 3µm, Phenomenex, Torrance, USA)	Acetonitrile –formic acid 0,5% (90:10, v/v, pH 3.5)	0.2 mL/ min	Propranolol hydrochloride	1.0 ng/mL for metoprolol and 0.1 ng/mL for simvas-tatin	Selvan, 2009 (9)
LC-MS/MS	Peerless basic C18 column (33mm x 4.6mm, 5µm, Chro-matopak, India)	Methanol –water containing 0.5% formic acid (8:2, v/v)	1 mL/ min	Hydrochloro thiazide	1.5 ng/mL for metoprolol and 1.25 ng/mLfor amlodipine	Sarkar, 2008 (10)
LC-MS/MS	Venusil MP - C18 column (100 mm x 4.6mm, 5µm)	Methanol – ammonium acetate – formic acid (50:50:0.05 v/v/v, pH 3.4)	0.8 mL/ min	Tramadol for metoprolol, 5-bromouracil for hydrochloro thiazide	3 ng/mL for both analytes	Gao, 2010 (11)
LC-MS/MS	C8 column (50mm x 3mm i.d., 3µm, Phenomenex, USA)	Methanol – 10mM ammonium formate (97:3, v/v)	1 mL/ min	Atenolol	5 ng/mL for both analytes	Gowda, 2007 (12)
LC-ESI-MS/MS	Atlantis C18 column (50 mm x 4.6mm, 3µm, Waters, USA)	0.2% formic acid in water – acetonitrile (25:75, v/v)	0.4-0.7 mL/ min	Phenacetin	0.53 ng/mL for metoprolol and 0.59 ng/mL for felodipine	Kallem, 2012 (13)
LC-MS/MS	Diamonsil C18 column (50 mm x 4.6mm, 5µm)	Methanol – water – formic acid (75:25:0.01, v/v/v)	0.3 mL/ min	Clenbuterol	0.2 ng/mL for both analytes	Chang, 2012 (8)
HPLC-MS	Purosphere STAR C8 column (125mm x 4mm, 5µm, Merck, Germany)	Ammonium acetate (1mM) with formic acid (0.2%) – methanol – acetonitrile (65:17.5:17:5, v/v/v)	0.65 mL/ min	Moxifloxacin	0.1 ng/mL for bisoprolol and 1.00 ng/mL for hydrochlo-ro thiazide	Tutunji, 2009 (14)
LC-MS/MS	Gemini C18 column (50 mm x 2mm i.d., 3µm) with a Security Guard C18 column (4 mm x 3mm i.d., Phenome-nex, Torrance, USA)	Acetonitrile – 0.05mM formic acid (50:50, v/v, pH 3.5)	0.25 mL/ min	Tamsulosin	0.01 ng/mL for nebivolol and 1.0 ng/mL for valsartan	Selvan, 2007 (15)
HPLC	Shim-pack cyanopropyl column (250 x 4.6mm i.d., 5µm, Shimadzu, Japan)	10mM KH_2PO_4 (pH 6) –methanol (70:30, v/v)	1mL/m in	Hydrochloro thiazide	0.2 ng/mL for atenolol and 0.129 ng/mL for chlorthali-done	Elgawish, 2011 (16)
LC-MS/MS-ESI	Hypurity Advance C18 column	Ammonium acetate– acetonitrile (15:85, v/v)	1.0 mL/ min	Carbamazepine	*	Kallem, 2013 (17)
LC-MS/MS	C18 column	*	*	Atenolol-d_7 for atenolol, amlodipine-d_4 for amlodipine	*	Kallem, 2013 (18)
HPLC	Cyanide column (250 x 4.6 mm i.d., 5µm, Phenomenex, UK)	Acetonitrile –water (35:65, v/v)	1.0 mL/ min	Guaifenesin	0.3 µg/mL for atenolol and 0.25 µg/mL for chlorthalidone	El-Gindy, 2008 (19)

* Not specified

CONCLUSIONS

The results of recent developments in the use of fixed dose combinations with selective beta-blockers and simultaneous determination of the components of these combinations in biological fluids facilitates the evaluation of pharmacokinetic parameters, respectively the evaluation of bioa-

vailability.

In the field of cardiovascular diseases, the methods used for simultaneous determination of selective beta-blockers from combinations with other substances are essential because of the continued need for therapeutic monitoring of plasma drug levels. The analysis methods were validated according to USFDA 2001 international guideline for validation of bioanalytical methods, by determining the following parameters: specificity, linearity, selectivity, accuracy, precision, robustness, stability, and limit of quantification. The described methods have proven high precision and accuracy in the quantitative determination of fixed combination with selective beta-blockers in human plasma, rat plasma and human milk breast. This facilitates application of the analysis methods in studies of bioavailability, bioequivalence, toxicological and clinical studies, and also for monitoring drug-drug interactions in human subjects.

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NEWS

MYOFIBROBLASTS IN HEALTH AND DISEASE

Myofibroblasts are present in organs with a high remodelling capacity such as kidneys, lungs and the periodontal ligament or during increased remodelling, such as in growth, development, inflammatory responses and the contraction of healing wounds. Myofibroblasts, as first described in 1971 cause the extracellular matrix to contract and are occupied in the regulation of proliferation and differentiation of neurogenic, vascular and epithelial cells. Myofibroblasts play a significant role in oncogenesis, inflammation, repair, organogenesis and fibrosis in various organs and tissues by the secretion of inflammatory and anti-inflammatory cytokines, both lipid and gaseous inflammatory mediators, chemokines, growth factors as well as extracellular matrix proteins and proteases. This smooth muscle like cells or myofibroblasts take part in development, growth and repair of normal and disease affected tissues and organs. Because of their presence everywhere in all tissues, myofibroblasts play significant roles in numerous diseases of the organs and probably in multisystem diseases also. Understanding the role of the stromal cells and extracellular matrix will allow us to identify more precise prognostic markers and potentially new therapeutic options and prevent various diseases caused by these miraculous multipotential cells. New studies can help us to use only beneficial effects of myofibroblasts and control their activation wherever they act hyperactive (Pallavi D. Shirol, Dayanand D. Shirol. Myofibroblasts in Health and Disease. International Journal of Oral & Maxillofacial Pathology. 2012; 3 (1): 23-27).

Doina Butcovan

ARMILLARIA MELLEA: PHENOLIC CONTENT, *IN VITRO* ANTIOXIDANT AND ANTIHYPERGLYCEMIC EFFECTS

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ARMILLARIA MELLEA: PHENOLIC CONTENT, *IN VITRO* ANTIOXIDANT AND ANTIHYPERGLYCEMIC EFFECTS (Abstract): *Armillaria mellea*, known as honey mushroom, has been used both as food and medicine. **Aim**: In this work, the ethanolic and hydromethanolic extracts from *Armillaria mellea* fruiting bodies were investigated for their phenolic content, antioxidant and antihyperglycemic effects (*in vitro* studies). **Material and methods**: The total phenolics were quantified using Folin-Ciocalteu assay. The antioxidant activity was evaluated by testing the free radical scavenging capacity, reducing power and ferrous ion chelating ability; in addition, the capacity to inhibit 15-lipoxygenase was also assessed. The antihyperglycemic activity was investigated by α-glucosidase assay. **Results**: Total phenolic contents of 21.68 ± 0.06 and 5.70 ± 0.28 mg/g were determined in the hydromethanolic and ethanolic extracts, respectively. The hydromethanolic extract showed higher free radical scavenging and reducing abilities (EC_{50} = 452.6 ± 2.7, 140.57 ± 1.45 and 129.45 ± 0.98 μg/mL in DPPH, ABTS and reducing power assays, respectively). The ethanolic extract proved to be more efficient in the ferrous ion chelation, 15-lipoxygenase and α-glucosidase inhibition assays (EC_{50} = 67.93 ± 0.35, 290.93 ± 2.05 and 8.54 ± 0.06 μg/mL, respectively). **Conclusions**: *Armillaria mellea* extracts showed antioxidant and antihyperglycemic potential in *in vitro* models and therefore they are promising candidates for the development of dietary supplements and pharmaceutical products. **Keywords**: *ARMILLARIA MELLEA*, FREE RADICALS, 15-LIPOXYGENASE, α-GLUCOSIDASE.

Since centuries wild edible mushrooms have been used both as food and medicine. Wild edible mushrooms are an important source of nutrients (proteins, essential aminoacids, fatty acids, carbohydrates, vitamins, mineral elements). In addition, they contain non-nutrient compounds such as phenolics, terpenes, steroids, polysaccharides. For many of these non-nutrients there have been reported different biological effects (antioxidant, antitumor, anti-inflammatory, antimicrobial, antidiabetic, hypocholesterolemic, anti-atherosclerotique, hypotensive effects) (1, 2).

Armillaria mellea (Vahl) P. Kumm. (Basidiomycota, honey mushroom) is a wild edible mushroom which grows on the stumps and roots of a wide variety of

woody and herbaceous plants. It has been traditionally used in the treatment of headache, neurasthenia, insomnia, infectious diseases and cancers (3-5). Different phytochemicals have been isolated/identified in the fruiting bodies of *A. mellea* from different geographic regions: carbohydrates (glucose, xylose, trehalose, D-mannitol, D-erythritol, glucans), peptides (prosomatostatin), sphingolipids (armillar-amide), sterols (ergosterol, ergosterol peroxide), fatty acids (oleic, linoleic, palmitic and stearic acids), sesquiterpenoids (protoilludan derivatives such as armillarin, melleolid, melledonal A, B and C), phenolics (*p*-hydroxybenzoic and cinnamic acids) and indole compounds (serotonin, tryptamine, tryptophan). Some of these phytochemicals (β-glucan with a peptide component, prosomatostatin, ergosterol peroxide) have been reported to possess anticancer properties (4, 5). Besides, extracts from the fruiting bodies of *A. mellea* showed anti-inflammatory (inhibition of the expression of inducible NO synthase, cyclooxygenase-2, prostaglandin E2, tumor necrosis factor-α, interleukin-4, -8) and immunomodulating effects [modulation of the expression of cluster of differentiation (CD) molecules, namely an increase in the expression of CD80, CD83, CD86, CD205 and a decrease in the expression of CD206, a decrease in the endocytic capacity of human dendritic cells] (*in vitro* studies) (3, 6).

A literature survey revealed antioxidant studies on extracts from Chinese and Portuguese samples of *A. mellea* (5). To the best of our knowledge, possible biological effects of Romanian samples of *A. mellea* have not been investigated yet. Besides, there are no literature reports on *A. mellea* capacity to scavenge ABTS radical cation and to inhibit 15-lipoxygenase and α-glucosidase. In this respect, the present study reports on the *in vitro* antioxidant and antihyperglycemic potential of *A. mellea* collected from Iasi region in relation to its phenolic content.

MATERIAL AND METHODS

Chemicals and reagents. All chemicals and reagents were of analytical grade.

Mushroom material. Samples of *A. mellea* were collected in Dobrovat forest (Iasi County) in October 2012. The identity of the mushroom material was confirmed by Prof. Catalin Tanase, PhD, (*Alexandru Ioan Cuza* University of Iasi). The mushroom material was cleaned without washing and air dried in shade. A voucher specimen is deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, "*Grigore T. Popa"* University of Medicine and Pharmacy – Iasi.

Extraction. Dried and powdered mushroom material (50 g) was extracted with 500 mL of 96% ethanol at room temperature for 3 h; the extraction was repeated once. The combined ethanolic extracts were evaporated at 40°C under reduced pressure giving the ethanolic extract. The residue was extracted with methanol:water mixture (1:1, v:v) using the same procedure; the combined extracts were evaporated at 40 °C under reduced pressure giving the hydromethanolic extract. Both extracts were kept at –18 °C until further studies.

Total phenolic content. The total phenolic content was determined by Folin-Ciocalteu method as previously described (7, 8).

DPPH radical scavenging activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was assessed according to Wangensteen *et al.* (7) except that the reaction time was 60 min. Quercetin was

used as positive control.

ABTS radical cation scavenging activity. ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging activity was evaluated according to the method described by Re *et al.* (9). Scavenging activity was evaluated after 6 min. reaction time. Quercetin was the positive control.

Reducing power. The assay was performed using the method described by Ferreira *et al.* (10) with slight modifications. Quercetin was used as positive control.

Ferrous ion chelating activity. The ability to chelate ferrous ions was determined according to the method described by Venditti *et al.* (11) with minor changes. EDTA (ethylenediaminetetraacetic acid) was the positive control.

15-Lipoxygenase inhibition. The 15-lipoxygenase inhibitory effects of both extracts were evaluated as previously described (7, 12). Quercetin was used as positive control.

α-Glucosidase inhibition. The α-glucosidase inhibitory activity was determined according to Liu *et al.* (13). Acarbose was used as positive control.

Statistical analysis. All assays were performed in triplicate. The results were expressed as mean ± standard deviation. The EC_{50} values were calculated by linear interpolation between the values above and below 50% activity except the reducing power assay; in this assay, the EC_{50} values were the effective concentrations giving an absorbance of 0.5 (10).

RESULTS AND DISCUSSION

Fruiting bodies of *A. mellea* from Iasi region (Dobrovat forest) were evaluated for their total phenolic content, antioxidant and antihyperglycemic effects (*in vitro* studies). As the extraction solvent affects the phenolic content and consequently, the biological effects, the fruiting bodies were sequentially extracted with 96% ethanol and methanol:water mixture (1:1, v:v). The antioxidant potential of the extracts was assessed by testing their free radical scavenging, reducing and ferrous ion chelating abilities; besides, the capacity to inhibit 15-lipoxygenase was also tested. To evaluate the antihyperglycemic potential, the inhibition of α-glucosidase was measured.

Total phenolic content. Successive extraction of the mushroom material with 96% ethanol and methanol:water mixture (1:1, v:v) gave 9.75 g of ethanolic extract and 19.31 g of hydromethanolic extract (yields: 19.5% and 38.64%, respectively). The Folin-Ciocalteu assay showed a higher total phenolic content in the hydromethanolic extract in comparison with the ethanolic extract (21.68 ± 0.06 *vs.* 5.70 ± 0.28 mg/g).

DPPH radical scavenging activity. Both extracts scavenged DPPH radical in a dose-dependent manner. It is worthy to note that the hydromethanolic extract showed stronger scavenging effects than the ethanolic extract at all concentrations tested. At the highest concentration (833.34 μg/mL), the hydromethanolic extract exhibited 80.61 ± 0.28% scavenging activity while the ethanolic extract showed only 20.69 ± 0.20% activity (tab. I); higher concentrations of ethanolic extract could not be tested due to their low solubility in the reaction medium. The hydromethanolic extract scavenged DPPH radical with an EC_{50} value of 452.6 ± 2.7 μg/mL, considerably higher than the EC_{50} value determined for quercetin (2.33 ± 0.06 μg/mL).Vaz *et al.* reported EC_{50} values of 17.13 ± 0.67 and 3.95 ± 0.16 mg/mL for the ethanolic and

water soluble polysaccharidic fractions isolated from *A. mellea* samples from Bragança-Northeast Portugal (5).

ABTS radical cation scavenging activity. *A. mellea* extracts showed dose-dependent ABTS radical cation scavenging effects. At 250 µg/mL, the ethanolic extract exhibited only 18.31 ± 0.17% activity. Therefore, higher concentrations (500 and 1000 µg/mL) were tested for EC_{50} calculation. With regard to EC_{50} values, the hydromethanolic extract was 5.6 times more active than the ethanolic extract (140.57 ± 1.45 *vs.* 781.3 ± 16.6 µg/mL) (tab. II). Both extracts were less active than the positive control, quercetin (EC_{50} = 1 ± 0 µg/mL).

TABLE I

DPPH radical scavenging activity of *Armillaria mellea* extracts

Sample	Concentration (µg/mL)	Activity (%)	EC_{50} (µg/mL)
Ethanolic extract	104.17	6.44 ± 0.35	n.d.
	208.34	9.16 ± 0.13	
	416.67	13.36 ± 0.07	
	833.34	20.69 ± 0.20	
Hydromethanolic extract	104.17	15.28 ± 0.30	452.6 ± 2.7
	208.34	26.88 ± 0.09	
	416.67	45.85 ± 0.30	
	833.34	80.61 ± 0.28	

n.d. – not determined

TABLE II

ABTS radical cation scavenging activity of *Armillaria mellea* extracts

Sample	Concentration (µg/mL)	Activity (%)	EC_{50} (µg/mL)
Ethanolic extract	62.5	5.34 ± 0.16	781.3 ± 16.6
	125	10.17 ± 0.65	
	250	18.31 ± 0.17	
	500	41.63 ± 0.62	
	1000	54.43 ± 0.28	
Hydromethanolic extract	62.5	24.27 ± 0.40	140.57 ± 1.45
	125	44.09 ± 0.51	
	250	78.97 ± 0.54	

Reducing power. As in the previous studies, the hydromethanolic extract was more active; its reducing capacity ranged from 0.18 ± 0.00 at 36.36 µg/mL to 0.86 ± 0.00 at 218.18 µg/mL. In the same concentration range, the reducing power of the ethanolic extract was insignificant (data not shown). At 266.67 µg/mL, the ethanolic extract showed a reducing power of 0.17 ± 0.00 which increased to 0.53 ± 0.01 at 800 µg/mL. According to the EC_{50} values, the hydromethanolic extract was almost 6 times more active than the ethanolic one (129.45 ± 0.98 *vs.* 759.02 ± 5.71

μg/mL) (tab. III). The positive control, quercetin, showed a remarkable reducing capacity (EC_{50} = 2.98 ± 0.12 μg/mL). Vaz *et al.* determined EC_{50} values of 7.53 ± 0.10 and 0.98 ± 0.00 mg/mL for Portuguese *A. mellea* samples (ethanolic and water soluble polysaccharidic fractions, respectively) (5).

TABLE III
Reducing power of *Armillaria mellea* extracts

Sample	Concentration (μg/mL)	Activity	EC_{50} (μg/mL)
Ethanolic extract	266.67	0.17 ± 0.00	759.02 ± 5.71
	333.34	0.31 ± 0.00	
	466.67	0.38 ± 0.00	
	600	0.43 ± 0.00	
	733.34	0.48 ± 0.01	
	800	0.53 ± 0.01	
Hydromethanolic extract	36.36	0.18 ± 0.00	129.45 ± 0.98
	72.72	0.31 ± 0.00	
	145.44	0.55 ± 0.00	
	218.18	0.86 ± 0.00	

Ferrous ion chelating activity. Ferrous ion chelating capacity of both extracts increased with concentration. According to the results, the most active was the ethanolic extract (EC_{50} = 67.93 ± 0.35 μg/mL); its activity was almost 11 times lower in comparison with EDTA, which is a very efficient metal chelator (EC_{50} = 6.34 ± 0.06 μg/mL). The hydromethanolic extract chelated ferrous ions with a higher EC_{50} value (2 ± 0 mg/mL) suggesting a very low metal chelating capacity (tab. IV).

TABLE IV
Ferrous ion chelating activity of *Armillaria mellea* extracts

Sample	Concentration	Activity (%)	EC_{50}
Ethanolic extract	36*	36.41 ± 0.36	67.93 ± 0.35*
	72*	51.24 ± 0.10	
	144*	85.33 ± 0.10	
	288*	92.96 ± 0.22	
	576*	95.27 ± 0.31	
Hydromethanolic extract	1.15**	21.43 ± 0.19	2 ± 0**
	2.3**	56.70 ± 0.38	
	4.6**	94.55 ± 0.54	
	9.2**	96.90 ± 0.29	

* μg/mL; ** mg/mL

15-Lipoxygenase inhibition. Only the ethanolic extract showed the ability to inhibit 15-lipoxygenase. Its activity increased from 3.14 ± 0.25% at 83.34 μg/mL to 93.31

± 0.44% at 666.67 µg/mL showing an EC_{50} value of 290.93 ± 2.05 µg/mL (tab. V). In the same assay, quercetin (EC_{50} = 19.5 ± 0.7 µg/mL) showed a higher 15-lipoxygenase inhibitory activity than the ethanolic extract.

TABLE V

15-Lipoxygenase inhibitory activity of *Armillaria mellea* extracts

Sample	Concentration (µg/mL)	Activity (%)	EC_{50} (µg/mL)
Ethanolic extract	83.34	3.14 ± 0.25	290.93 ± 2.05
	166.67	15.29 ± 0.51	
	333.34	58.57 ± 0.44	
	500.00	85.87 ± 0.58	
	666.67	93.31 ± 0.44	
Hydromethanolic extract	inactive		

α-Glucosidase inhibition. The ethanolic extract showed a considerably higher α-glucosidase inhibitory activity than the hydromethanolic extract. At 16.67 µg/mL the ethanolic extract had 87.09 ± 0.14% inhibitory effect on α-glucosidase while the hydromethanolic extract exhibited only 5.38 ± 0.31% α-glucosidase inhibition at 833.34 µg/mL (tab. VI). It is worthy to note that the ethanolic extract (EC_{50} = 8.54 ± 0.06 µg/mL) showed a higher activity than the positive control, acarbose (EC_{50} = 70.7 ± 0.3 µg/mL).

TABLE VI

α-Glucosidase inhibitory activity of *Armillaria mellea* extracts

Sample	Concentration (µg/mL)	Activity (%)	EC_{50} (µg/mL)
Ethanolic extract	1.67	8.62 ± 0.03	8.54 ± 0.06
	8.34	48.66 ± 0.17	
	12.5	71.49 ± 0.23	
	16.67	87.09 ± 0.14	
Hydromethanolic extract	833.34	5.38 ± 0.31	n.d.

n.d. – not determined

The hydromethanolic extract was more efficient as free radical scavenger and reducing agent than the ethanolic extract. As the hydromethanolic extract contained almost 4 times more phenolic compounds, it is obvious that these constituents are mainly responsible for the higher free radical scavenging and reducing effects of the hydromethanolic extract. On the other hand, the ethanolic extract gave better results in the ferrous ion chelating, 15-lipoxygenase and α-glucosidase inhibition assays. Ferrous ions are strong pro-oxidants; they stimulate lipid peroxidation by different mechanisms (generation of hydroxyl radical *via* the Fenton reaction, decomposition of lipid hydroperoxides into radicals that propagate the chain reactions

such as peroxyl and alkoxyl radicals) (14). Therefore, ferrous ion chelation is an important mechanism in reducing oxidative stress. Lipid peroxidation is also mediated by 15-lipoxygenase; the enzyme catalyzes low density lipoproteins oxidation thus being involved in the pathogenesis of atherosclerosis (12). Besides these two antioxidant effects, the ethanolic extract showed a remarkable inhibitory activity on α-glucosidase, a carbohydrate hydrolysing enzyme; its activity was 8 times higher than that of acarbose, a well-known antidiabetic drug. It seems that other compounds than phenolics are involved in the ferrous ion chelating, 15-lipoxygenase and α-glucosidase inhibitory effects of ethanolic extract.

CONCLUSIONS

This study demonstrated that the hydromethanolic extract from *Armillaria mellea* fruiting bodies has higher free radical scavenging and reducing abilities than the ethanolic extract. The ethanolic extract is an effective ferrous ion chelator and inhibitor of 15-lipoxygenase and α-glucosidase. Overall, it can be concluded that *Armillaria mellea* fruiting bodies can be used in the development of products (dietary supplements, pharmaceutical products) with antioxidant, anti-atherosclerotique and antihyperglycemic (decrease of postprandial hyperglycemia) effects. It is obvious that further studies are needed to evaluate the *in vivo* relevance of *in vitro* activities.

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NEWS

GASTRORETENTIVE FLOATING SYSTEM CONTAINING ENALAPRIL MALEATE

Oral administration is the most convenient and preferred means of any drug delivery to the systematic circulation. Drugs that are easily absorbed from the gastrointestinal tract and have short half-lives are eliminated quickly from the systemic circulation. To avoid this limitation, the development of gastroretentive floating systems is an attempt to release and maintain an effective drug concentration in the systemic circulation for a long time. Floating systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at the desired rate and reduces fluctuation in plasma drug concentration. Based on these aspects, a group of researchers from College of Pharmacy, Chikhli, India, prepared and evaluated the gastroretentive dosage form using enalapril maleate to increase oral bioavailability and increase gastric residence time. Enalapril maleate is an angiotensin-I converting enzyme inhibitor (ACE inhibitor) and is used in the treatment of hypertension and angina pectoris. The drug has an oral bioavailability of 40-60% possible due to narrow absorption window in the upper part of the small intestine. There were prepared eight formulations using different ratios of natural polymers by direct compression technique. Each tablet contained 20 mg of enalapril maleate, polymers and sodium bicarbonate, carbopol 934p, microcrystalline cellulose, magnesium stearate, talc, and had a total weight of 200 mg. There were used carrageenan and carbopol 934p because both have adhesive properties to the gastrointestinal wall. Xanthan gum (floating capacity) and guar gum (binder and gelling properties) were also used. The gastroretentive floating tablets were evaluated and analyzed according to the official method in Indian Pharmacopoeia. The gastroretentive floating tablet (enalapril maleate : carrageenan : carbopol 934p : xhanthan gum : guar gum : sodium bicarbonate : microcrystalline cellulose : magnesium stearate 20 : 23 : 2 : 23 : 23 : 4 : 99 : 99 : 3) had a floating time of more than 15 h; its floating lag time was found to be 69 s. The *in vitro* dissolution studies were carried out using USP apparatus type II at 50 rpm. Aliquots of dissolution medium (0.1 N HCl maintained at 37 ± 0.5°C) were withdrawn at predetermined intervals and the content of enalapril maleate was determined spectrophotometrically at 207 nm. Drug release was found to be 99.19 % (Nikan MS, Kale RH, Deshmane SV, Biyanik R. Preparation and evaluation of gastroretentive floating tablet containing Enalapril Maleate. *Int J Curr Pharm Res* 2014; 6 (3): 45-47)

Simona Gherman

THE EFFECTS OF MAGNESIUM NANOVESICLE FORMULATIONS ON SPATIAL MEMORY PERFORMANCE IN MICE

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THE EFFECTS OF MAGNESIUM NANOVESICLE FORMULATIONS ON SPATIAL MEMORY PERFORMANCE IN MICE (Abstract). **Aim:** The study investigates the effects of magnesium nanovesicles on the memory processes performance in mice. **Materials and methods:** L-a-phosphatidylcholine was used to obtain nano formulations as lipid vesicles systems stabilized thereafter with chitosan. The experiment was carried out on white Swiss mice, divided into 3 groups of 7 animals each, treated orally 7 consecutive days: Group I (Control): 0.1 mL/10g distilled water; Group II (Mg): 1 mmol/kbw magnesium chloride; Group III (Mg-vesicles): 1 mmol/kbw magnesium nanovesicles. The spatial memory performance was assessed by recording spontaneous alternation behavior in Y-maze test. Each animal was placed at the end of one arm and allowed to move freely through the maze during a single 8 min session. Alternation was defined as a consecutive entry in three different arms. The alternation percentage was computed according to the formula: (number of alternations/total number of arm visits – 2) x 100. Data were analyzed using *SPSS 17.0* software. Experimental protocols were implemented according to the recommendations of the University Committee for Research and Ethical Issues. **Results:** New carrier formulations entrapping magnesium chloride were designed: their mean size was 129.56 nm and the mean Zeta potential was +36.1 mV, indicating a moderate stability of the solution. Oral administration of magnesium vesicles resulted in a significant increase of spontaneous alternation percent in Y-maze test ($p<0.01$), which suggests an improvement of short-term memory. **Conclusions:** Using magnesium chloride entrapped in lipid vesicles induced an enhancement of cognitive functions in mice especially by facilitation of learning extinction. **Keywords:** MAGNESIUM, NANOVESICLES, MEMORY PERFORMANCE, COGNITIVE

Magnesium is one of the fourth main cations in the body (together with Ca^{2+}, Na^{+} and K^{+}), being the second most abundant intracellular cation after K^{+} (1, 2). It is involved in a various metabolic processes catalyzing more than 300 enzymatic reactions. One of the most important implications of this ion consists of modulation of the spinal cord and brain functions (3). Electrophysiology investigations

show that the activity of motor nerves carrying messages by electrical impulse from the brain to the muscles is mediated by the presence of magnesium (4, 5). Considerable evidence has been accumulated, definitely postulating that this cation is a significant factor in the brain's reactivity to metabolic and physiological stress (3). Magnesium ions (Mg^{2+}) possess a well-known depressant effect on the central nervous system and on neuromuscular transmission (5). Literature date reveals that the variation of intracellular magnesium concentration may represent a decisive aggression factor in acute and chronic disturbances in the central nervous system (2).

Nanoparticles consist of systems in size ranging from 10 to 1000 nm in which the active principle is dissolved, entrapped, encapsulated or attached. Among the carriers for active substances delivery, vesicles are self-assembled colloidal particles with broad application in modified drug-release from various pharmaceutical dosage forms (6).

Lipid nanovesicles are hollow spheres, commonly used to encapsulate labile hydrophilic molecules within their interior, and are important to be studied for developing new drug delivery carriers to facilitate the targeted delivery of active substances, to sustain drug effect in various tissues, to decrease its adverse effects (7). The advantages of using nanovesicles as drugs carriers are associated to their physicochemical properties, such as ultra small size, large surface area to mass ratio and high reactivity (8).

We aimed to investigate the effects of nanovesicles containing magnesium chloride on memory performances, after oral administration in mice.

MATERIAL AND METHODS

Materials: Substances were purchased from Sigma-Aldrich Company: the lipid used was egg yolk L-α-phosphatidylcholine (L-α-lecithin), approx. 99% pure; chitosan (N-deacetylation grade 79.7%, mean molecular weight M_w=310,000g/mol, polydispersity index = 3.26); magnesium chloride. The 0.5 (*w*/*w*) chitosan solutions were prepared in a 0.5 % (*v*/*v)* acetic acid. The other solutions were prepared in distilled water.

The soft matter vesicles were obtained after magnesium chloride immobilization inside lipid vesicles by dissolving the lipid in chloroform and removing the solvent by evaporation which lead to dry lipid film formation. The film was then hydrated by adding 12 mg/mL concentration of magnesium chloride aqueous solution. The obtained vesicles were stabilized afterwards with a 0.5% chitosan solution (9). The obtained nanovesicles containing magnesium were characterized using laser diffraction method with Shimadzu-SALD-7001 Laser Diffraction Particle Size Analyzer and Nikon E600 optical microscope. The present experiment was carried out on white Swiss mice (20-25 g), distributed into 3 groups of 7 animals each, treated orally (using an eso-gastric device), during 7 consecutive days, as follows: group 1 (Control): 0.1 mL/10 g body weight distilled water; group 2 (Mg): 1 mmol/kbw magnesium chloride; group 3 (Mg-vesicles): 1 mmol/kbw magnesium chloride entrapped in nanovesicles.

Methods: To evaluate the possible contribution of magnesium in maintenance of spatial cognition, the present study explored the effects of entrapped and non-entrapped magnesium on memory performances of mice in Y-maze test. This stand-

ardized model is used as a tool for investigation the neurobehavioral modifications of cognitive processes based upon spatial memory (10). The apparatus consists of 3 similar arms (40 x 9 x 16 cm) positioned at 120 degree from each others, and a central triangular area. Each arm has walls with specific designs all over the inner part allowing animals to differentiate it from the others. This experimental model was used to test if the mouse recognized the arm it had just explored and would consequently enter one of the other arms of the device (11).

Each animal was placed at the end of one arm and allowed to circulate freely through the maze during an 8 minutes session. The first 2 minutes were for habituation, and the last 6 minutes for the alternation between arms counted with photo beam breaks positioned at midpoint of each arm. Repeated activation of the same detector did not represent an arm-entry until the detector in another arm was activated (12).

Spontaneous alternation was considered as entry to an arm that was least occupied in recent times. Spontaneous percentage alternation was calculated as the ratio of actual to possible alternation (defined as the total number of arm entries minus two) multiplied by 100, according to the following formula: alternation % = number of alternations / total number of arm visits – 2) x 100 (13). Latency to leave the start arm, latency of first arm visit, number of arm visited, alternate arm returns, and the same arm returns were also registered. Supplementary, a video camera connected to a computer was used to allow us to record the animal behavior without distressing it. All trials were videotaped and analyzed by an independent observer. The experiments were performed between 08:00 and 13:00 a.m.

The obtained data were expressed as mean ± standard deviation (SD) in graphic charts. All values were analyzed using the ANOVA method implemented in *SPSS 17.0* software for Windows. P-value less than 0.05 were considered statistically significant compared to the Control group.

The experimental protocol was implemented following the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues guidelines for handling and use of experimental animals, according to the ethical standards of the European Community (14).

RESULTS

In a previous study we developed new carrier formulations that entrapped magnesium chloride in lipid vesicles with a moderate stability. The polymer chitosan binds to the vesicles forming a rigid bilayer decreasing the size of unilamellar systems.

The magnesium nanovesicles investigated were found to have a mean size of 129.56 nm and a mean zeta potential of +16.6mV. We appreciate that the systems correspond to the criteria of nanosuspensions and the presence of chitosan resulted in positively charged, more confined vesicles, generating repulsion forces, thus increasing stability.

In the behavioral model used (Y-maze test), spontaneous alternation or free-running represents the natural tendency of a laboratory animal to impulsively choose alternate arms.

Arm exploration was defined as entering at least the first third of an arm. The alternation percentage of control mice was 16.83 ± 0.75 (fig. 1).

Oral administration of magnesium-nanovesicles for 7 consecutive days resulted in an increase of arm entries number (mean ±SEM number of arm entries =

21.17±1.72), statistically significant compared to Control animals ($p<0.05$), relatively with a similar intensity compared to non-entrapped magnesium chloride (21.33±1.21) (tab. I, fig. 1).

In the Y-maze test spontaneous alternation represents the natural animal tendency to decide the arm in which to enter; spontaneous alternation observed for the Control group was 47.33±1.37.

The obtained results showed that the alternation percent of magnesium chloride in treated mice (59.50±1.05) was statistically higher than in Control rats ($p<0.05$), effect that corresponds to a facilitation of extinction learning in Y-maze test (fig. 2).

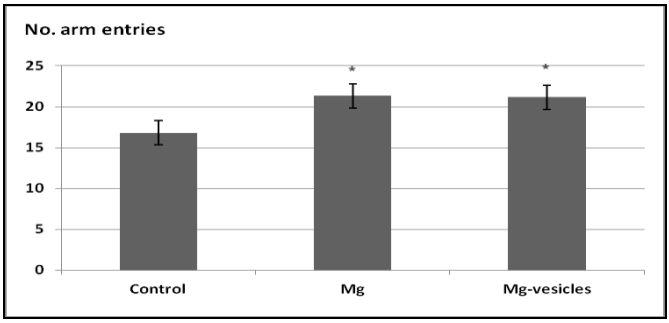

Fig 1. Effects of magnesium nanovesicles administration on the number of arm entries in Y-maze. Values were expressed as mean ± S.E.M of spontaneous alternation rate. *$p<0.05$ *vs.* control.

TABLE I

Variable dependent. Number of arm entries (Tukey HSD), (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
MgCl2	.20000	.131	**.049***	.1148	.4016
MgCl2-vesicles	.25000	.142	**.044***	.1286	.4022

*mean difference is significant at the .05 level.

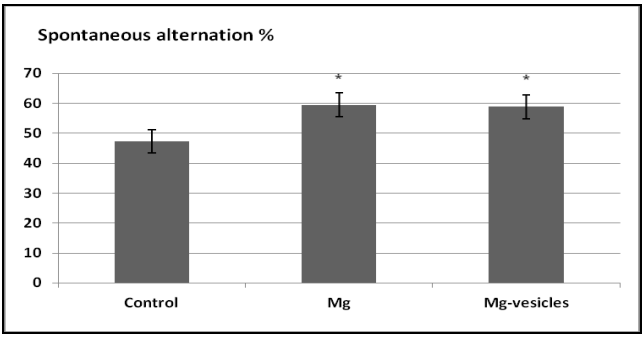

Fig 2. Effects of magnesium nanovesicles administration on spontaneous alternation percent in Y-maze. (values expressed as mean ± SEM of spontaneous alternation rate; *$p<0.05$ *vs.* Control).

TABLE II
Variable dependent. Spontaneous alternation %
(Tukey HSD), (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
MgCl2	.35700	.121	**.038***	.2076	.4146
MgCl2-vesicles	.31500	.136	**.047***	.2112	.4114

* mean difference is significant at the .05 level.

The treatment with magnesium-nanovesicles during 7 consecutive days was associated with a statistically significant increase of spontaneous alternation percent (58.83 ± 2.32, $p<0.05$) compared to Control group, suggesting an improvement of short-term spatial memory. The effects of magnesium-nanovesicles were slightly less intense, but statistically non-significant than those of non-entrapped magnesium chloride (tab. II, fig. 2).

No modifications of exploration capacity were observed after administration of entrapped, non-entrapped magnesium chloride or saline.

Also, no significant changes were noticed neither of the latency of leaving the start arm, the latency of first arm visit, and the number of arm visited, alternate arm returns, nor in the same arm returns between groups treated with magnesium chloride, magnesium-nanovesicles and saline solution.

DISCUSSION

Literature reports limited studies about the use of magnesium chloride designed in nanovesicles (15).

Our work was focused on the integration of vesicles with a biopolymer as a route to create systems with increased stability. Using an original methodology we achieved the entrapment of magnesium chloride within the lipid vesicles stabilized with chitosan. Multiple studies on the biomedical use of chitosan as a drug carrier have been communicated; its physicochemical properties, such as intra- and intermolecular hydrogen bonding and cationic change in acidic medium makes the polymer appropriate for nanoparticulate system design.

Chitosan can persist longer in the gastrointestinal tract, being a suitable candidate for formulation of modified release drug delivery systems. It also can be cross-linked to different grades to modulate drug diffusion and consequently to realize prolonged release of the active substance (16).

Our experimental study proved that these new obtained formulations could be used as carriers for magnesium chloride in the organism.

Various experimental researches and clinical trials have revealed a strong correlation between the presence of stressful conditions, the blood level of magnesium and different pathological states such as anxiety and depression (17).

Also, several clinical studies have revealed low serum magnesium level in depressed patients. The results indicate that magnesium induces the antidepressant and anxiolytic-like effects without developing tolerance to these activities, which corresponds to a potential antidepressant and anxiolytic activity of magnesium in these disorders in humans. It has been suggested

that magnesium may be exerting its antidepressant effect through anxiolytic activity, and much more, a correlation between the severity of anxiety or depression and low plasma magnesium concentrations was observed (11).

It has been proved that magnesium deficiency increases susceptibility to different types of neurologic and psychological stressors in rodents and also in patients. Magnesium deficiency in laboratory animals was associated with a diminution of offensive aggressive manifestations, but with an increase of defensive aggressive behavior (2, 6). On the other hand, a lot of evidence suggests that the administration of magnesium proved a neuro-protective potential in different pathologic conditions, increasing learning capacity and enhancing the behavioral response to stimulants (1, 5, 14).

Some studies reveal that mice receiving a low magnesium diet for several weeks displayed increased immobility time in the forced swim test, indicating enhanced depression-like behavior. In addition, the partial magnesium-depletion increased anxiety-related behavior in the light/dark and open field test, while loco motor activity or motor coordination was not influenced. In the mouse magnesium induces an increase in motive agitation at low dose (100 mg/kg) and, on the contrary, a very clear reduction of this agitation at high dose (200 mg/kg). Experimental researches have proved that doses of 20 and 30 mg magnesium/kg were able to reduce immobility time in forced swim test exerting antidepressant-like activity (17). In the elevated plus-maze test, magnesium at the same doses produced an anxiolytic-like effect. The doses of magnesium active in both tests did not affect loco motor activity.

CONCLUSIONS

The present research indicates that oral administration during 7 consecutive days of soft magnesium nanovesicles was associated with enhancement of cognitive functions in mice. This was proved to be more efficient in facilitating extinction learning and a facilitation of short-term memory performances. The Y-maze test used to assess aspects of cognitive function and learning in rats showed an improvement of spatial memory in mice treated with magnesium chloride entrapped or non-entrapped in nanovesicles, indicated by an increase of spontaneous alternation rate compared to Control group, suggesting significant effects on short-term memory.

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NEWS

DIETARY POTASSIUM INTAKE AND RISK OF VASCULAR DISEASES

The presence of potassium in the daily diet has many benefits such as prevention of vascular disease, lowering blood pressure and reducing the risk of coronary disease. A recent study followed the relationship between a diet rich in potassium *versus* risk of stroke and death. The study group included 90,137 postmenopausal women, with or without hypertension, which ingested potassium-rich foods (average 2,611 mg potassium/day). The results showed that high dietary potassium intake lowers stroke risk and mortality. The risk of stroke was 12% lower in women with a diet rich in potassium, while the risk of death was 10% lower. Also, researchers found that the potassium-rich diet did not decreased stroke risk in postmenopausal women with hypertension, but has decreased mortality. These findings show that a diet rich in fruits and vegetables with high content of potassium (bananas, potatoes, tomatoes, leafy greens, white beans) can help to prevent vascular disease (Seth A, Mossavar-Rahmani Y, Kamensky V et al. Potassium intake and risk of stroke in women with hypertension and nonhypertension in the Women's Health Initiative. *Stroke*. 2014. DOI: 10.1161/STROKEAHA.114.006046).

Cătălina Luncă

POLYPHENOL-RICH EXTRACT FROM *PINUS SYLVESTRIS* L. BARK – CHEMICAL AND ANTITUMOR STUDIES

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POLYPHENOL-RICH EXTRACT FROM *PINUS SYLVESTRIS* L. BARK – CHEMICAL AND ANTITUMOR STUDIES (Abstract): *Pinus sylvestris* L. is a very important timber tree in Romania, the bark being the main waste from the wood processing. **Aim**: The aim of this study was to investigate the chemical composition and antitumor effects against HeLa cells of a polyphenol-rich extract from *Pinus sylvestris* L. bark. **Material and methods**: The polyphenolic content and profile were analyzed by means of spectrophotometry and RP-HPLC-UV/ESI-MS in the negative ion mode. The antitumor activity was investigated using two *in vitro* assays: MTT and flow cytometric apoptosis assays. **Results**: Pine bark extract contained a high total phenolic content (48.16±0.29%). RP-HPLC-UV/ESI-MS analysis allowed the identification of taxifolin, a taxifolin-hexoside and several procyanidins (two monomers, three dimers and three trimers). At 200 µg/mL, pine bark extract exhibited a high cytotoxicity against HeLa cells (82.10±1.95%). Flow cytometry revealed the ability of pine bark extract to induce apoptosis: 71.97±0.96% apoptotic cells were determined in HeLa cells after a 48 h incubation with pine bark extract (200 µg/mL). **Conclusions**: The ability of pine bark extract to reduce viability and induce apoptosis in HeLa cells suggests the presence of compounds with antitumor properties and encourages further studies for their isolation and characterization. **Keywords**: *PINUS SYLVESTRIS* L., RP-HPLC-UV/ESI-MS, CYTOTOXIC ACTIVITY, PRO-APOPTOTIC ACTIVITY

Besides its economic importance, *Pinus sylvestris* L. (Pinaceae, Scots pine) is used for therapeutic purposes; pine shoots, pine needle oil and purified turpentine oil are used in the inflammation of upper and lower respiratory tract as well as in rheumatic and neuralgic ailments (1). In Northern Europe *Pinus sylvestris* L. bark has been used for centuries both as food and folk medicine. In Sweden Sami people collected the inner bark and ate it fresh, dried or roasted (2). Recently, Scots pine bark extract was found to be a rich source of polyphenols with antioxidant and anti-inflammatory effects. Procyanidins from monomers through higher polymers and

other phenolic compounds (taxifolin, taxifolin-3'-*O*-β-D-glucoside, dihydro-coni feryl alcohol, pinoresinol, matairesinol, glucosides of *p*-hydroxybenzoic acid, vanillic and ferulic acids, vanillin, β-hydroxypropiovanillone) have been identified in Finnish Scots pine bark and phloem (3, 4). Extracts from Finnish Scots pine bark and phloem were also reported to inhibit lipid peroxidation, liposome and low-density lipoproteins oxidation and the production of inflammatory mediators nitric oxide and prostaglandin E_2 (4, 5, 6).

Pinus sylvestris L. is a very important timber tree in Romania, the bark being the main waste from the wood processing. A literature survey showed no studies on the chemical composition and possible biological effects of bark extracts from trees growing in Romania. The aim of this study was to investigate the phenolic content and profile and antitumor effects against HeLa cells (cervical adenocarcinoma) of a polyphenol-rich extract from the bark of a mature Scots pine tree growing in Calimani Mountains (Romania). This study is part of a larger, extensive research aiming to explore strategies for the therapeutic valorization of conifer bark wastes.

MATERIAL AND METHODS

Plant material. *Pinus sylvestris* L. bark was collected in Calimani Mountains (Romania) on February 20, 2008. Patches of bark were removed from a mature Scots pine tree which was randomly selected. The species was identified by specialists from *Anastasie Fatu* Botanical Garden, Iasi, Romania. The bark was shade-dried at room temperature for two weeks and powdered in a knife mill. A herbarium voucher sample is deposited in the Discipline of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy *Grigore T. Popa*-Iasi, Romania.

Materials. All reagents and solvents were of analytical grade.

Extraction. Pine bark powder (150 g) was extracted with 3 × 1500 mL of 80% methanol using a magnetic stirrer, each time for 3 h. The combined extracts were taken to dryness by evaporation under reduced pressure followed by lyophilization resulting in 26.03 g of raw extract (yield: 17.35%). The extract was stored at -20ºC until used.

Total phenolic content. Total phenolic content was determined by Folin-Ciocalteu method (7). The result was expressed as g of gallic acid equivalents (GAE) in 100 g extract.

RP-HPLC analysis. Reversed-phase high performace liquid chromatography (RP-HPLC) analysis was conducted on a Perkin-Elmer liquid chromatograph consisting of a SCIEX API 365 LC/MS/MS mass spectrometer connected to a Series 200 HPLC system with UV/VIS detector and Analyst Software 1.1 data system. RP-HPLC coupled with electrospray ionization-mass spectrometric detection (ESI-MS) in the negative ion mode was carried out using the method of Karonen et al. (2) with slight modifications. Separations were done on a Merck Superspher 100 RP-18 column (75 mm×4 mm, i.d. 4 μm). The mobile phase consisted of (A) acetonitrile and (B) water and formic acid (99:1, v/v). The elution profile was: 100% B (0-3 min, isocratic); 0-30% A in B (3-30 min, linear gradient); 30-70% A in B (30-33 min, linear gradient); 70% A in B (33-42 min, isocratic); 70-0% A in B (42-45 min, linear gradient); 100% B (45-60 min, isocratic). The flow rate was 1 mL/min. The detection wavelength was set at 280 nm.

MTT assay. MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed according to Mosman (8) after a 48 h incubation of pine bark extract (50-200 μg/mL) with HeLa cells. Etoposide (200 μg/mL) was the positive control.

Flow cytometric apoptosis assay. The effects on apoptosis were evaluated using a Cell Lab Quanta TM SC MPL flow cytometer (Beckman Coulter, Fullerton, USA). Two fluorochromes were used to identify live, dead, apoptotic and pre-apoptotic cells: annexin V conjugated with fluorescein isothiocyanate (FITC) and propidium iodide (PI). Annexin V binds to phosphatidylserine which moves from the inner leaflet of plasma membrane to the outer leaflet in apoptotic and pre-apoptotic cells. Conjugation with FITC increases annexin V affinity for the membrane. PI is used to distinguish live cells from dead cells: it penetrates only the membrane of dead cells and binds to DNA. The assay was performed after a 48 h incubation of HeLa cells with pine bark extract (100 and 200 μg/mL). Etoposide (200 μg/mL) was used as positive control. Data analysis was performed with Flowing Software (Cell Imaging Core, Turku Center for Biotechnology, Åbo Akademi University, Finland) (9, 10).

Statistics. All analyses were carried out in triplicate. Results were expressed as mean ± SE.

RESULTS AND DISCUSSION

Phenolic content and profile. Spectrophotometric quantification showed a high phenolic content (48.16±0.29 g% GAE) in pine bark extract (PBE). Main polyphenolic constituents in PBE were identified on the basis of their MS fragmentation pattern, namely characteristic deprotonated molecules and fragment ions which were compared with those mentioned in literature (2, 3). A taxifolin-hexoside, taxifolin and procyanidins (two monomers, three dimers and three trimers) were identified as major polyphenols in PBE. The mass spectrum of taxifolin-hexoside (retention time=17.39 min) showed $[M-H]^-$ and $[2M-H]^-$ deprotonated molecules at *m/z* 465.2 and 931.5 together with two fragment ions: $[A]^-$ fragment ion at *m/z* 302.8 corresponding to the deprotonated aglycone part of taxifolin-hexoside and $[A-H_2O]^-$ fragment ion at *m/z* 284.8. Several taxifolin-*O*- and *C*-glucosides have been isolated from different species (Finnish Scots pine, *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., *Smilax glabra* Roxb., *Agrimonia pilosa* Ledeb., *Polygonum orientale* L., *Garcinia epunctata* Stapf) (4, 5, 11, 12). In our study, the identity of the hexose could not be established. In case of glycosides, ESI-MS analysis allows only the identification of the aglycone and the class of carbohydrates (hexose, pentose) the components of the sugar chain belong to; it cannot provide information on the identity of the hexose or position of aglycone-sugar linkage. The mass spectrum of taxifolin (retention time=17.62 min) showed $[M-H]^-$ and $[2M-H]^-$ deprotonated molecules at *m/z* 302.9 and 607.3 and $[M-H_2O]^-$ fragment ion at *m/z* 284.8. The fragmentation pattern of taxifolin is in agreement with MS spectral data reported by Karonen et al. (2, 3). In addition, $[M-H]^-$ deprotonated molecules of well separated procyanidin peaks corresponding to monomer (*m/z* 289.0), dimer (*m/z* 577.3) and trimer (*m/z* 865.3) structures were detected. In each spectrum, additional $[2M-H]^-$ deprotonated molecules and/or fragment ions were also detected. Two monomers were identified on the basis

of their specific MS fragmentation pattern: $[M-H]^-$ and $[2M-H]^-$ deprotonated molecules at *m/z* 289.0 and 579.4 and a fragment ion at *m/z* 245.1 generated by the loss of a hydroxyethylene moiety from $[M-H]^-$ at *m/z* 289.0. According to literature data, (+)-catechin (retention time=13.09 min), having a *2R,3S*-stereochemistry, elutes earlier than (-)-epicatechin (retention time=13.41 min), having a *2R,3R*-stereochemistry (3). Three dimers (retention times=12.29, 12.81 and 16.97 min) and three trimers (retention times=13.61, 13.89 and 16.27 min) were also identified. Isomeric dimers had similar fragmentation patterns. The mass spectrum of a dimer (RT=12.81 min) consisted of $[M-H]^-$ and $[2M-H]^-$ deprotonated molecules at *m/z* 577.3 and 1155.5 and fragment ions formed through quinone methide cleavage of the interflavonoid bond (*m/z* 286.9, *m/z* 289.0), retro-Diels-Alder fragmentation (*m/z* 425.3), sequential elimination of water from *m/z* 425.3 (*m/z* 407.4) and heterocyclic ring fission (*m/z* 451.1). Similar mass spectra were obtained for isomeric trimers. For example, a trimer (RT=13.61 min) exhibited a very abundant $[M-H]^-$ deprotonated molecule at *m/z* 865.3 and fragment ions indicating quinone methide cleavage of the upper interflavonoid bond (*m/z* 286.8, 577.2), retro-Diels-Alder fragmentation (*m/z* 713.6), heterocyclic ring fission (*m/z* 739.4) and further retro-Diels-Alder fragmentation of *m/z* 577.2 (*m/z* 425.3). Mass spectral data of the major polyphenolic constituents in PBE are shown in tab. I. An unresolved hump corresponding to procyanidin polymers eluted between 20 and 30 min (fig. 1).

MTT assay. PBE reduced the viability of HeLa cells in a concentration-dependent manner. At 200 μg/mL, PBE (82.10± 1.95%) exerted a cytotoxicity comparable to that of etoposide (87.16±2.72%) (tab. II).

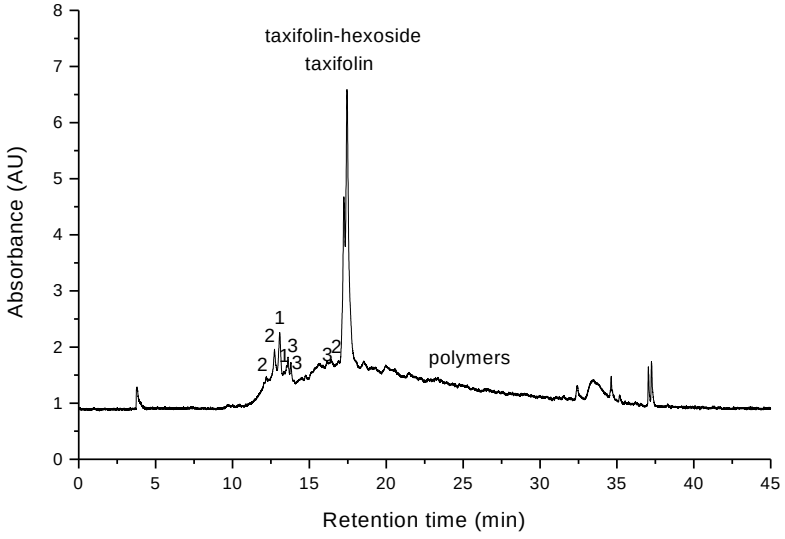

Fig. 1. RP-HPLC-UV trace (280 nm) of pine bark extract (PBE) (1-monomer; 2-dimer; 3-trimer)

TABLE I

Mass spectral data of polyphenolic constituents in pine bark extract (PBE)

Taxifolin-hexoside	**Mass spectral data** (*m/z*)		
	$[M-H]^-$	$[2M-H]^-$	Fragment ions
	465.2	931.5	302.8[a]; 284.8[b]
Taxifolin	302.9	607.3	284.8[c]
Procyanidins			
monomer	289.0	579.4	245.1[d]
dimer	577.3	1155.5	286.9, 289.0[e]; 425.3[f]; 451.1[g]; 407.4[h]
trimer	865.3	-	286.8, 577.2[e]; 713.6[f]; 739.4[g]; 425.3[i]

[a]$[A]^-$; [b]$[A-H_2O]^-$; [c]$[M-H_2O]^-$; fragment ions formed through [d]loss of CH_2=CH-OH from $[M-H]^-$ at *m/z* 289.0; [e]quinone methide cleavage of the interflavonoid bond; [f]retro-Diels-Alder fragmentation; [g]heterocyclic ring fission; [h]sequential elimination of water from *m/z* 425.3; [i]further retro-Diels-Alder fragmentation of *m/z* 577.2. A=aglycon.

TABLE II

Cytotoxic activity of pine bark extract (PBE) against HeLa cells (MTT assay)

PBE (μg/mL)	**Cytotoxic activity** (%)
50	27.92 ± 6.00*
100	61.99 ± 1.40**
200	82.10 ± 1.95**
Etoposide (μg/mL)	**Cytotoxic activity** (%)
200	87.16 ± 2.72**

(*p < 0.01; **p < 0.001)

Flow cytometric apoptosis assay. Defective apoptosis is a major cause in the development and progression of cancer. As apoptosis is down-regulated in cancer cells, identification of drugs having the ability to induce apoptosis is a very promising treatment option in antitumor therapy as such drugs will selectively affect tumor cells (13). At both concentrations tested (100 and 200 μg/mL), PBE significantly increased the percentage of apoptotic cells to 70.17±8.11% and 71.97±0.96%, respectively in comparison with the control (3.18±0.56%). The percentage of apoptotic cells reached only 41.00±3.02% under treatment with etoposide (200 μg/mL) (tab. III). Etoposide is a well-known apoptosis-inducing agent; its activity is based on caspase activation and nuclear fragmentation (14).

TABLE III

Effects of pine bark extract (PBE) on apoptosis in HeLa cells

	Cells (%)			
	Live	**Dead**	**Apoptotic**	**Pre-apoptotic**
Control	91.70 ± 3.23	4.61 ± 0.77	3.18 ± 0.56	0.51 ± 0.01
PBE				
100 μg/mL	14.02 ± 0.60**	7.75 ± 2.00*	70.17 ± 8.11**	8.05 ± 0.35**
200 μg/mL	3.90 ± 0.37**	23.29 ± 1.29**	71.97 ± 0.96**	0.84 ± 0.02**
Etoposide				
200 μg/mL	25.41 ± 3.69**	30.97 ± 5.02**	41.00 ± 3.02**	2.61 ± 0.01**

(*p < 0.01; **p < 0.001)

Taxifolin and procyanidins might be partially responsible for the cytotoxic and pro-apoptotic effects of PBE. Taxifolin has been reported to inhibit the proliferation of human cervical cancer HeLa cells; the antiproliferative activity is mainly based on inducing apoptosis by upregulating the expression of pro-apoptotic proteins p53 and p21. Procyanidin extract from *Pinus koraiensis* bark inhibited the growth of HeLa cells by increasing the expression of pro-apoptotic protein Bax and reducing the expression of anti-apoptotic proteins Bcl-2 and survivin (15). Besides taxifolin and procyanidins, other compounds might be also involved in the antitumor activity of PBE. Conifer barks contain a large variety of phytochemicals including polyphenols, terpenes, resinic acids, fatty acids, free or esterified alcohols. It is worthy to note that certain serratane-type triterpenes, resinic acids and their derivatives were reported to reduce the tumor-promoting potential of 7,12-dimethyl-benzanthracene and 12-*O*-tetradecanoylphorbol-13-acetate. Monoterpenes, lanostane and serratane-type triterpenes isolated from *Abies* and *Pinus* species showed antiproliferative effects in different human tumor cell lines: A549 (alveolar adenocarcinoma), COLO 205 (colon adenocarcinoma), QGY-7703 (hepatocellular carcinoma) and THP-1 (monocytic leukemia) (16-18). In view of these literature data, it is obvious that extensive chemical studies are to be done for the identification and isolation of antitumor compounds in PBE.

CONCLUSIONS

The polyphenol-rich extract from *Pinus sylvestris* L. bark effectively reduced viability and induced apoptosis in human cervical cancer HeLa cells. Our findings clearly show that pine bark extract is a good candidate for the isolation of compounds with apoptosis-inducing effects.

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LAUDATIO

UNIVERSITY PROFESSOR DOCTOR EMANOIL GRIGORESCU AT 90 YEARS

Prof. Dr. Emanoil Grigorescu is an outstanding personality with remarkable scientific achievements in the field of medicinal plants, one of the foremost exponents of the Romanian School of Pharmacognosy.

Prof. Dr. Emanoil Grigorescu was born on the 10th of May 1923 in Oltenita (Ilfov county). He has graduated from the Faculty of Pharmacy, Institute of Medicine and Pharmacy in Bucharest in 1948. Additionally, he was a student of the Faculty of Sciences, Physical – Chemistry section which he graduated in 1947 (without state examination). In 1964 Prof. Dr. Emanoil Grigorescu defended his Ph.D. thesis *Contributions to the pharmacognostic and phytochemical study of indigenous Hippophaë rhamnoides L.*, initially supervised by Prof. Dr. Elemér Kopp and then by Prof. Dr. Teodor Goina.

Prof. Dr. Emanoil Grigorescu started his academic didactic career after his university graduation. Between 1948-1953 he was trainer and then assistant in the Department of Biochemistry, Faculty of Pharmacy, Institute of Medicine and Pharmacy in Bucharest. In October 1953 he started his work as an assistant in the Department of Pharmacognosy. Ten years later, in 1963, he became lecturer in the same department. Meanwhile, he served as scientific secretary (July 1961-February 1963) and deputy scientific director (February-October 1963) in the Institute for State Control of Drugs and Pharmaceutical Research. In October 1963, lecturer Emanoil Grigorescu was appointed substitute associate professor in the Department of Pharmacognosy of the Institute of Medicine and Pharmacy in Iasi. The most prolific years in his didactic and research career were the years spent in this department. In 1965 he became associate professor and three years later, professor and Ph.D. advisor. Between 1993-1997 he was consulting professor. He was also involved in administrative activities being vice dean (February-October 1965) and dean (October 1965-February 1975) of the Faculty of Pharmacy.

Prof. Dr. Emanoil Grigorescu had a fruitful scientific activity focused on the valorization of medicinal plants used in traditional medicine, scientific justification of traditional uses of medicinal plants and identification of new medicinal plants with potential therapeutic uses. He studied with passion and tenacity not only indigenous medicinal plants, but also plants used in the traditional medicine in several African and Asian countries. The list of studied species is so long that it covers almost all letters in the alphabet starting with *Aesculus hippocastanum* and ending with *Withania somnifera*. Prof. Dr. Emanoil Grigorescu was interested not only in the isolation and chemical characterization of phytochemicals from different plants, but also in the possibility of using phytochemicals in the semi/synthesis of new drugs. His investigations have led to the isolation and characterization of isorhamnetin, malvidin, narco-

tine, rheadine, galanthamine, reserpine, berberine, ursolic and oleanolic acids, beta-sitosterol, usnic acid and different polysaccharides. He intensively collaborated with colleagues from the chemistry departments to develop new drugs from phytochemicals. This collaboration led to the development of several new semisynthetic derivatives with higher bioavailability such as rutin-cysteine, rutin-ε aminocaproic acid, berberine-ε aminocaproic acid and podophyllotoxin-tryptophan complexes. In addition to phytochemistry, Prof. Dr. Emanoil Grigorescu approached other areas of research. He studied the biosynthesis of certain phytochemicals (antraquinones, coumarins, anthocyanidins, carotenoids, thioglycosides, lisergic alkaloids) using *in vitro* tissue cultures. He developed phytobiological methods as a preliminary step for toxicity evaluation before animal testing. His innovative research is materialized in more than 200 published works, 12 original drugs in microproduction (Rutin-S, Stomadex, Septazulen, Hipotens, Cerebrodil, Bilogal, Lactoflux), 7 original drugs in industrial production (Variterp, Rutin-S, Perasin, Plavobil, Plavocorona) and 56 patents. He was awarded three times by the Romanian Ministry of Education and Teaching (1963, 1968, 1971). In 1991 he was awarded the Title of Elite Inventor class IV. The research activity of Prof. Dr. Emanoil Grigorescu is undoubtedly a reference point for the young researchers in the fields of pharmacognosy, phytochemistry, phytotherapy and plant biology.

Prof. Dr. Emanoil Grigorescu supervised the activity of 14 Romanian and 5 foreign Ph.D. students. He has authored and coauthored several books in the field of medicinal plants: *Din ierburi s-au născut medicamentele* (*Drugs derived from plants*, Ed. Albatros, Bucharest, 1984); *Burundi: oameni, fapte, locuri* (*Burundi: people, deeds, places*, Ed. Albatros, Bucharest, 1984); *Index fitoterapeutic* (*Phytotherapeutic index*) in collaboration with Prof. Dr. Ursula Stănescu and Prof. dr. Ioan Ciulei (Ed. Medicală, Bucharest, 1986); *Plante medicinale, fitochimie şi fitoterapie* (*Medicinal plants, phyto-chemistry and phytotherapy*) in collaboration with Prof. dr. Ioan Ciulei and Prof. Dr. Ursula Stănescu (Ed. Medicală, Bucharest, 1993); *De la etnomedicină la fitoterapie – tezaurul verde al medicinei* (*From ethnomedicine to phytotherapy – the green treasure of medicine*) in collaboration with Pharm. Dr. Felix Silva (Ed. Spiru Haret, 1997); *Strămoşii plantelor medicinale – legende pierdute* (*The ancestors of medicinal plants – lost legends*, Ed. Cantes, 1998); *Index fitoterapeutic* (*Phyto-therapeutic index*) in collaboration with Prof. dr. Mihai Ioan Lazăr, Prof. dr. Ursula Stănescu, Prof. dr. Ioan Ciulei (Ed. Cantes, 2001). *Plante medicinale, fitochimie şi fitoterapie* has received the Iuliu Hatieganu Award of the Romanian Academy in 1993.

It is noteworthy that in 1979 Prof. Dr. Emanoil Grigorescu became United Nations Organisation (UNO) expert for medicinal plants. As UNO expert he took part in the organisation of three UNIDO (United Nations Industrial Development Organization) international courses partly held in the Department of Pharmacognosy in Iasi. He participated in two UNIDO missions in Burundi (1979) and Rwanda (1984). Later, scientists from Burundi, Rwanda and Yugoslavia were trained in the Department of Pharmacognosy under the supervision of Prof. Dr. Emanoil Grigorescu.

Prof. Dr. Emanoil Grigorescu was president of Section of Pharmacy of the Society

of Physicians and Naturalists, Iasi (1968-1975), Commission of Medicinal Plants in the Association of Scientists Iasi (since 1990) and Romanian Society of Phytotherapy (since 1993). He was member (1976-1990) and then vicepresident (since 1990) of the Commission of Medicinal Plants of the Romanian Academy and member in several professional and scientific commissions (Medicinal Plants Commission for Elaboration of the Romanian Pharmacopoeia, Drug and Pharmacovigilance Commission, Research Commission for the Valorization of Medicinal and Aromatic Plants, Medicinal Plants Commission of the International Federation of Pharmacy). In recognition of his outstanding merits, in 1991 Prof. Dr. Emanoil Grigorescu became member of the Academy of Medical Sciences.

Prof. Dr. Emanoil Grigorescu built his impressive didactic and scientific career on extensive work, dedication and passion. At his 90th anniversary we wish him a long life in good health.

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CONTRIBUTIONS TO THE PHYTOCHEMICAL STUDY OF *BIDENS TRIPARTITAE HERBA* FROM ROMANIA I. TANNINS.

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CONTRIBUTIONS TO THE PHYTOCHEMICAL STUDY OF *BIDENS TRIPARTITAE HERBA* FROM ROMANIA. I.TANNINS (Abstract): **Aim:** To analyze qualitatively and quantitatively tannins in the native plant, collected during the whole vegetation period from different areas of the country, and in its different organs (flower, stem, leaf). **Material and methods**: For quantitative analysis, the plant product was extracted by repeated maceration (3 days) with 80% methanol. Proanthocyanidins in the extract were quantified by spectrophotometric methods. **Results**: condensed tannins were present while hydrolyzed tannins were absent. Chromatographic analysis showed that tannins spectrum is similar in all plant organs and in plants collected at different times throughout the vegetation period. The differences are only quantitative. The maximum amount of tannins was found during the flowering stage (10.32%). **Conclusions**: In terms of tannin content, flowering is the best time to collect. However, collection throughout the whole vegetation period is acceptable. **Keywords:** MEDICINAL PLANTS, *BIDENS TRIPARTITA L.*, TANNIN.

Bidenstripartita L. (family *Asteraceae*), usually named Burr marigold and Water agrimony, is an erect annual plant, growing 15 to 100 cm high. (1, 2, 3, 4, 5, 6).

The plant is known and used since ancient times, especially for treatment of dermal diseases and wound healing (2). In popular medicine, it is orally administered as a diuretic, diaphoretic, febrifuge, antidiarrhoeal, antiallergic, anti-inflammatory, anthelminthic, choleretic and as a kidney tonic (1, 5, 6, 7). All these effects were confirmed by pharmacological tests or clinical studies. Moreover, good results have been reported in treating chronic dysentery, acute and chronic enteritis, ulcerative colitis, bladder and kidney problems and some forms of cancer (1, 8).

The chemical composition is not well studied. Some authors even specify this. In the last URSS pharmacopoeia the product is standardized according to polyholosides, but their presence does not account for all therapeutic properties of the plant.

It is known that the dried aerial parts contain: flavones (luteolin, cynaroside), tannins, polyacetylenes, coumarins (umbelliferone, scopoletin and aesculetin), 0.05-0.11% essential oil (eugenol, ocimene and cosmene), 4.51-4.65% saccharides (arabinose, galactose, glucose, rhamnose and xylose), organic acids, carotene, vitamin C and microelements (manganese) (1, 3, 4, 8, 9).

In the literature we found almost noth-

ing about tannins in this plant. Considering the therapeutic properties of tannins (astringent, anti-inflammatory, antioxidant, anti-microbial and others) we can presume that they are extremely important components responsible for the numerous therapeutic uses of the product *Bidenstripartitaeherba*.

Also unknown is the best time for collecting the plant material. Some authors recommend the period of flowering, others budding and/or flowering (1, 3), or just budding (5, 6, 9). Some of them admit the later collection, but only the side branches, with flower buds (10), others are very strict about insisting that the product collected after the beginning of flowering has no curative properties (4).

For this reason, we decided to collect the native plant during the whole vegetation period in different regions of the country. Our aim wasto study tannins qualitatively and quantitatively in the collected plant material, and their content in different plant organs (flower, stem, leaf).

MATERIAL AND METHODS

The vegetative material originated from Iasi area and Ceahlau massif and was collected in the summer of year 2012, from late June to early October, for approximately equal time periods (10-15 days). The collection was made at different times during the afternoon. The plant was identified based on macro- and microscopic characters (1, 3) and dried in our laboratory.

For the qualitative analysis the air-dried samples of raw plant material were cut into 2-3 mm particles and then extracted using water: 5 g of dried herb was extracted with 100.0 ml boiling water on water bath for 15-20 min. The obtained extract was filtered through filter paper and was used to carry out tannins specific qualitative reactions (11, 12).

For the TLC analysis we chose some more representative samples. We prepared a 1:10 ethanolic extract for each analyzed sample. The extracts were subjected to chromatographic separation.

From each sample 10 µl of extractive solution was spotted on the chromatographic plate. The working conditions are:

- stationary phase - Silica gel G F_{254}-precoated TLC plates (Merck, Germany);
- mobile phase: n-buthanol / glacial acetic acid / water (40 / 12 / 28, v/v/v);
- detection: spaying with 1% vanillin in concentrated HCl (12).

For quantitative analysis, 2 g of crushed plant material taken from each sample has been extracted by repeated maceration method (3 days) with 25 ml of 80% methanol. For comparison, a sample extraction was performed by repeated maceration and stirring (magnetic stirrer).The crushed product was extracted with 3 equal portions of 80% methanol in 3 one-hour cycles.

Hydrolysis of proanthocyanidin was performed as described by Porter et al. (13, 14). The iron reagent was a 2% (w/v) solution of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2M HCl. 0.5 ml of analyzed extract, 3.0 ml of n-butanol solution and conc. HCl (95:5 v/v) and 0.1 ml of iron reagent were added to the glass tube sealed with a cap. The reaction mixture was then heated for 1 h at 95^0C on water bath and then red at 550 nm in a 10 mm lath-length glass cell.

The results were expressed as milligrams of cyanidin equivalents per gram of extract using ε = 17,360 L mol-1 cm-1 and MW = 287.24 (15).

RESULTS

The results of qualitative reactions for the

tannins: with lead acetate-precipitate (tannins), with basic zinc acetate-green precipitate (tannins), with $FeCl_3$soln-green precipitate (condensed tannins), with iron alum-dark green precipitate (condensed tannins), with styassny reagent-red precipitate (condensed tannins), filtrate gave no precipitate with iron alum (no hydrolysable tannins).

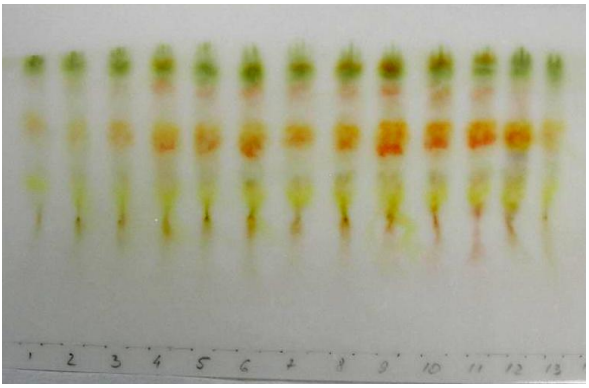

Fig. 1. Chromatogram for tannins during the vegetation period. Sample numbers correspond to those in tab. I.

TABLE I

Tannins concentration depending on collection time

No sample	**Time of collection**	**Location of collection**	Observations	Concentration %
1	17.06	Ciric area		6.80
2	27.06	-„-		3.81
3	11.07	-„-		7.03
4	23.07	-„-	Water deficiency	5.02
5	-„-	-„-	Optimal growth conditions	3.91
6	-„-	-„-	Heavily pigmented plants	4.75
7	16.08	-„-		6.52
8	21.08	Bahlui		4.21
9	22.08	Ceahlau		6.59
10	11.09	Ciric area	Flowering plants	10.32
11	-„-	-„-	Plants with fruits	6.17
12	2.10	Osoi	Only leaves	4.11
13	27.06	Ciric area	Maceration with agitation	3.98

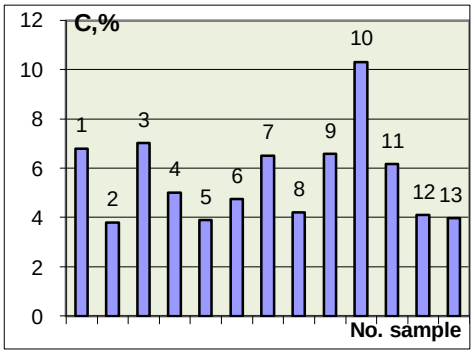

Fig. 2. Tannins concentration depending on collection time.

TABLE III

Tannins concentration in the organs of *B. tripartitaeherba*.

No	1	2	3	4
Plant organ	flower	stem	leaf	herba
Conc., %	2.57	1.46	8.91	4.98

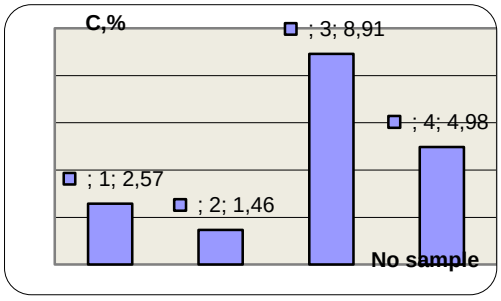

Fig. 3. Tannins concentration in the organs of *B. tripartitaeherba*.

DISCUSSION

From the results of qualitative reactions (tab.I) we conclude that in this plant product the condensed tannins are present while hydrolysed tannins are missing. This confirms the results of thin layer chromatography. Moreover, chromatographic analysis showed that tannins spectrum is similar in all plant organs and in plants collected at different times throughout the vegetation season. The differences are only quantitative (fig. 2,3).

Samples 4, 5 and 6 were collected on the same day (afternoon of July 23), from the same area but in locations with different environmental conditions. Sample 4 was collected from the banks of dried up pond. Plants were small size (about 25 cm), highly pigmented and at an advanced stage of development. Many specimens were in fruiting stage (we did not collect them because of indications in the literature on optimal collection period). Samples 5 and 6 were collected from an area intensively irrigated by a creek. Sample 5 had a reduced pigmentation, while sample 6 was heavily pigmented. The determinations showed that sample 5 is less rich in tannins than the other two samples. Sample 7 also included some plants in budding and flowering stage. Samples 8 and 9 were collected on two consecutive days. Sample 8 was collected from a wetland, partially shaded on the banks of Bahluiriver (altitude less than 100 m above sea level). Sample 9 was collected from Ceahlau massif (approx. altitude 800 m). Due to drastic reduction of flow and stream channels narrowing, some plants grew on rocky soil, exposed to direct sun light and away from water. Sample 9 was obviously richer in tannins. This is probably accounted for by altitude, but other factors that might influence the biosynthesis of tannins should not be ignored. Samples 10 and 11 were collected on the same day and location. Sample 10 included only flowering plants. Sample 11 included only the plants with fruits. Sample 10 contained the highest amount of tannins of all samples. Although Sample 11 contained less tannins, it exceeded the average for all study samples. This finding allows us

to conclude that the opinion according to which after flowering the plant should not be collected is unfounded, at least in terms of tannin concentration.

Sample 12 included only leaves, the other parts being removed because most of the collected plants had lignified stems. These are not usually used for the preparation of a drug. Results fit, however, to the levels of evidence with the lowest concentrations. So it is possible to even use them.

Sample 13 is similar to sample 2, but extracted by shaking with slight heating magnetic stirrer. The results are very close. We chose the classic repeated maceration because it was easier to implement.

The differences in concentration might be due to the fact that we collected samples from different areas around Iasi with different habitat conditions. It is known, for example, that tannins biogenesis is dependent on the intensity of photosynthesis (11). This happened because the populations of previously collected plants were destroyed by human activities.

CONCLUSIONS

The *Bidenstripartitaeherba* plant product contains condensed tannins.

The average concentration throughout the vegetation period is 5.77%.

Tannins are present in all plant organs. The highest amount was found in the leaves, the lowest in the stem. During the vegetation period the highest amount of tannins was at flowering. In terms of tannin content flowering is the best time to collect.

Somewhat lower values were found throughout the vegetation period, fruiting period included. Thus, the collection throughout the vegetation period may be acceptable.

The obtained results allow us to conclude that water deficit increases the concentration of tannins. We also found that heavily pigmented specimens contain a larger amount of tannins. Plants collected at high altitudes were also rich in tannins, but they were collected from drier places and were intensely pigmented.

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NEWS

EPOTHILONE A AND EPOTHILONE B, PROMISING SUBSTANCES FOR TREATMENT OF HUMAN OVARIAN CANCER

Epothilones, a class of microtubule-targeting agents, are secondary metabolites of myxobacteria. A new study on epothilone A and epothilone B showed that they could be good candidates for ovarian cancer treatment. Epothilones have a taxane-like mechanism of action, but are more effective against P-glycoprotein overexpressing cancer cells. The effects of epothilone A and epothilone B, obtained from myxobacterium Sorangium cellulosum, were investigated on SKOV-3 human ovarian carcinoma cell line and the results were compared with those of paclitaxel. The study demonstrated that epothilone A and epothilone B had higher cytotoxic effects (IC50 = 20.4 nM and 57.5 nM, respectively) than paclitaxel (IC50 = 126.2 nM) in MTT assay. In addition, they induced apoptosis in ovarian carcinoma cell line through reactive oxygen species (ROS) production. This could be considered one of the mechanisms involved in epothilones cytotoxicity. The oxidative stress caused by ROS determine disruption of mitochondrial membrane function and has as consequence the induction of apoptosis. The results showed that epothilone A and epothilone B caused greater depolarization of the mitochondrial membrane than paclitaxel. Preliminary data of this study indicate that epothilone A and epothilone B can be used in the near future in ovarian cancer treatment with better results than those of paclitaxel (Rogalska A, Marczak A, Gajek A et al. Induction of apoptosis in human ovarian cancer cells by new anticancer compounds, epothilone A and B. Toxicol in Vitro 2013; 27: 239-249).

Cristina Lungu

VALUE OF SCOTS INDEX IN A SCHOOL COMMUNITY FROM IASI

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VALUE OF SCOTS INDEX IN A IASI TOWN SCHOOL COMMUNITY (Abstract): The SCOTS version of the Developmental Defects of Enamel Index was designed for use in field settings as an add-on to caries prevalence examinations in order to monitor prevalence of enamel defects. **Aim:** The aim of this study was to determine the prevalence of developmental dental anomalies (DDE) using the SCOTS index and to statistically analyze the distribution of these anomalies. **Material and methods:** The study included 240 children aged 7-10 years old, from Iasi, Romania. DDE have been evaluated using SCOTS index. **Results:** DDE prevalence was 9.2% with the highest rate represented by diffuse opacities (SCOTS score 2), followed by demarcated opacities and hypoplasia (SCOTS scores 1 and 3). Most affected were the male children. 61.9% of all affected teeth had an extension defect of less than 1/3 of the tooth surface and 66.7% of lesions were asymmetrical. There was not a statistically significant correlation between the SCOTS score value and children age or sex. Socio-economic status can be a potential risk factor for DDE. **Conclusions:** DDE prevalence was quite low compared with other studies. SCOTS index is suitable for epidemiological screenings and highlights the public health significance of DDE. **Keywords:** CHILDREN, DEVELOPMENTAL DEFECTS ENAMEL (DDE), SCOTS INDEX.

Despite the relatively low incidence in child oral pathology, developmental defects of enamel (DDE) trigger a special concern, attested by the considerable amount of papers in specialized literature as well as the numerous studies in recent years.

SCOTS index is a modified version of the DDE index and was designed for use in field evaluations as an add-on to caries prevalence examinations in order to monitor prevalence of enamel defects. It was proposed by Davies and Pitts in 1990 - '91 initially for Scottish Health Board, and used in studies conducted under time pressure and poor equipment. Its purpose is to monitor the prevalence of enamel defects and thus to determine the public health significance of enamel defects (1).

SCOTS is an abbreviation that means (2):

S - Scotland. It was initially adopted by the Scottish Health Boards and used in epidemiological studies in Scotland.

C - Child. Implies evaluation of children from any community in order to determine the presence of DDE.

O - Opacity. Description of opacity or hypoplasia according to the screening criteria of the modified DDE index version.

T - Third. Records the extent of the lesion, the tooth surface being previously divided into three zones (thirds).

S - Symmetry. Records lesions symmetry.

For the examination, assessment was made of developmental defects of the upper four incisor teeth, examined wet and without prior cleaning and anomalies smaller than 1mm were excluded (3).

The aim of this study was to determine the epidemiological features of enamel defects in permanent dentition in an Iasi town school community (Romania), a prevalence study of enamel defects, and their correlation with the socio-economic status of families and type of nutrition at birth.

MATERIAL AND METHODS

The study series included 240 children aged 7-10 years attending an Iasi town secondary school, in which the SCOTS (tab. I), as a public health index of development defects of enamel, was applied to the 4 upper incisors.

TABLE I

Defect type and corresponding code in SCOTS index (1)

Description	*Codes*
A. Type of lesion	
Normal	0
Demarcated opacities: white-cream or yellow-brown	1
Diffuse opacities	2
Hypoplasia	3
Demarcated and diffuse opacities (codes 1&2)	4
Demarcated and hypoplastic opacities(codes 1&3)	5
Diffuse and hypoplastic opacities(codes 2&3)	6
Others (defects which cannot be classified under codes 1-6, for ex.: discromia)	7
All demarcated, diffuse and hypoplastic opacities(codes 1&2&3)	8
Cannot be examined (ex.: missed or with brackets)	9
B. Surface extent of the lesion	
Less than one third of the tooth, from the incisal edge, affected	1
Between one and two-thirds of the tooth, from the incisal edge, affected	2
More than two-thirds of the tooth, from the incisal edge, affected	3
C. Lesion symmetry	
No symmetrical defects	1
Only one type of defect, and it is symmetrical	2
More than one type of defect, all symmetrical	3

Clinical examination was performed by a single dentist in daylight without prior teeth drying or cleaning. Tooth surfaces were visually inspected and the suspect surfaces explored with the dental probe to assess the contours and surface defects (4). When there were doubts about the diagnosis, the dental surface was classified as normal (SCOTS =

0), and when the defect was obvious and could not be promptly included in one of the category of defects provided by SCOTS index, it was included in the category "other defects" (5). Data about the socio-economic status of children families and type of nutrition at birth were obtained through a questionnaire filled out by the parent at the same time with signing a consent to participate in the study. Statistical processing of the obtained data was performed using *SPSS 16.0*®

RESULTS AND DISCUSSION

The series structure shows a slightly higher frequency of boys (52.5%), sex ratio = 1.1: 1 and children aged 8 years (39.2%) (tab. II).

TABLE II

Sex and age distribution of study series

Age (years)	Female		Male		Total	
	n	%	n	%	n	%
7	14	5.8	16	6.7	30	12.5
8	42	17.5	52	21.6	94	39.2
9	40	16.7	32	13.3	72	30.0
10	18	7.5	26	10.8	44	18.3
Total	114	47.5	126	52.5	240	100.0

Following clinical examination, DDE were found in 28 children; after lesions smaller than 1 mm, and lesions located on other teeth than upper incisors were excluded, 22 of the study children (9.2%) remained in the study (tab. III).

Diffuse opacities (SCOTS = 2) were most common (54.5%), and the combination of diffuse opacity and hypoplasia (SCOTS = 6) the rarest (9.1%). Distribution of children according to SCOTS score is shown in fig. 1.

TABLE III

Children with developmental defects of permanent teeth according to SCOTS classification

Defect	Nr.of children	% of total defects	% of total series
SCOTS 1	4	18.2	1.7
SCOTS 2	12	54.5	5.0
SCOTS 3	4	18.2	1.7
SCOTS 6	2	9.1	0.8

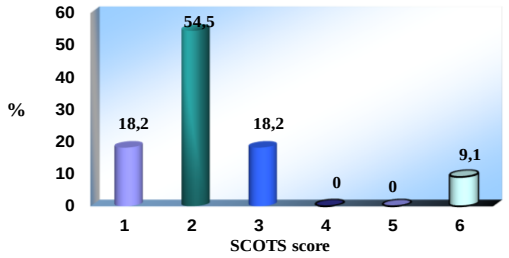

Fig. 1. Distribution of children according to SCOTS score

In the studied cases, the SCOTS score ranged between 1 and 6, with an average value of 2.32 ± 1.25. The average, minimum and maximum values of SCOTS score depending on DDE location are shown in table IV.

TABLE IV

SCOTS score values depending on the location of enamel defect

Enamel defect	Nr. of children	%	min	max	$\bar{x} \pm \sigma$
Maxilla	18	7.5	1	6	2.17 ± 1.03
Maxilla + mandible	4	1.7	2	6	3.47 ± 1.66

Average value of SCOTS score is significantly higher if the defect is found simultaneously on the maxilla and mandible than if the defect location is only maxillary (t-Student = 2.21, GL = 9, P <0.05).

In the 22 children enrolled in the study, 88 incisors were examined, and only 42 (47.7%) had various forms of developmental defects of enamel (table V). Most affected were the central incisors (30, 71.4%) and the upper left hemi jaw (26 central incisors and left lateral incisors, 61.9%).

TABLE V

Defect prevalence depending on the affected tooth

Score / Tooth	SCOTS 1	SCOTS 2	SCOTS 3	SCOTS 6	Total
1.1	2	8	-	2	12 (28.6%)
1.2	-	4	-	-	4 (9.5%)
2.1	4	8	6	-	18 (42.9%)
2.2	-	6	2	-	8 (19.0%)
Total	6 (14,3)	26 (61,9%)	8 (19,0%)	2 (4,8%)	42

Of the 42 affected teeth, 61.9% had an extension defect on more than one third of the tooth surface, and about 9.5% had an injury over two thirds of the tooth surface (fig. 2).

We noticed that 66.7% of affected teeth (n = 28) had asymmetric lesions, and that asymmetric diffuse opacities (38.1%, n = 16) were most common. Single symmetrical lesions were present on 33.3% (n = 14) of incisors. There was no record of multiple symmetrical lesions on the index teeth (tab. VI).

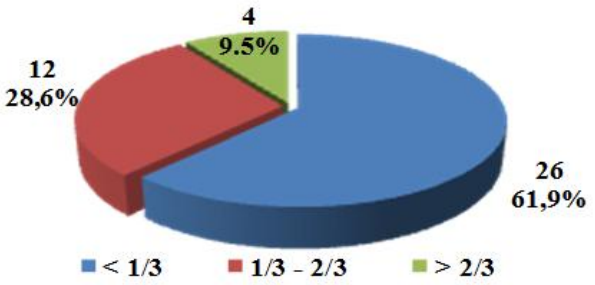

Fig. 2. Extension of surface defect

TABLE VI

Share of defect depending on the affected tooth

Defect / Symmetry	SCOTS1	SCOTS2	SCOTS3	SCOTS6	Total
Not symmetrical defects	4 (9.5%)	16 (38.1 %)	6 (14.3%)	2 (4.8%)	28 (66.7%)
Only one type of defect, and it is symmetrical	2 (4.8%)	10 (23.7%)	2 (4.8%)	-	14 (33.3%)

In the 22 children who presented defects on upper incisors no statistically significant gender and age-related differences have been identified ($p > 0.05$). Unfavorable socio-economic status proved to be a potential risk factor in the developmental defect of enamel ($p = 0.034$) (tab. VII).

In our study, there was a correlation between the prevalence of developmental defects of enamel and a potential risk factor represented by socio-economic status of children families; statistically significant differences in DDE prevalence between children from lower middle class (34.2%) and lower class families (10%) ($p < 0.001$) were found. Thus family socio-economic status was confirmed by logistic regression as a potential risk factor in DDE (fig. 3).

By correlation with the type of nutrition at birth, we noticed that artificially fed children tend to show more affected teeth and even more affected tooth surfaces (4%) than naturally fed children (2%), but the difference is not statistically significant ($p > 0.05$) (fig. 4).

TABLE VII

Epidemiological features depending on SCOTS score

Parameter	SCOTS ≤ 2	SCOTS > 2	Statistical significance
Gender			
Male	10	4	$\chi^2 = 0.10$; GL = 1; p = 0.752
Female	6	2	
Age group			
7 - 8 yers	10	2	$\chi^2 = 0.55$; GL = 1; p = 0.458
9 - 10 yers	6	4	
Socio-economic status			
Lower middle class	8	6	$\chi^2 = 4.50$; GL = 1; p = 0.034
Lower class	8	0	

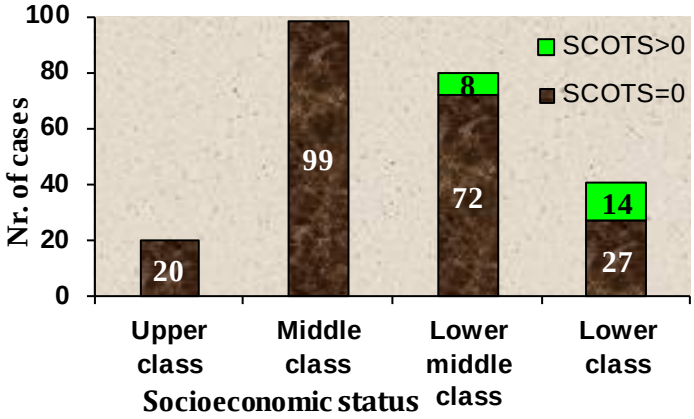

Fig. 3. Trend of DDE in children according to socio-economic status

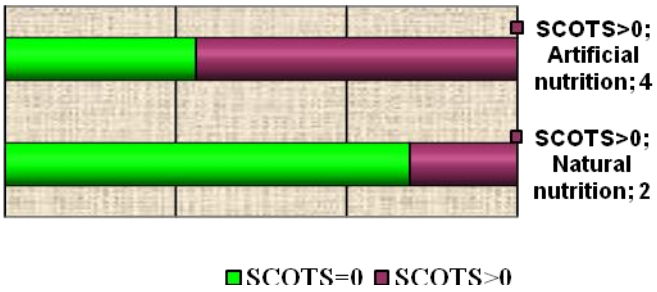

Fig. 4. Correlation between the prevalence of enamel defects and type of nutrition at birth

CONCLUSIONS

In our study in children aged 7 to 10 years the prevalence of enamel development defects assessed by SCOTS index was 9.2 percent.

SCOTS index restricts the clinical examination to the four upper incisors which makes it suitable for screening studies, but provides a lower value than the real number of defects, which requires examination of all teeth on the arch.

The highest frequency was recorded for opacities (72.7%; 54.5% diffuse opacities and 18.2% demarcated opacities), followed by hypoplasia (18.2%) and combinations of defects (9.2%).

A highly significant prevalence ($p<0.001$) of enamel development defects was noticed and correlated to socio-economic status as risk factor.

There was no statistically significant difference in the value of SCOTS scores and children gender or age

By correlation with the type of nutrition at birth, we did not observe any statistically significant differences between children fed naturally or artificially.

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FUNCTIONAL ANATOMY OF THE TEMPOROMANDIBULAR JOINT (I)

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FUNCTIONAL ANATOMY OF THE TEMPOROMANDIBULAR JOINT (I). (Abstract): Jaw movement is analyzed as the action between two rigid components jointed together in a particular way, the movable mandible against the stabilized cranium. Jaw articulation distinguishes form most other synovial joints of the body by the coincidence of certain characteristic features. Its articular surfaces are not covered by hyaline cartilage as elsewhere. The two jointed components carry teeth the shape, position and occlusion of which having a unique influence on specific positions and movements within the joint. A fibrocartilaginous disc is interposed between upper and lower articular surfaces; this disc compensates for the incongruities in opposing parts and allows sliding, pivoting, and rotating movements between the bony components. These are the reasons for our review of the functional anatomy of the temporomandibular joint. **Key words**: TEMPOROMANDIBULAR JOINT, JAW MUSCLES, MANDIBLE POSTURES AND MOVEMENTS

The craniomandibular articulation is a peculiar, bilateral, roving fulcrum around which moments of force turn into a complex jaw-lever system.

Each joint involves the articular fossa above and the mandibular condyle below (1). The articular eminence, a transversly elliptical region sinuously curved in the sagittal plane and tilted forward at 25° to the occlusal plane, forms most of the articular surface of the articular fossa. Its steepness is variable and becomes flatter in the edentulous. Its anterior limit is the summit of the articular eminence, a transverse ridge that extends laterally out to the zygomatic arch as far as the articular tubercle. Articular tissue extends anteriorly beyond the articular summit and on to the preglenoid plane. Posteriorly it extends behind the depth of the fossa as far as the squamotympanic fissure. A postglenoid tubercle (at the root of the zygomatic arch, just anterior to the fissure) is usually poorly developed in human skulls (2).

The articular surface of the mandibular condyle is slightly curved and tilted forward at about 25° to the occlusal plane. In coronal plane its shape varies from that of a gable to roughly horizontal in the edentulous.

Fibrous capsule

The lower part of the joint is surrounded by tight fibers which attach the condyle of the mandible to the disc. The upper part of the joint is surrounded by loose fibers which attach the disc to the temporal bone. Thus, the articular disc is attached separately to

the temporal bone and to the mandibular condyle forming what could be considered two joint capsules. Longer fibers joining the condyle directly to the temporal bone may be regarded as reinforcement.

Ligaments

Sphenomandibular ligament is medial to, and normally separate from the capsule. It is a flat, thin band that descends from the spine of the sphenoid and widens as it reaches the lingual of the mandibular foramen. With the jaw closed, there is about 5 mm slack within the ligament, but it becomes taut when the jaw is about half open. Lateral pterygoid and auriculotemporal nerve are lateral relations: chorda tympani nerve lies near its upper end and medial pterygoid is an infero-medial relation.

Stylomandibular ligament is a thickened band of deep cervical fascia that stretches from the apex and adjacent anterior aspect of the styloid process to the angle and posterior border of the mandible. Its position and orientation indicate that it cannot mechanically constrain any normal movements of the mandible and does not seem to warrant the status of a ligament of the joint.

Temporomandibular (lateral) ligament is attached above to the articular tubercle on the root of the zygomatic process of the temporal bone. It extends downwards and backwards at an angle of 25° to the horizontal, to attach to the lateral surface and posterior border of the neck of the condyle, deep to the parotid gland. It appears to be poorly developed in the edentulous.

Synovial membrane lines the inside of the capsule of the joint but does not extend to cover the disc or the articular surfaces.

Articular disc

The transversal oval articular disc is composed predominantly of dense fibrous connective tissue (3). It has a thick margin which forms a peripheral annulus and a central depression in is lower surface that accommodates the articular surface of the mandibular condyle. The depression probably develops as a mechanical response to pressure from the condyle as it rotates inside the annulus.

The functions of the articular disc remain controversial. It is generally believed that the disc helps to stabilize the temporomandibular joint. The articulating surfaces of the mandibular condyle and the articular fossa fit together poorly and are therefore separated by an irregular space. Muscle forces control the position of the mandible, and therefore of the condyle, in relation to the articular eminence, and these in turn set the shape and thickness of the irregular space (4).

Posturess

The term posture is preferable, since it emphasizes the disposition of the parts of the body with reference to each other, which is precisely the feature to be analyzed. The most commonly discussed of these postures are usually labeled rest position and hinge position, along with centric and other occlusal position (5). Unfortunately, the terminology of some of these positions is confusing and controversial in the current literature. Here only the simplest and least equivocal terms will be used.

Resting posture (rest position). The resting posture of the mandible is the position the lower jaw assumes when the mandibular muscles are at „rest", provided that the individual stands or sits at ease in the upright posture and holds the head so that the gaze is toward the horizon. This provi-

so is essential because it specifies that the entire head and neck should also be in normal rest position. If the head is flexed forward, the bunched-up soft structures between chin and chest tend to shove the mandible forward from its rest position. If the head is extensed backward, the opposite displasement occurs. Then such structures as skin, fascia, and facial muscles (for example, platysma) are stretched and pull the mandible down and back form the rest position.

Therefore it must be clearly understood that the term „resting posture" does not imply a fixed or static position (6). The position varies continuously depending on numerous factors, including momentary bodily postures, immediately preceding activities, fatigue, and perhaps even the time of the day. That any such position is precisely reproductible with any degree of confidence is wholly an illusion.

In the resting posture the teeth are clearly not in contact. The space between upper and lower teeth is called the free-way space, or interocclusal clearance. This normally measures from 2 to 5 mm between incisors. In this position the lips touch lightly. Thus, it is also clear that rest position is entirely independent of the number, form, position, or even the presence or absence of teeth. Instead, rest position is entirely dependent on the resting tonus of the mandibular musculature and gravity. No muscle is ever totally atonal (except perhaps under the influence of certain drugs, deep anesthesia, or unconsciousness). The residual tension of a muscle „at rest" is termed resting tonus, but this too must be defined with care.

Resting tonus is due both to the innate turgor and elasticity of muscular and fibrous tissue and to the discontinuous contractions of muscle bundles in response to an alert nervous system. But in addition, in antigravity muscles, such as those of the jaw, intermittent reflex contraction of a number of muscle fibers is always present (5). Thus, as some fibers become fatigued, others take up the tension so that a given percentage of the fibers maintains, at all time, firm contact between articulating joint surfaces and thus ensures the integrity of the articulation. This mechanism becomes especially critical in all highly movable joints where the stability of the articulation cannot be greatly dependent on other features of joint structure (for example, the shoulder joint). Thus, in normal rest position of the jaw, the antero-superior articular surfaces of the mandibular condyles are pulled toward the posterior slopes of the articular eminences of the temporal bone. As shown, these surfaces are protected by articular coverings. The thinnest parts of the articular discs intervene between these surfaces, and all are held in these relations by the conditions of resting tonus mentioned above.

This is simply one instance of the general physiologic principle in which the proper relations of all body parts are maintained by muscle tone. Though considerably lower during sleep, tonus is even then not entirely lost. Obviously, as muscle tonus is constant under constant conditions, so are the rest positions of the mandible. In aberrant conditions, such as disease, exhaustion, or nervous tension, these relationships may change. Certainly, it is well understood that „constancy" in a living organism means simply that the range of variation is small but constant.

Clinically, the range of the resting posture is of great significance because it specifies the crucial limits to any „bite-

raising" prosthetic procedures. If the bite is „opened" (raised) as far as the rest position (meaning that the teeth touch in this position), the mandibular musculature is severely stressed. Constant contact of the teeth, however light, causes the neural end organs in the periodontium to signal this contact to the motor nucleus of the fifth nerve in the brainstem. This disrupts the normal, long established firing pattern. The disrupted pattern then deprives the muscle fibers of their normal resting sequences. Trauma to teeth, supporting structures, and joint structures, as well as muscle spasm and pain, are the obvious consequences.

Hinge posture of the jaw joint is one that can be located with some accuracy. It is the posture in which the condyles rest at the most retruted limit, against the thick back rims of the discs below the front of the fossa when the cusps of the teeth are just cleared of contact. It is from this position that a practically „pure" hinge raising and lowering of the jaw can be made. The simple hinge swinging of the mandible describes a small arc and is accomplished only when the mandible is forcefully retruded. The posterior most retrusion that the condyle can reach is determined by the lenght of the tensed inner horizental band of the craniomandibular ligament. Since the lenght of this band is constant, the clinical registration of this position can be repeated accurately. The hinge movement rotates around a common horizontal axis, which runs approximately through the centers of both condyles. Because of the appreciable asymmetries between both sides of the skull, it is highly unlikely that the axis will ever run exactely through a juncture of horizontal and frontal planes. The location of this position is said to be useful in some clinical procedures. One features of this is obviously significant; it specifies that the normal rest position of the condyle must be some short distance anterior to the hinge position.

Centric occlusal posture signifies some sort of contact between upper and lower teeth. Centric occlusion denotes a concept of normal mandibular posture in which the dentition is occluded with all teeth fully interdigitated at the same time that all other kinetic components of the oral apparatus are in „harmonious balance". The condyles are slightly rotated backward and are at or slightly retruded from the level of their position at rest. Ideally, that is the position the jaw should reach if snapped shut from the open position when the head and neck are in the upright posture. This is the most likely to occur in healthy young adults with a full complement of teeth and what is classically considered a normal occlusion. It has been shown that, in the overwhelming majority of cases, the mandible can be retruded from this position some 0.5 to 1 mm, if the teeth are barely freed. As noted above, it is obvious that the normal centric occlusal posture must be slightly forward of the position that the masticatory musculature can actually achieve. Thus, the ability to move the mandible slightly backward from the centric occlusal relation is at least one distinct sign of a well-balanced oral apparatus. The hinge position is an extreme position, and it is entirely contrary to well-known principles of biologic constructs that any normal joint be habitually postured in such a strained position.

Protrusive occlusal relation is the po-

sition of occlusion in which the incisal edges of the four lower incisors contact the incisal edges of the upper centrals and sometimes the laterals. Normally, all the other teeth are not in occlusion. The condyles are slightly forwardly rotated and moved downward and forward to a level at or near the peak of the articular eminence.

Lateral occlusal relation is the position when the upper and lower posteriors of the ipsilateral side contact along the line of the crests of the buccal and lingual cusps. In unworn teeth the cusps bulge in curves so that the contacts are at spots where opposing contours meet. In worn teeth the contours are flattened, and contact lines may be almost continuous. In the natural dentition, the teeth of the controlateral side are not in occlusion.

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EFECTUL UNOR ELICITORI BIOTICI ASUPRA PRODUCȚIEI DE FLAVONE ÎN CULTURI CELULARE DE *DIGITALIS LANATA*

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EFFECT OF SOME BIOTIC ELICITORS ON FLAVONOIDS PRODUCTION IN *DIGITALIS LANATA* CELL CULTURES (Abstract): A method to improve the secondary metabolites production in plant cell cultures is the use of elicitors (biotic or abiotic). In this paper we investigated the effect of two biotic elicitors, *Botrytis* and *Sclerotinia,* on the flavonoids production, an important category of secondary metabolites, in *Digitalis lanata* suspension cultures. **Material and method**: Were used two cell lines: line 11 and line 13C 100 (aged 10 days). Were used two concentrations of elicitors (1 ml and 2 ml/100 ml suspension, respectively). Flavones were determined by a spectrophotometric method, according to Romanian Pharmacopoeia no. X (*Cynarae Folium* monography). **Results**: For both cell lines and for both elicitors, flavonoids accumulation grew with elicitation time and elicitor concentration. The highest flavonoids production took place after 96 hours elicitation time for the highest elicitor concentration (2ml/100ml suspension). So, line 11 accumulated 1000 mg% flavonoids with *Botrytis* and 999.81 mg% with *Sclerotinia*. Line 13C 100 accumulated 1051,65 mg% flavonoids with *Botrytis* and 1025,43 mg% with *Sclerotinia*. For all experiments, *Botrytis* (both concentration) induced a higher flavonoids accumulation for line 11 than *Sclerotinia*. Line 13C 100 responses to elicitor action is stronger for *Sclerotinia* for higher elicitor concentration than for same concentration of *Botrytis*. **Key words**: BIOTIC ELICITORS, *BOTRYTIS, SCLEROTINIA,* FLAVONOIDS

Elicitorii reprezintă, în general, compuşi de origine biologică implicaţi în interacţiunea plantă-agent patogen. În contextul acumulării de metaboliţi în culturi celulare vegetale, elicitorii sunt mediatori ai stresului microbian (elicitori biotici) sau sunt agenţi de stres cum ar fi: lumina UV, presiunea osmotică sau ioni ai metalelor grele (elicitori abiotici) (1).

Răspunsul dat de culturile vegetale *in vitro* la expunerea la elicitori a fost studiat prima dată pentru a aprofunda cunoştinţele despre chimia şi biochimia produşilor de elicitare, fitoalexinele, compuşi antimicrobieni sintetizaţi de plantă, componenţi ai sistemului de apărare al plantei (2). S-a descoperit astfel că elicitarea este un proces inductiv care are ca rezultat creşterea nivelelor ARN-m pentru diferite enzime (calcon sintetaza, calcon izomeraza), fiind primul

răspuns într-o serie de procese biochimice ulterioare. Consecutiv creşterii activităţii enzimatice se produce sinteza fitoalexinelor (3).

În cazul elicitorilor biotici, experimentele se realizează folosind culturi complexe de omogenate de origine bacteriană sau fungică sau cu fracţiuni ale acestor omogenate. Au fost folosiţi agenţi patogeni (*Phytophthora, Botrytis, Verticillium*), precum şi material nepatogen (*Aspergillus, Micromucor, Rhodotorula*) sau condiţionat patogen (*E.coli*) (2).

O abordare recentă cu rezultate foarte bune este folosirea unor combinaţii de elicitori biotici şi abiotici sau combinaţia elicitori biotici şi înnoirea mediului pentru obţinerea unor producţii sporite de metaboliţi secundari (4, 5).

Sinteza flavonelor, o categorie importantă de fitoalexine (6), în planta *Digitalis lanata* sau în culturi celulare de *Digitalis,* a fost puţin studiată (7). În lucrări anterioare am studiat efectul unor elicitori abiotici asupra producţiei unor metaboliţi secundari: glicozide cardiotonice (8, 9) şi flavone (sub tipar) în culturi celulare de *Digitalis lanata*. Rezultatele obţinute au fost foarte bune.

În lucrarea de faţă am studiat efectul elicitării culturilor de suspensii celulare de *Digitalis lanata* cu preparate obţinute din ciupercile patogene *Botrytis cinerea* şi *Sclerotinia tulipifera* asupra acumulării de flavone.

MATERIAL ŞI METODE

Materialul vegetal: Au fost luate în studiu două linii celulare de *Digitalis lanata*: linia 11 şi linia 13C 100 obţinute anterior (10). Suspensiile celulare au fost crescute într-un mediu lichid Murashige-Skoog (MS) (11) suplimentat cu 1 mg/l acid 2,4-diclorfenoxiacetic şi 2 mg/l 6-benziladenină, în vase Erlenmeyer de 300 ml ce conţineau 50 ml mediu nutritiv. Ele au fost subcultivate la un interval de 14 zile, raportul dintre inocul şi mediul proaspăt fiind de 1:6. Vasele de cultură au fost menţinute pe un agitator rotativ orizontal (100 rpm) la o fotoperioadă de 16 ore şi la o temperatură de 25°C.

Preparatele (omogenatele) fungice: pentru obţinerea lor ciupercile *Botrytis* şi *Sclerotinia* au fost izolate de pe diferite organe infestate ale plantelor şi cultivate pe un mediu Williams şi Ellis (12) în cutii Petri. După dezvoltarea miceliului pe suprafaţa mediului, o porţiune de circa 1 cm^2 a fost detaşată şi inoculată în 100 ml mediu lichid MS, fără fitohormoni. Vasele au fost agitate pe agitatorul rotativ la semiîntuneric timp de 6 zile. Miceliile dezvoltate, împreună cu mediul, au fost omogenizate cu un omogenizator de tip Polytron şi autoclavate. Omogenatele obţinute au fost inoculate în vasele conţinând suspensiile celulare de *Digitalis lanata* aflate în ziua a 10-a de cultură în concentraţii de 1 ml (concentraţia I) şi respectiv, 2 ml/100 ml suspensie (concentraţia II). Recoltarea probelor s-a făcut după 10, 24, 48, 72 şi respectiv, 96 de ore.

Analiza cantitativă a flavonelor s-a efectuat spectrofotometric conform FR X (monografia *Cynarae folium*) folosind $AlCl_3$ ca reactiv şi o curba-etalon de rutozidă (13). Concentraţia flavonelor a fost exprimată în mg/100g s.u. (mg%).

REZULTATE

Rezultatele obţinute sunt prezentate în tabelele I, II şi în figurile 1, 2, 3, 4.

TABELUL I

Producția de flavone, exprimată în mg%, linia 11

ORE	*BOTRYTIS*		*SCLEROTINIA*	
	Conc. I	**Conc.II**	**Conc. I**	**Conc.II**
10	474.57	724.9	480.6	730.5
24	586.2	750	524.65	763.31
48	612.61	840	582.31	832.41
72	715.79	886.95	650.48	893.53
96	871.79	1000	730.6	999.81

TABELUL II

Producția de flavone, exprimată în mg%, linia 13C 100

ORE	*BOTRYTIS*		*SCLEROTINIA*	
	Conc. I	**Conc. II**	**Conc. I**	**Conc.II**
10	568	730.75	520.41	751.62
24	592.85	803.57	550.46	811.1
48	640	870.17	623.85	883.81
72	718.58	944.82	690.98	961.75
96	976.99	1051.65	780.61	1025.43

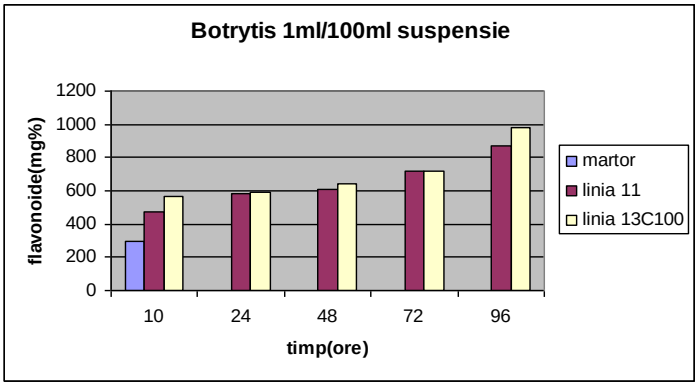

Fig. 1. Producția de flavone, elicitor *Botrytis*, concentrația I

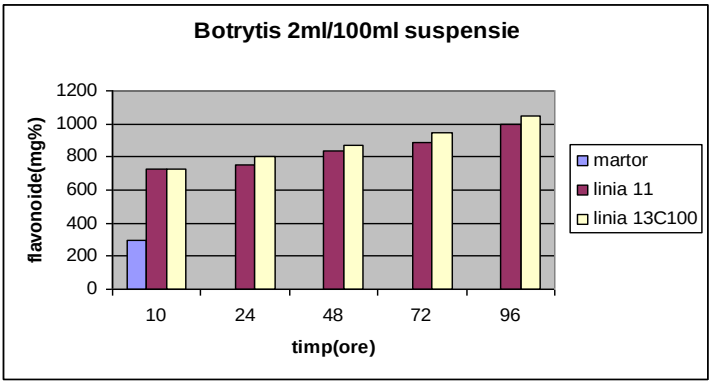

Fig. 2. Producția de flavone, elicitor *Botrytis*, concentrația II

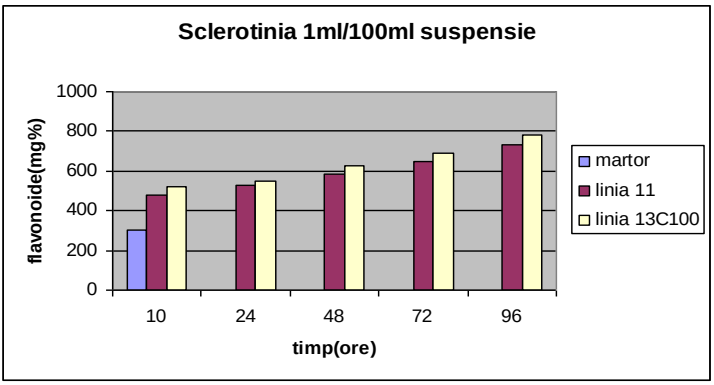

Fig. 3. Producţia de flavone, elicitor *Sclerotinia*, concentraţia I

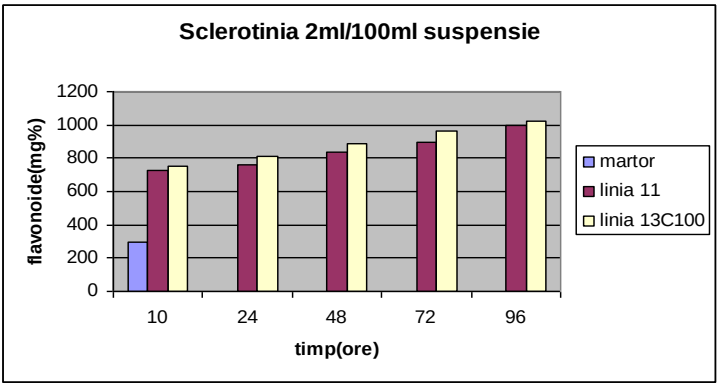

Fig. 4. Producţia de flavone, elicitor *Sclerotinia*, concentraţia II

DISCUŢII

Pentru testarea efectului elicitării biotice asupra culturilor celulare de *Digitalis lanata* s-au luat în calcul mai multe criterii şi anume: vârsta culturii, durata elicitării şi concentraţia elicitorului (14). Aceşti parametri au fost stabiliţi pe baza experimentelor anterioare efectuate de noi (vârsta culturii, durata elicitării) (8, 9) şi a datelor existente în literatura de specialitate (concentraţia elicitorului) (15, 16).

Modificările ce s-au produs în urma elicitării au depins de durata de contact dintre elicitor şi suspensia celulară, de tipul şi concentraţia elicitorului şi de linia celulară folosită.

Pentru ambele linii celulare, cantitatea de flavone acumulată a crescut direct proporţional cu timpul de elicitare şi cu concentraţia elicitorului. Aceste date sunt în concordanţă cu datele din literatura de specialitate (17). Acumularea cea mai mare de flavone se produce după 96 de ore de tratament, pentru concentraţia II a elicitorului, atât pentru linia 11, cât şi pentru linia 13C 100. Astfel, linia 11 sintetizează 1000 mg% flavone folosind *Botrytis* concentraţia II şi 999,8 mg% flavone folosind *Sclerotinia* concentraţia II (tab.I, fig. 2, 4). Linia 13C 100 acumulează 1051,65 mg% flavone folosind *Botrytis* concentraţia II şi 1025,43 mg% flavone folosind

Sclerotinia concentraţia II (tab. II, fig. 2, 4).

Aceste rezultate sunt în concordanţă cu cele obţinute în culturi de *Brugmansia candida*, elicitate cu diferite omogenate fungice, unde producţia de scopolamină a crescut de 7 ori folosind concentraţia cea mai mare de elicitor, după timpul maxim de elicitare (7 zile) (18). De asemenea, în culturi de *Ononis arvensis* elicitate cu *Aspergillus terreus*, cantitatea cea mai mare de flavone se acumulează după 7 zile de la adăugarea elicitorului, respectiv perioada maximă de elicitare (19). În schimb, în culturi de *Bellis perenis* elicitate cu omogenate de *Candida utilis*, producţia cea mai mare de flavone a fost obţinută după 48 de ore de elicitare, folosind concentraţia cea mai mare de elicitor (20), iar în culturi de *Catharanthus roseus*, elicitate cu omogenate de *Aspergillus niger*, cantitatea cea mai mare de ajmalicină s-a acumulat după 48 de ore de tratament (14). Similar, în culturi de *Ononis arvensis* elicitate cu *Escherichia coli*, producţia de flavone a fost maximă după 48 de ore de la adăugarea elicitorului (19).

Comparând cele două linii celulare luate în studiu, se observă că linia 13C 100 este mai sensibilă la acţiunea elicitorilor şi sintetizează cantităţi mai mari de flavone, pentru ambele tipuri şi concentraţii de elicitori, precum şi pentru toţi timpii de elicitare (tab. I, II).

În acest experiment tipul elicitorului nu influenţează semnificativ producţia de flavone, cele două linii celulare răspund aproximativ egal la acţiunea celor două tipuri de omogenate fungice, cu un uşor avantaj pentru *Sclerotinia* (tabele I, II şi figurile 3, 4).

Analizând rezultatele, merită subliniat faptul că elicitorul *Botrytis* la concentraţia I stimulează cel mai puternic producţia de flavone la ambele linii celulare. Astfel, pentru linia 11, după 10 ore de tratament, cantitatea de flavone este de 474,57 mg%, iar după 96 de ore ea creşte la 871,79 mg%, mai mare cu 83%. Linia 13C 100 are o comportare asemănătoare. După 10 ore de elicitare producţia de flavone este de 568 mg%, iar după 96 de ore aceasta creşte la 976,99 mg%, mai mare cu 71% (fig. 1). Rezultate asemănătoare au fost obţinute în culturi de *Ononis arvensis*, elicitate cu *Pseudomonas aeruginosa*, unde se observă o stimulare a producţiei de flavone cu 83% folosind concentraţia cea mai mică de elicitor, după 7 zile de tratament (19).

După evaluarea tuturor rezultatelor obţinute se observă că producţia maximă de flavone, 1051,65 mg%, a fost indusă de elicitorul *Botrytis*, concentraţia II, timp de elicitare 96 de ore, linia celulară 13C 100 (tab. I, fig. 2).

CONCLUZII

Cercetările noastre au evidenţiat că elicitorii biotici utilizaţi în acest experiment, la fel ca şi cei utilizaţi în experimentele anterioare, stimulează puternic producţia de flavone, în culturi celulare de *Digitalis lanata*, la nivel de laborator. Aceste rezultate ne demonstrează că experimentele pot continua cu transpunerea lor la scară de bioreactor.

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SUSCEPTIBILITY TESTING OF *STREPTOCOCCUS PNEUMONIAE* AND *VIRIDANS STREPTOCOCCAL* ISOLATES AGAINST: QUINOLONES, OXAZOLIDINONES AND GLYCOPEPTIDES

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SUSCEPTIBILITY TESTING OF *STREPTOCOCCUS PNEUMONIAE* AND VIRIDANS STREPTOCOCCAL ISOLATES AGAINST: QUINOLONES, OXAZOLIDINONES AND GLYCOPEPTIDES (Abstract): The **aim** of this study was to test the susceptibility of 100 strains (pneumococci and viridans streptococci) isolated from oral and respiratory tract infections and their complications, against one antibiotic of each of the following classes: quinolones, oxazolidinones and glycopeptides. **Material and methods:** The Etest has been used in order to investigate the susceptibility of the isolates against levofloxacin, linezolid and vancomycin. **Results and Conclusions**: As expected, the results of the study indicated that all isolates were susceptible to linezolid and vancomycin. In contrast to the pneumococcal isolates, which were all susceptible to levofloxacin, 10% of the viridans strains showed resistance to this quinolone. When fluoroquinolones are needed as an alternative to the beta-lactam antibiotics in infections in which oral streptococci are involved, the *in vitro* susceptibility testing is required. **Key words:** STREPTOCOCCUS PNEUMONIAE, ORAL STREPTOCOCCI ANTI-MICROBIAL SUSCEPTIBILITY.

The first place in the aetiology of the bacterial respiratory infections is conferred to the pneumococci. Based on molecular studies, *Streptococcus pneumoniae* should be considered one of the species of *S. mitis* group, which belongs to the oral streptococci, but due to its special role in human pathology is treated separately (1, 2, 3, 4). The oral streptococci are represented by the streptococci that normally inhabit the oral cavity. The term "viridans streptococci" is also commonly used for these bacteria, but never for *S. pneumoniae*. Even if oral streptococci belong to the normal body flora (predominantly to the flora of the mouth and upper respiratory tract), they are sometimes involved in infections, as unique microbial agents or mostly, in association with other microorganisms (5). Several recent studies have brought scientific evidence also regarding the role of these streptococci as pathogens in respiratory infections (6). Numerous authors have evidenced the emergence of antibiotic resistance in both Streptococcus pneumoniae and viridans streptococci. Moreover, it is well-known the possibility of gene transfer from viridans streptococci to pneumococci (7).

MATERIAL AND METHODS

Fifty pneumococcal and 50 oral streptococcal strains stored on cryobiles (AES Laboratoire, France) at -70^{0}C, at the laboratory of the Microbiology Chair, Faculty of Dentistry, University of Medicine and Pharmacy "Carol Davila", Bucharest, have been tested for their susceptibility to: levofloxacin, linezolid and vancomycin. These isolates originated from paediatric patients among those diagnosed during 2010 with different oral and respiratory tract infections at the following hospitals in Bucharest: the Clinical Hospital of Infectious and Tropical Diseases "Dr. Victor Babeş", the Emergency Clinical Hospital for Children "Grigore Alexandrescu", the Ear-Nose-Throat Department - "Sfanta Maria" Hospital and "Prof. Dr. Dan Theodorescu" Hospital.

The speciation of the 50 isolates of viridans streptococci by the Rapid ID 32 STREP system (BioMérieux, France) indicated that the strains belonged to the following species: *S. anginosus*, *S. mitis*, *S. oralis*, *S. sanguinis* and *S. parasanguinis* (previously communicated data).

The investigation of the susceptibility of the 100 isolates to the 3 antibiotics mentioned above was done using the Etest (BioMérieux, France/AB Biodisk, Sweden). The reference strain *S. pneumoniae* ATCC 49619 was used as quality control. The bacterial inoculum was prepared in Müller-Hinton broth (Sanimed, Romania) and standardized at 0.5 McFarland turbidity (or 1 McFarland for mucoid strains), growth on Müller-Hinton agar supplemented with 5% sheep blood (Sanimed, Romania). The Petri plates were incubated in 5% CO_2 atmosphere at 37^{0}C for 24h. The interpretation of the minimum inhibitory concentration (MIC) values has been done according to the Clinical Laboratory Standards Institute (CLSI) recommendation (8).

RESULTS

The MIC values ranged between: 0.094 – >32 μg/ml for levofloxacin, 0.125 – 0.75 μg/ml for linezolid and 0.125 – 1 μg/ml for vancomycin (Table I). The 50 isolates of *S. pneumoniae* were sensitive to the tested antimicrobial agents. All the viridans streptococcal strains were susceptible to linezolid and vancomycin, but only 45 of them were sensitive to levofloxacin (Fig.1.). The 5 isolates of *S. oralis* which were resistant to the respective fluoroquinolone showed high values of MIC (≥32 μg/ml).

TABLE I

The minimum inhibitory concentration (MIC) values of the antibiotics tested against the pneumococcal and viridans streptococcal isolates

BACTERIA (no. of isolates)	ANTIBIOTIC	VALUES OF MIC (μg/ml)			
		MIC_{min}*	MIC_{max}**	MIC_{50}***	MIC_{90}****
S. pneumoniae **(n = 50)**	Levofloxacin	0.19	1.5	0.38	0.50
	Linezolid	0.125	0.75	0.38	0.75
	Vancomycin	0.125	1	0.50	0.75
Viridans streptococci (n = 50)	Levofloxacin	0.094	>32	0.5	1.5
	Linezolid	0.19	0.75	0.38	0.50
	Vancomycin	0.38	1	0.75	1

*The lowest value of MIC;
** the highest value of MIC;
***the MIC value inhibiting 50% of the isolates;
****the MIC value inhibiting 90% of the isolates

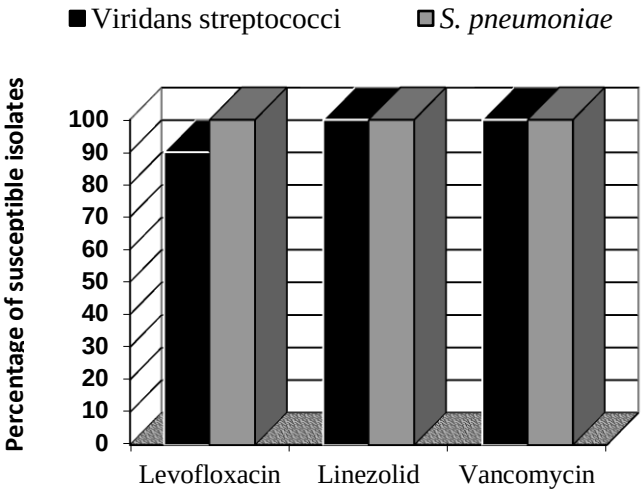

Fig.1. The percentage of the susceptible isolates to the 3 tested antibiotics

DISCUSSION

It has been already noticed that resistance of pneumococci to beta-lactam antibiotics, macrolides and trimethoprim-sulfamethoxazole in pediatric patients with respiratory tract infections is increasing on an annual basis (9). There are some articles reporting isolates of *S. pneumoniae* resistant to quinolones too. For example, Karlowsky et al. found a percentage of resistance to levofloxacin of 0.1% among the pneumococcal strains isolated from pediatric patients in the 2000-2001 and 2001-2002 respiratory illness season surveillance studies in the United States (8). These authors stated that monitoring of fluoroquinolones susceptibility will be increasingly important in future surveillance studies, if these antibiotics become a treatment option for pediatric patients.

Another research team, from South Africa, presented data which referred to 12 cases of invasive pneumococcal disease, with 5 deaths, due to levofloxacin non-susceptible strains in pediatric patients under 15 years old, and a high prevalence of nasopharyngeal carriage (of 89%) of *S. pneumoniae* strains non-susceptible to this fluoroquinolone, also in children aged under 15 years. The results of their study suggested that the treatment with fluoroquinolones of multidrug-resistant tuberculosis in pediatric patients has led to the emergence of invasive pneumococcal infection caused by levofloxacin non-susceptible strains and to its nosocomial spread (10).

In the present study, none of the pneumococcal isolates were resistant to levofloxacin. In contrast to *S. pneumoniae*, among the isolates belonging to oral streptococci there have been found some strains exhibiting a high resistance level to levofloxacin (MIC of $\geq$32 μg/ml). These fluoroquinolone resistant strains had been identified as *S. oralis*. Resistance to levofloxacin in case of viridans streptococcal clinical isolates has been reported in other articles too (11, 12). In a retrospec-

tive study referring to a period of over 30 years, Prabhu et al. observed that the percentage of viridans streptococcal strains resistant to levofloxacin isolated from patients with infective endocarditis was 0 in the period: 1971-1986, and raised to 9 in the period: 1994-2002 (13). Another research team noticed that levofloxacin prophylaxis in patients receiving chemotherapy for hematological malignancies produced an increasing resistance among the viridans streptococci during prolonged administration (14).

Even if fluoroquinolones are reasonably safe in pediatric patients, due to their specific adverse reactions, they are used in infections treatment in children only when no other effective and safe alternative exists (15).

CONCLUSIONS

As expected, linezolid and vancomycin were fully active on both pneumococcal and viridans streptococcal isolates. The percentage of levofloxacin resistance found in the viridans streptococcal isolates indicates the necessity of the *in vitro* susceptibility testing whenever the fluoroquinolones are required as an alternative to the beta-lactam antibiotics for the treatment of infections in which oral streptococci are involved. Further investigation regarding the detection of quinolone resistance mechanism is recommended too.

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NOUTĂȚI

TEHNICA IMUNOCROMATOGRAFICĂ – METODĂ RAPIDĂ ŞI ACCESIBILĂ DE IDENTIFICARE A COMPLEXULUI *MYCOBACTERIUM TUBERCULOSIS*

Detecția rapidă a noilor cazuri de tuberculoză depinde de rapiditatea identificării bacteriologice. OMS a aprobat obținerea culturii micobacteriene prin însămânțarea produsului patologic în mediu lichid pentru a îmbunătăți sensibilitatea diagnosticului. Cu toate acestea, identificarea rapidă şi precisă a *Mycobacterium tuberculosis* în culturi pozitive este esențială pentru diagnostic şi tratament, în special când cultura în mediu lichid izolează micobacterii nontuberculoase (NTM). Identificarea este dificilă deoarece: metodele biochimice convenționale de identificare sunt lente, necesitând subcultura pe mediile solide, iar metodele de amplificare a acizilor nucleici depăşesc capacitatea financiară a laboratoarelor mai ales în țările cu resurse limitate. Testele de identificare rapidă imunocromatografică (TIC) de tipul truselor comerciale MPT64 au fost dezvoltate pentru a detecta complexului *Mycobacterium tuberculosis* (MTBC) în cultură pe mediu lichid. Ele sunt mai ieftine şi mai simplu de utilizat şi, astfel, pot reprezenta o alternativă în aceste cazuri. Brent et all. au comparat performanțele MPT64 TIC cu cele ale truselor de identificare genotipică de tip line probe assay (LPA) pentru identificarea MTBC în cultură pe mediu lichid în sistem BACTEC MGIT. Sensibilitatea, specificitatea, valoarea predictivă pozitivă şi valoarea predictivă negativă au fost: 97,6% (95% CI, 91.8 - 99.7), 100% (97.1 - 100), 100% (95,7 la 100) şi respectiv 98,4% (94.4 - 99.8). Nu a existat nici o diferență semnificativă statistic între LPA şi MPT64 TIC în precizia diagnosticului (P = 0.500). Aceste rezultate recomandă utilizarea TIC pentru identificarea precisă a MTBC în vederea stabilirii rapide a diagnosticului şi respectiv a tratamentului tuberculozei. (Brent AJ, Mugo D, Musyimi R, Mutiso A. Performance of the MGIT TBc identification test and meta-analysis of MPT64 assays for identification of the *Mycobacterium tuberculosis* complex in liquid culture. *J Clin Microbiol* 2012; 49(12):4343-6).

Cătălina Luncă

STUDIU CLINICO-EPIDEMIOLOGIC ASUPRA UNUI FOCAR DE GASTROENTERITĂ ACUTĂ ÎNREGISTRAT ÎNTR-O GRĂDINIŢĂ CU PROGRAM PRELUNGIT, DIN MUNICIPIUL IAŞI, ÎN LUNA MAI 2009

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CLINICAL EPIDEMIOLOGICAL STUDY OF ACUTE GASTRO-ENTERITIS OUTBREAK RECORDED IN A CHILDREN COMMUNITY, IN IASI, DURING MAY, 2009 (Abstract): Acute diarrheal disease affect all demographic structures and it is considered a major cause of morbidity and mortality for young age groups; underreporting is due to clinical and epidemiological diversity, everywhere, including the developed countries. **Material and methods.** The authors present an epidemic outbreak of acute gastroenteritis by rotavirus in a community of 240 children. Processing and interpretation of collected data was done by statistical, mathematical and computerized methods. **Results. A number of** 17 cases of acute gastroenteritis caused by rotaviruses were detected and confirmed, mostly in children older than 5 years (92%), with a tendency to focus on large group "A ", 40% . All cases showed mild forms evolving medium. **Conclusions.** The 17 cases of infection with rotaviruses were the second episode of acute gastroenteritis with the same etiology, reported over two months. Extensivity of the epidemic outbreak was moderate, with the predominant age group affected over six years (58%). Developing and implementing preventive measures included daily cleaning and decontamination standards. **Key words**: EPIDEMIOLOGY, ACUTE GASTROENTERITIS, ROTAVIRUS, CHILDREN COMMUNITY.

Boala diareică acută (BDA) este o maladie care poate afecta toate structurile demografice, cu o evoluţie clinică şi epidemiologică polimorfă, implicată în creşterea semnificativă a solicitărilor pentru asistenţa primară şi a absenteismului şcolar şi ocupaţional (1, 2, 3). Atât în România, cât şi la nivel mondial, BDA este considerată o cauză majoră de morbiditate şi de mortalitate pentru grupurile mici de vârstă, iar diversitatea manifestărilor clinice şi epidemiologice determină subraportarea, inclusiv în ţările dezvoltate (4, 5, 6, 7). Datele clinico-epidemiologice, provenind din cele mai variate arii geografice, atestă că BDA este o afecţiune cu evoluţie autolimitantă, la cei mai mulţi dintre bolnavi, cu reale posibilităţi de diagnostic, terapie şi prevenţie.

Infecţiile determinate de rotavirusuri (Rv), cu o incidenţă de peste 50%, domină etiologia gastroenteritelor acute înregistrate la copiii cu vârsta de până la 5 ani (8, 9, 10).

MATERIAL ŞI METODE

Studiul a inclus un lot de 240 copii de la grădiniţa cu program prelungit nr. 16 din Iaşi, în care a evoluat, în luna mai, 2009, un episod epidemic de infecţie cu rotavirusuri, similar cu cel din luna martie a aceluiaşi an, confirmat epidemiologic, clinic şi cu laboratorul. Informaţiile au fost obţinute de la Direcţia de Sănătate Publică Iaşi – Compartimentul de Epidemiologie a Bolilor Transmisibile şi urmare a evaluării rezultatelor obţinute în laboratorul Spitalului Clinic de Boli Infecţioase „Sf. Parascheva" Iaşi, unde s-au investigat probele biologice recoltate de la subiecţii din focarul epidemic de gastroenterită acută. Prelucrarea şi interpretarea informaţiilor acumulate au fost realizate cu ajutorul metodelor statistico-matematice clasice şi computerizate (MS Excel, EPI/INFO).

REZULTATE

1. Date epidemiologice. În intervalul 11-15 mai 2009, au fost depistate şi confirmate epidemiologic, clinic şi cu laboratorul, 17 cazuri de gastroenterită acută cauzată de rotavirusuri. Episodul epidemic a debutat brusc, cu 4 cazuri din grupuri de vârstă diferite. Numărul dominant (modal) de cazuri s-a înregistrat în zilele I, III şi IV, cu un număr de patru îmbolnăviri iar în ziua a IV-a, trei dintre copii aparţineau aceleiaşi grupe de preşcolari (fig. 1).

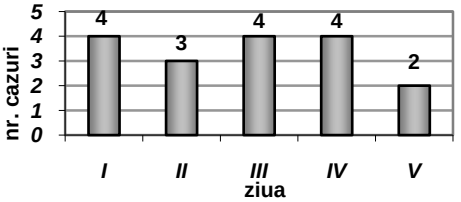

Fig. 1. Distribuţia cazurilor de gastroenterită acută cauzată de rotavirusuri, în relaţie cu data debutului clinic

Distribuţia cazurilor în relaţie cu grupurile de vârstă afectate, a arătat predominanţa îmbolnăvirilor la copiii cu vârsta de peste 5 ani (92%) (fig. 2).

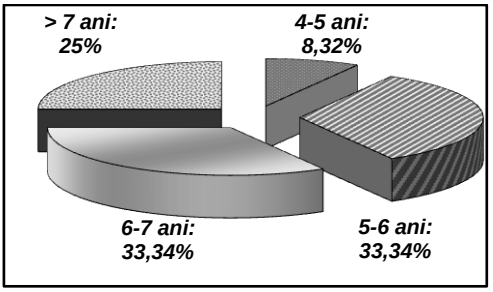

Fig. 2. Distribuţia cazurilor de gastroenterită acută cauzată de rotavirusuri în raport de grupul de vârstă afectat

Îmbolnăvirile au interesat cazuri din şase grupe de preşcolari din cele şapte existente, fiind distribuite în relaţie cu grupa din care provin, în valori de incidenţă relativ apropiate, dar cu tendinţă de concentrare la grupa mare „A" (40% dintre bolnavi), cu afectarea în special a sexului masculin (58,3%) (fig. 3).

2. Date clinice. Toţi copiii au prezentat simptomatologia caracteristică bolii, constând în scaune diareice, vărsături şi dureri abdominale; febra a fost semnalată la debut în 3 cazuri, iar alte semne clinice (mialgii, cefalee şi anorexie) au fost raportate la câte 1 caz. Pacienţii au prezentat o evoluţie de gravitate uşoară sau medie, iar 2 cazuri (16,7%) au necesitat spitalizare (internare de zi) (fig. 4).

3. Date de laborator. În laboratorul Spitalului Clinic de Boli Infecţioase "Sf. Parascheva" Iaşi, au fost investigate materiile fecale de la 49 de copii şi 21 adulţi reprezentând personalul de îngrijire. Probele au fost recoltate în primele 5 zile de la debut si examinate citologic, parazitologic, micologic, virusologic (pentru rotavirus, metoda

latex aglutinare) şi bacteriologic (pentru *Salmonella* şi *Shigella*). Rezultate pozitive pentru Rv au fost înregistrate numai la copii (34,7%) (fig. 5).

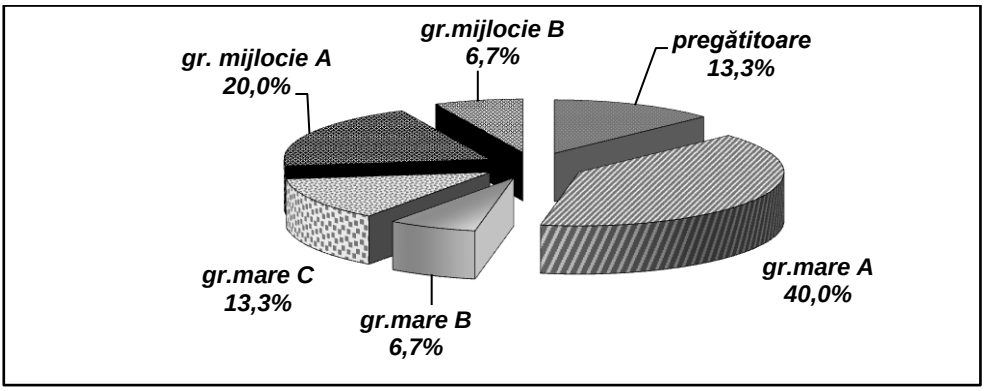

Fig. 3. Distribuţia cazurilor în raport de grupele de preşcolari

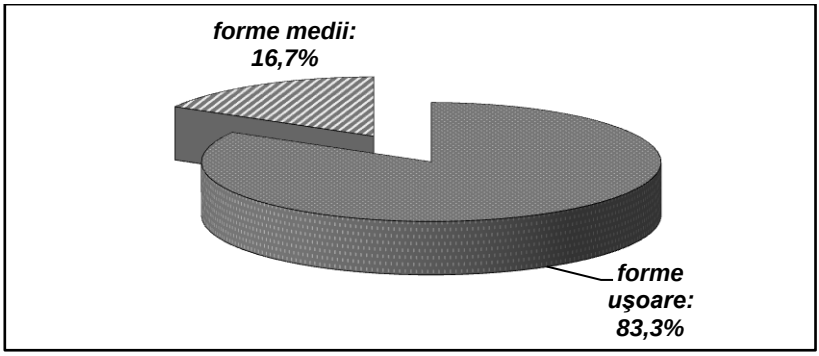

Fig. 4. Distribuţia cazurilor în relaţie cu forma clinică

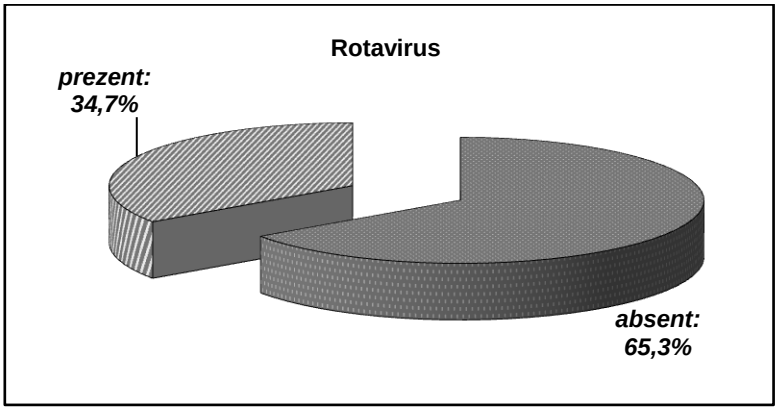

Fig. 5. Rezultatele investigaţiilor de laborator obţinute la 49 copii din grădiniţa luată în studiu

DISCUŢII

Estimări realizate în populaţia generală din SUA au indicat că ar exista, anual, aproximativ 211 milioane cazuri de BDA, iar numărul cazurilor pediatrice spitalizate a fost apreciat la 210.000 anual, din care aproximativ 10.000 decese. Din punct de vedere financiar, aceste internări au reprezentat 4% din costul tuturor internărilor, de orice cauză. La copiii sub 5 ani, în SUA, au fost raportate, anual, peste 500.000 consultaţii, cu 55.000 spitalizări şi peste 500 decese (11). În Australia, anual, 15000–20000 de copii cu vârsta sub 5 ani sunt spitalizaţi pentru gastroenterită acută gravă (12).

Un studiu de tip caz-martor, realizat în Olanda (13), având drept suport un sistem de tip „santinelă" de supraveghere a gastroenteritelor acute, a estimat impactul acestei afecţiuni la nivelul reţelei de medicină de familie, estimându-se că, anual, 128000 de persoane solicită consultaţii pentru gastroenterită acută şi că 80 persoane din 10000 prezintă un episod de gastroenterită acută. În acelaşi studiu, a fost remarcat faptul că este cel mai întâlnit agent etiologic a fost *Campylobacter* (10% din cazuri), urmat de *Giardia lamblia* (5%), *Rotavirus* (5%) Norwalk-like virus (5%) şi *Salmonella* (4%). Datele au prezentat variaţii semnificative în funcţie de teritoriul supravegheat (incidenţa mai scăzută în Nord şi în mediul rural). În Germania, potrivit primului studiu care a utilizat RT-PCR pentru precizarea etiologiei BDA la copiii spitalizaţi (14), s-a constatat că infecţiile cu rotavirus ocupă 79% din spectrul etiologic.

În unităţile de îngrijire a copiilor, dar şi în familiile în care cresc mulţi copii, s-au identificat o parte din factorii care potenţează contagiozitatea infecţiilor gastrointestinale: aglomeraţia (numărul mare de copii în îngrijire); apariţia unui caz (sau mai multe) de BDA, cu reţinerea copiilor bolnavi în colectivitate (fără izolare); acelaşi pat folosit de mai mulţi copii; suprafeţe comune pentru schimbarea scutecelor; lipsa complianţei personalului de a purta şi schimba mănuşi sau pentru spălarea mâinilor înainte şi după toaleta fiecărui copil; pardoseli acoperite cu mochetă sau covor (material textil); jucării şi alte obiecte / rechizite comune (6, 15).

CONCLUZII

Cele 17 cazuri de infecţie cu rotavirusuri înregistrate în Grădiniţa cu program prelungit nr. 16, din Municipiul Iaşi, au reprezentat un al doilea episod de gastroenterită acută cu aceeaşi etiologie, semnalat pe durata a două luni de zile. Cei mai afectaţi au fost subiecţii de sex masculin (58,3%) în vârstă de peste 5 ani (92%). Formele clinice uşoare, cu simptomatologie digestivă (scaune diareice, vărsături şi dureri abdominale) au fost înregistrate la 83,3% dintre cazuri. Probele de materii fecale au fost examinate citologic, parazitologic, micologic şi bacteriologic, inclusiv metoda latex aglutinare pentru rotavirusuri.

Extensivitatea procesului epidemiologic a avut intensitate medie, cu afectarea predominantă a grupului de vârstă peste 6 ani (58%). S-au elaborat şi aplicat măsuri de prevenţie incluzând, predominant, igienizarea cotidiană şi decontaminarea realizate cu exigenţă.

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CARCINOAMELE CUTANATE ALE FEȚEI - FORME CLINICE ŞI HISTOPATOLOGICE. UN STUDIU RETROSPECTIV PE 308 CAZURI

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CUTANEOUS CARCINOMA OF THE FACE. CLINICAL AND HISTOPATHOLOGICAL FORMS. RETROSPECTIVE STUDY OF 308 CASES. (Abstract): Cutaneous carcinomas are the most frequent malignant tumors, the majority of them being located on the face, scalp and back. **Aim:** To assess the clinical presentation and pathology aspects of the patients with face skin carcinomas admitted to our clinic. **Material and methods**: The retrospective study was carried out on a series of 308 patients diagnosed and treated for cutaneous carcinoma of the face in the Department of Oral and Facial Surgery of Iasi over a period of 5 years (January 2006- December 2010). The following parameters were analyzed: demographic data, size and location of the tumor, clinical presentation, and pathology aspects. **Results**: Basal cell carcinoma accounted for 75,97% of the cases, the nodular, superficial and morphea types being the most common. As to the microscopic aspects, the nodular/solid, superficial, and basosquamous (metatypical) types were the most frequent. The squamous-cell carcinoma represented 18,18%, mainly in the ulcero-vegetant and nodular forms. The moderately and well differentiated forms prevailed. Carcinomas of the skin adnexes as well as Merkel cell carcinomas were the most rare (4,22% and 0,65%, respectively). **Conclusions:**. The face is the most common location of the cutaneous carcinomas requiring early diagnosis and treatment as a key factor to favorable long term outcome. **Key words**: CUTANEOUS CARCINOMAS, FACE, BASAL CELL, SQUAMOUS-CELL CARCINOMA

Carcinoamele cutanate se situează pe primul loc ca frecvență dintre toate tumorile maligne, fapt deloc surprinzător dacă luăm în considerare că tegumentul este cel mai întins organ al corpului uman, expus direct şi constant la agenții carcinogenetici din mediul înconjurător. Majoritatea leziunilor sunt localizate la nivelul feței, scalpului şi spatelui. Carcinoamele cutanate îşi au originea în elementele celulare ce alcătuiesc cele două straturi distincte dar interdependente ale tegumentului, epidermul şi dermul, cât şi în structurile anexe ale pielii. Se pot dezvolta pe tegumentul aparent sănătos, sau prin transformarea malignă a unor leziuni cutanate preexistente (leziuni precanceroase). Implicarea factorilor carcinogenetici externi în etiopatogenia acestora este certă, cei mai importanți fiind radiațiile solare, radiații ionizante, unele substanțe chimice şi papiloma virusul uman (1).

Pornind de la aceste date, ne-am propus identificarea celor mai frecvente forme clinice şi anatomo-patologice ale carcinoamelor cutanate ale feței. În acest scop am întreprins un studiu retrospectiv pe o peri-

oadă de 5 ani (ianuarie 2006 - decembrie 2010).

MATERIAL ŞI METODĂ

În intervalul ianuarie 2006- decembrie 2010 au fost internați și diagnosticați în Clinica Chirurgie Orală şi Maxilo-facială Iași un număr de 308 pacienți cu carcinoame cutanate ale feței. Pentru fiecare din cazurile incluse în lotul de studiu am reținut următoarele date: sex, vârstă, mediul de proveniență, localizarea tumorii, diagnosticul clinic şi cel anatomo-patologic.

Studiul clinic s-a bazat pe precizarea localizării şi a formei clinice a carcinomului în momentul spitalizării. Pentru studiul histopatologic am avut în vedere următorii parametri: tipul carcinomului cutanat (CBC – carcinom bazocelular, CSC – carcinom spinocelular, de structură anexa a pielii, cu celule Merkel, metastază de carcinom cu altă localizare - MTS); forma histopatologică a carcinomului bazocelular; gradul de diferențiere a carcinomului scuamocelular (bine diferențiat; moderat diferențiat; slab diferențiat; nediferențiat).

REZULTATE

În intervalul ianuarie 2006 - decembrie 2010 au fost spitalizați 308 pacienți cu carcinoame cutanate, din care 56,16% au fost pacienți de sex masculin, femeile reprezentând 43,84%. Majoritatea pacienților (69,80%) au provenit din mediul rural (tab. I).

Carcinoamele cutanate au predominat la pacienți cu vârste cuprinse între 71-80 de ani (32,79%) şi 61-70 de ani (26,62%) (tab.II). Vârsta medie de apariție a bolii a fost de 69,16 ani.

TABELUL I

Distribuția anuală a pacienților în funcție de sex şi mediul de proveniență

An	Femei	Bărbați	Mediu rural	Mediu urban
2006	18	28	31	15
2007	25	48	45	28
2008	32	35	59	8
2009	30	25	37	18
2010	30	37	43	24
Total	**135 (43,84%)**	**173 (56,17%)**	**215 (69,8%)**	**93 (30,2%)**

TABELUL II

Distribuția pacienților pe decade de vârstă

Decadă vârstă	Nr/ (%)
< 40 ani	7 (2,29%)
41-50 ani	16 (5,19%)
51-60 ani	56 (18,18%)
61-70 ani	82 (26,62%)
71-80 ani	101 (32,79%)
> 81 ani	46 (14,93%)

Din cei 308 pacienți luați în studiu, în 82,46% din cazuri au fost prezente carcinoame cutanate cu o singură localizare. Localizările predilecte de dezvoltare a leziunilor cutanate maligne au fost piramida nazală – 28,57% şi regiunea geniană – 23,70%. Alte localizări (regiuni mentonieră, palpebrală, cervicală, periauriculară, parietală etc) au fost reprezentate proporții mult reduse, cuprinse între 2,59% şi 0,39%) (tab.III).

TABELUL III
Localizarea leziunilor carcinomatoase cutanate ale feței

Localizare	Nr/ (%)
Localizare unică	**254 (82,46%)**
Piramida nazală	88 (28,57%)
Regiune genianã	73 (23,70%)
Regiune temporală	31 (10,06%)
Regiune frontală	28 (9,09%)
Regiune maseterină	25 (8,11%)
Regiune labială	24 (7,79%)
Alte localizări	39 (12,68%)
Localizare multiplă	**54 (17,54%)**

Carcinomul bazocelular s-a situat pe primul loc ca frecvență, reprezentând 75,97%, urmat de carcinomul scuamocelular (18,18%) (fig. 1). Alte tipuri de carcinoame cutanate întâlnite, dar în proporții semnificativ mai reduse, au fost carcinoamele cu celule Merkel (0,65%) şi carcinoamele dezvoltate pe structuri anexe al tegumentului, precum şi metastaze cutanate ale unor carcinome cu alte localizări (sân, colon) (tab.IV).

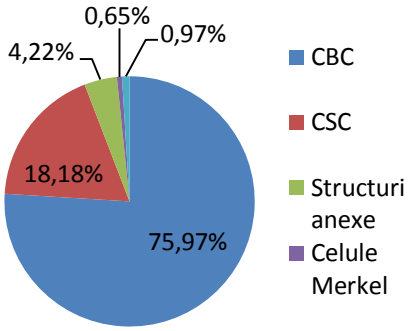

Fig. 1. Distribuția procentuală a carcinoamelor cutanate.

TABELUL IV
Tipuri de carcinoame cutanate

Sex	CBC (nr./%)	CSC (nr./%)	Structuri anexe (nr./%)	Celule Merkel (nr/%)	MTS (nr/%)
F	106 (45,30%)	26 (46,43%)	2 (15,39%)	0	1 (33,34%)
B	128 (54,70%)	30 (53,57%)	11 (84,61%)	2 (100%)	2 (66,66%)
Total	234	56	13	2	3

Din cele 234 cazuri de carcinoame bazocelulare, 54,70% au fost diagnosticate la pacienți de sex masculin, restul de 45,30% fiind reprezentat de sexul feminin. În cadrul carcinoamelor bazocelulare, am întâlnit toate tipurile clinice descrise în literatură: tipul nodular (69,23%), tipul superficial (20,08%), tipul morphea (5,55%) şi pigmentat (5,14%). Examenul histopatologic a confirmat prezența carcinomului bazocelular tip nodular sau solid pe primul loc ca frecvență, dar şi alte forme anatomopatologice, cum ar fi carcinomul bazoscuamos sau metatipic (3,84%), carcinomul bazocelular micronodular (3,41%) sau carcinomul bazocelular keratotic (2,56%). Tipurile polimorfe de carcinoame bazocelulare au fost întâlnite în 3,41% din cazuri (tab. V).

Carcinoamele scuamocelulare au reprezentat 18,18% din totalul leziunilor, cele mai frecvente fiind: ulcero-vegetantă (50%), nodulară (17,85%), verucoasă (17,85%). În funcție de gradul de diferențiere, carcinoamele scuamocelulare bine diferențiate au fost prezente în procent de 32,14%, formele

moderat diferențiate – 42,85%, iar cele slab diferențiate 16,07% (tab. VI).

TABELUL V
Forme clinice şi histopatologice carcinoame bazocelulare

Forme clinice	**Nr (%)**
Nodular	162 (69,23%)
Superficial	147 (20,08%)
Morphea	13 (5,55%)
Pigmentat	12 (5,14%)
Forme histopatologice	**Nr (%)**
Nodular/solid	156 (66,66%)
Superficial	34 (14,52%)
Bazoscuamos	9 (3,84%)
Micronodular	8 (3,41%)
Polimorf	8 (3,41%)
Keratotic	6 (2,56%)
Infiltrativ	5 (2,13%)
Diferențiere anexială	5 (2,13%)
Fibroepitelial	3 (1,34%)

TABELUL VI
Forme clinice şi histopatologice de carcinoame scuamocelulare

Forme clinice	**Nr (%)**
Ulcero-vegetantă	28 (50%)
Nodular	10 (17,85%)
Verucos	10 (17,85%)
Keratozic	8 (14,3%)
Forme histopatologice	**Nr (%)**
Bine diferențiat	18 (32,14%)
Moderat diferențiat	24 (42,85%)
Slab diferențiat	9 (16,07%)
Nediferențiat	5 (8,94%)

Carcinoamele structurilor anexe ale pielii au fost slab reprezentate în lotul de studiu, însumând doar 13 cazuri, adică 4,22%, din care 7 cazuri au fost carcinoame cu diferențiere sebacee, 2 cazuri cu diferențiere ecrină, 2 cazuri cu diferențiere apocrină şi 2 cazuri cu diferențiere foliculară (tab. VII).

TABELUL VII
Carcinoamele structurilor anexe ale pielii

Tipul diferențierii celulare	**Nr (%)**
Sebacee	7 (53,84%)
Apocrină	2 (15,38%)
Ecrină	2 (15,38%)
Foliculară	2 (15,39%)

Carcinomul cu celule Merkel s-a întâlnit în 0,65% din pacienți, iar metastazele cutanate de la carcinoame cu alte localizări în 0,97% din cazuri (fig. 1).

DISCUȚII

Carcinoamele cutanate sunt cele mai frecvente tumori maligne şi datorită creşterii continue a incidenței reprezintă o problemă importantă de sănătate publică. Numărul total al carcinoamelor cutanate diagnosticate anual în Statele Unite este de peste un milion, echivalent cu totalul cazurilor de cancere cu alte localizări (1). În Marea Britanie sunt raportate anual peste 46 000 de cazuri noi de cancer cutanat (2). De asemenea, incidențe foarte mari sunt întâlnite în Australia -1170/100 000 (3). În România cancerul cutanat a reprezentat 10,8% din totalul cancerelor în 2005 (4).

În etiopatogenia carcinoamelor cutanate sunt implicați mai mulți factori. Peste 90% din carcinoamele cutanate ale feței sunt cauzate de expunerea la radiațiile solare (1). În studiul nostru, cei mai mulți pacienți, reprezentând un procent de 69,92%, proveneau din mediul rural, majoritatea cu profesii care presupun expunerea îndelungată la soare (agricultori, grădinari, etc.).

Carcinoamele cutanate se dezvoltă în special la indivizi între a 4-a şi a 8-a decadă de viață, cu o uşoară predilecție pentru sexul masculin. În lotul nostru vârsta medie a fost de 69,16 ani, cele mai multe cazuri (32,79%) aparținând decadei vârstă

71-80 de ani. Raportul bărbaţi/femei a fost de 1,28, apropiat de cel înregistrat în Suedia sau Marea Britanie (5).

Majoritatea autorilor au apreciat că cea mai frecventă localizare a carcinoamelor cutanate este reprezentată de piramida nazală (25-30%), urmată de alte regiuni ale feţei, scalpului şi gâtului (6,7). În studiul nostru, piramida nazală s-a situat pe primul loc – 28,57% (88 cazuri), urmată regiunea genianã – 23,70% (73 cazuri).

Carcinoamele cutanate cu localizări multiple se dezvoltă cel mai adesea pe leziuni cutanate preexistente. Tranziţia de la tegumentul normal la leziunea precanceroasă şi ulterior la neoplazia cutanată se desfăşoară lent, pe durata a mai multor ani. Imunosupresia cronică poate contribui la apariţia tumorilor cutanate multiple, adeseori având o evoluţie agresivă. Într-o serie de afecţiuni determinate genetic, se remarcă o reală predispoziţie pentru dezvoltarea de tumori cutanate maligne multiple, la vârste semnificativ mai tinere decât la indivizii normali. Nevomatoza bazocelulară multiplă, sindromul Rambo, sindromul Bazex, xeroderma pigmentosum, sindromul Cowden includ în tabloul clinic prezenţa carcinoamelor bazocelulare multiple (4,8). În studiul nostru, 54 de pacienţi, reprezentând 17,54% au prezentat leziuni multiple. În 4 cazuri, carcinoamele s-au dezvoltat probabil pe fondul imunosupresiei cronice, deoarece 3 pacienţi au prezentat în antecedente transplanturi renale şi unul dintre ei a urmat tratament cronic cu imunosupresoare timp îndelungat pentru lupus eritematos sistemic. Examenul histopatologic a stabilit diagnosticul de carcinom scuamocelular moderat diferenţiat cu keratinizare redusă, focare de necroză şi ulceraţie întinsă. Cea mai mare parte a carcinoamelor multiple însă s-au dezvoltat pe leziuni preexistente, în special keratoza actinică. Cazurile de carcinoame bazocelulare multiple din lotul de studiu nu au apărut pe fondul unei afecţiuni genetice.

Carcinomul bazocelular se dezvoltă din keratinocitele bazale ale epidermului şi structurilor anexe ale pielii (foliculi piloşi, ductele glandelor sudoripare). Este cea mai frecventă tumoră malignă cutanată, reprezentând aproximativ 75% din carcinoamele cutanate nonmelanocitare (8). În lotul nostru procentul a fost de 75,97%.

Carcinomul bazocelular are mai multe forme clinice, care variază ca aspect clinic şi potenţial malign. Cea mai frecventă este cea nodulară, reprezentând 62-70%, urmată de forma superficială (10%). Forme mai puţin întâlnite sunt carcinomul bazocelular sclerozant sau forma morphea**,** cea mai agresivă formă clinică, precum şi carcinomul pigmentat (7,10). O atenţie specială trebuie acordată carcinomului bazoscuamos sau metatipic, care a constituit de-a lungul timpului subiectul a numeroase controverse. Confuzia derivă din aspectul său histopatologic, cu prezenţa caracteristicilor comune celor două tipuri, bazocelular şi scuamocelular la nivelul aceleiaşi leziuni, de unde şi caracterul imprevizibil al prognosticului şi comportamentului tumorii (8).

În ceea ce priveşte formele clinice ale carcinomului bazocelular, am observat prezenţa în lotul de studiu a tuturor tipurilor clinice citate în literatură, cel mai frecvent întâlnite forme fiind cea nodulară (69,23%) şi superficială (20,08%). De asemeni, am remarcat polimorfismul histopatologic al acestui carcinom, fapt de altfel consemnat în literatura de specialitate. În ordinea descrescătoare a frecvenţei, pe primele locuri am înregistrat următoarele forme histopatologice: carcinom bazocelular nodular/solid (66,66%), carcinom ba-

zocelular superficial (14,52%), carcinom bazoscuamos (3,84%).

Carcinoamele scuamocelulare sunt tumori maligne cu originea în keratinocitele epidermice, dezvoltate cel mai frecvent pe leziuni precursoare, cum este keratoza actinică. Ocupă locul al 2-lea ca frecvență după carcinomul bazocelular, reprezentând peste 20% din totalul tumorilor maligne ale pielii (7). În lotul luat în studiu, carcinoamele scuamocelulare au reprezentat 18,18%. Cele mai întâlnit forme clinice au fost forma ulcerativă (50%), forma nodulară (17,85%) şi forma verucoasă (17,85%). Studiul histopatologic al carcinomului scuamocelular a evidențiat predominența formelor moderat diferențiate (42,85% din cazuri), urmată de forma bine diferențiată (32,14%) şi cea slab diferențiată (16,07%). Aceste date sunt asemănătoare celor desprinse din alte studii (11).

Carcinoamele anexelor pielii prezintă adeseori reale dificultăți de diagnostic pozitiv şi diferențial. În funcție de tipul de diferențiere celulară, tumorile structurilor anexe se clasifică în tumori cu diferențiere apocrină, ecrină, foliculară şi sebacee (12).

Carcinoamele glandelor sudoripare sunt relativ rar întâlnite (2-3 cazuri la 100.000 indivizi) şi manifestă un comportament clinic agresiv (7). În lotul nostru le-am întâlnit în 4 cazuri, reprezentând 30,76% din carcinoamele structurilor anexe ale pielii şi 1,29% din totalul carcinoamelor cutanate. Carcinomul sebaceu este o tumoră agresivă rară cu originea în țesutul epitelial al glandelor sebacee. Tumora apare la indivizii în vârstă, de obicei în decadele 6 şi 8 de viață, având o incidență aproximativ egală la cele două sexe (13,14). În lotul studiat a repezentat 53,84% din carcinoamele structurilor anexe ale tegumentului şi 2,27% din totalul carcinoamelor cutanate luate în studiu.

Carcinomul cu celule Merkel sau carcinomul neuroendocrin cutanat primar este un tip rar de cancer cutanat dezvoltat din celulele Merkel din stratul bazal al epidermului, cu rol de receptori tactili. Incidență este estimată la 0,44 din 100 000 indivizi (6, 15). Am identificat doar 2 cazuri de carcinoame cu celule Merkel, reprezentând 0,65% din totalul carcinoamelor cutanate.

Metastazele cutanate, deşi rare, pot reprezenta primul semn clinic al unei malignități viscerale. Frecvența metastazelor cutanate este corelată la fiecare sex cu tipul tumorii primare. Femeile cu metastaze cutanate prezintă următoarea distribuție a carcinoamelor primare: sân 69%; colon 9%; melanom 5%. La bărbați, distribuția este următoarea: plămân 24%; colon 19%; melanom 13% (16). Am identificat 3 cazuri de metastaze cutanate, în două cazuri tumora primară fiind la nivelul sânului şi într-un caz cu localizare la nivelul colonului.

CONCLUZII

Carcinoamele cutanate sunt cele mai frecvente tumori maligne ale organismului, reprezentând prin morbiditatea crescută şi prin gravitatea potențială o problemă de sănătate publică. Educarea populației pentru cunoaşterea factorilor de risc, cât şi depistarea şi tratamentul precoce al cancerelor cutanate şi al leziunilor precanceroase sunt elemente decisive în managementul acestor leziuni, asigurând rate de vindecare crescute.

Datorită polimorfismului clinic al carcinoamelor cutanate, examenul histopatologic este indispensabil pentru stabilirea diagnosticului de certitudine, în unele cazuri fiind necesare investigațiile imunohistochimice, în special în cazurile cu structură histopatologică particulară.

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SCREENINGUL SCOLIOZEI PRIN INTERMEDIUL ANALIZEI PRESIUNII PLANTARE

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SCOLIOSIS SCREENING USING PLANTAR PRESSURE ANALYSIS (abstract) : Earlier scoliosis detection is one of the orthopedics and family doctor challenge. Normally, the first observation about a child deformity should be done by the family doctor in order to announce or not the need of special investigations by a specialist. Usually, the so called idiopathic scoliosis is determined by some factor that interfere during the growing period and most of the time a wrong body attitude static and dynamic during daily activities (learning, sleeping, playing, sitting) and/or angular discrepancy or limb length discrepancy. **Material and method :** All those factors effects can be seen in a plantar pressure analysis evaluation witch soft give us information about the center of gravity of the body and the pressure on the foot that can be correlate in order to discover any articulation imbalance, even the spine deformity. **Results** : It has been demonstrated through algorithms and case studies that a specific pattern of the plantar pressure analysis image is characteristic for the scoliotic persons. **Key words** : SCOLIOSIS, PODOLOGY, PRESSURE

Scolioza reprezintă deformările şi încurbările coloanei vertebrale în forma literei S sau a literei C. Devierea se petrece în plan frontal, dar se şi poate răsuci în jurul axului. Mulţi oameni au deviaţii uşoare ale coloanei vertebrale, dar nu sunt considerate a fi scolioze în cazurile în care curburile au mai puţin de 10 grade. Scolioza apare îndeosebi la nivelul coloanei toracice (în partea de mijloc a spatelui) sau la nivelul coloanei lombare (în partea de jos a spatelui). Dacă deviaţiile sunt mari, atunci pot apărea dureri disconfortante şi dificultăţi mari în respiraţie. La copii, deformările coloanei vertebrale care se concretizează cu timpul în scolioze, se produc în timpul copilăriei, între 9 şi 11 ani. Medicii specialişti susţin că deformările coloanei vertebrale sunt în strânsă legătură cu primii ani de şcoală, adică primii ani de stat în bancă perioade mai lungi de timp. Aceştia afirmă că scolioza este determinată de poziţia pe care o adoptă copilul în bancă în timpul orelor petrecute la şcoală.

La ora actuala evaluarea coloanei se face de către medicul ortoped la solicitarea parintelui sau a medicului de familie care observă o deformaţie a coloanei masurabilă cu scoliometrul sau radiologic. Nu există metodă de evaluare fără a dezbraca pacientul şi a-l observa. În al doilea rând, nici o altă metodă nu corelează inegalităţi ale sistemului osteoarticular cu prezenţa scoliozei (1).

MATERIAL ŞI METODĂ

În această lucrare se urmăreşte diagnosticarea scoliozei utilizând analiza plantară, o metodă modernă şi, în acelaşi timp eficientă.

Analiza plantară computerizată reprezintă una din tehnicile performante de identificare a zonelor de presiune de la nivelul piciorului (2,3). Testarea se realizează în statică şi/sau dinamică, prin contactul cu o platformă cu senzori de presiune, care preia

informaţiile de la nivelul piciorului şi le transpune prin intermediului unui soft specializat într-o imagine (1 : 1). Complexitatea programului ne permite stocarea unei baze de date a pacienţilor testaţi, putându-se analiza nu numai amprenta plantară ci şi mersul, uzura încălţămintei, postura întregului corp, facilitându-se, astfel, urmărirea evoluţiei pacienţilor (4,5).

În studiul prezentat în această lucrare, metoda de evaluare, a pacienţilor cu scolioză, presupune folosirea unei o plăci matriceale tip LorAn Engineering Sistema EPS-C, cu senzori de presiune şi soft de redare a presiunilor la nivelul plantei cu posibilitatea trasării graficului de repartiţie a centrului de greutate a corpului, la nivelul poligonului de susţinere.

Placa de presiune are o suprafaţă de 50cm $\times$50 cm cu 2500 de senzori cu timp de înregistrare setabil de minim 5 secunde. Poziţia centrului de greutate poate fi vizualizată atât statistic prin cumul total, cât şi punctual, în regim dinamic frame by frame.

În scopul diagnosticării scoliozei s-a realizat un algoritm de studiu, care constă în evidenţierea repartiţiei presiunilor plantare cae să fie caracteristică acestrui tip de deformare a coloanei vertebrale.

S-au luat în calcul modelele de deviaţie a centrului de greutate a corpului în funcţie de diferite inegalităţi ale sistemului osteoarticular.

S-a ţinut cont de deviaţiile în cele trei axe ale segmentelor corpului, în cazul diferitelor tipuri de scolioză, interpretarea luând în consideraţie deviaţiile în ambele sensuri, de sus în jos şi de jos în sus.

În regim static, scolioza este asociată cu deviaţiile clasice cele mai frecvente care stau la baza dezvoltarii acesteia : valg călcâi unilateral, metatars adductus unilateral, valg/var genunchi unilateral, rotaţie de gambă, rotaţie de femur, anteversie col femural, scurtare reală de membru, antecedente în articulaţia şoldului (displazie de şold unilaterală incomplet redusă), dezechilibru muscular între adductori şi abductori.

În regim dinamic, factorii care redau o scolioză, dar care nu stau la baza unor inegalităţi osteoarticulare, sunt parezele musculare şi hemiplagiile.

Deviaţiile datorate deformaţiei coloanei sunt : translaţie de bazin, înclinare de trunchi, modificare a poziţiei centurii scapulare, modificarea curburii costale pe diagonală, înclinaţia bazinului, rotaţia trunchiului faţă de bazin, false scurtări.

PROTOCOL EXPERIMENTAL

S-au efectuat 520 de amprente plantare la copii cu vârste cuprinse între 4 şi 18 ani. S-a ţinut cont de vârsta de 4 ani considerată vârsta pâna la care deviaţiile în ax ale piciorului sunt normale. În acest sens, s-au efectuat 50 de analize pe copii cu vârsta sub 4 ani, care s-au dovedit neconcludente datorită incapacităţii de a menţine copilul cu ambele picioare pe placă, (peste 50% dintre copii mişcau unul din membre în timpul înregistrării).

Pentru comparaţie, toţi pacienţii au fost verificaţi cu scoliometrul şi prin teste specifice care să determine deviaţia coloanei : metoda verificării cu firul de plumb, evaluare vizuală din poziţia aplecat la 90 de grade, radiografii (pentru cazurile mai grave).

S-au luat în calcul toate deviaţiile osteoarticulare posibile, astfel încât pacienţii suspectaţi că ar avea şi alte deviaţii decât cele ale piciorului şi coloanei, au fost verificaţi la nivelul genunchiului şi soldului prin măsurători goniometrice şi dinamice.

Primele caracteristici ale prezenţei scoliozei au fost determinate prin observarea unei diferenţe de presiuni plantare între membrul inferior stâng şi cel drept şi între raportul de reparţitie al presiunii atât între calcâiul drept şi cel stang, cât şi între antepiciorul drept şi cel stang.

REZULTATE ŞI DISCUŢII

Vor fi analizate, în cele ce urmează,

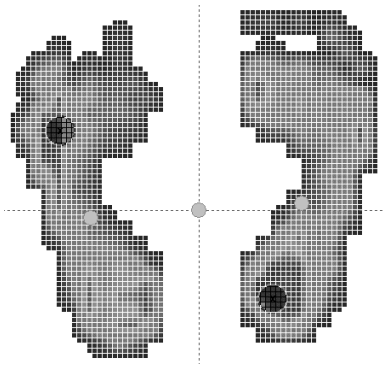

Fig. 1. Distribuţia de presiuni în urma analizei plantare (E. B. 8 ani ♀). Valorile maxime, ale presiunii, se înregistrează la nivelul antepicioarului stâng şi retropiciorului drept (cercurile mari)

două modele ale distribuţiei de presiuni la nivel plantar, corespunzătoare la două cazuri care sunt concludente în stabilirea diagnosticului de scolioză. Masurătorile au scos în evidenţă fasptul ca la nici unul dintre cele două cazuri nu sunt prezente deformaţii la nivelul articulaţiilor şoldului, genunchiului şi gleznei.

Un prim caz (E. B. 8 ani ♀) ale cărui rezultate, în urma analizei plantare, sunt ilustrate în fig. 1, relevă faptul că pacientul este perfect compensat, în sensul în care diferenţa presiunilor medii (în procente din valoarea medie a presiunii pe întreaga suprafaţă de sprijin) dintre piciorul stâng şi cel drept, este aproximativ egală: 49,5%/50,5%, (fig. 1.B). De asemenea, se observă că suprafeţele de contact diferă cu 1 cm^2 (105,0 cm^2 stanga şi 104,0 cm^2 dreapta) iar presiunile medii au valorile 0,9 kgf/cm^2 (stânga) şi 1 kgf/cm^2 (dreapta). Se poate trage concluzia că forţa echivalentă care acţionează pe piciorul stâng este de 94,5 kgf (927 N) iar pe piciorul drept este de 104 kgf (1020 N), astfel, se poate trage concluzia că că glezna dreaptă este mai încărcată decât glezna stângă. Cu alte cuvinte centrul de greutate al corpului va fi deplasat spre dreapta-posterior, coloana fiind curbată în aceeaşi direcţie.

Dacă, în schimb, se ia în consideraţie

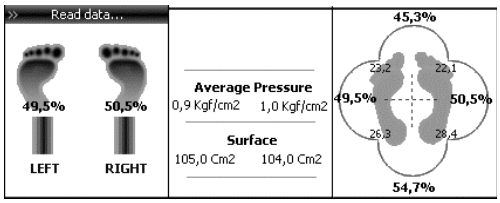

Fig. 2. Distribuţia presiunilor, în valori procentuale şi în valori reale, pentru piciorul stâng şi piciorul drept, şi valorile presiunilor în procente pentru fiecare antepicior şi retropicior în parte, corespunzătoare cadranului suprafeţei de sprijin (E. B. 8 ani ♀).

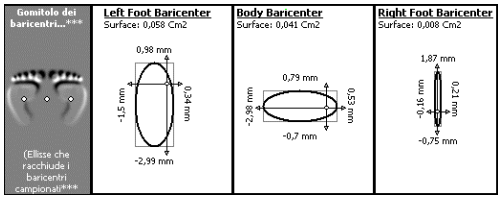

Fig. 3. Poziţia baricentrelor pentru: piciorul stâng, întreaga suprafaţă de sprijin şi piciorul drept (E. B. 8 ani ♀).

repartiţia presiunilor defalcată pe zonele reprezentate de retropicior şi antepicior se observă că distribuţia acestora se situează pe două diagonale: valorile mari pe o diagonală, valorile mici pe cealaltă diagonală, altfel spus, valorile mici sunt pe diagonala calcâi stâng şi antepicior drept şi valorile mari sunt pe diagonala ale calcâi drept şi antepicior stâng (fig. 1). Dacă se face suma presiunilor pentru un picior pe axul retropicior–antepicior, se observă că diferenţa este destul de mică de 1% (49,5%/50,5%), dar dacă se calculează pe cele două diagonale, se obţine o diferenţă semnificativă de 3,2% (48,4%/51,6%), ceea ce ilustrează, de asemenea, un dezechilibru (fig. 2).

O altă variabilă, care poate da informaţii cu privire la prezenţa scoliozei, este graficul rezultat prin unirea punctelor poziţiei baricentrelor corespunzătoare: piciorului sting, a întregului corp corp, picior drept, care este reprezentat de o linie descendentă

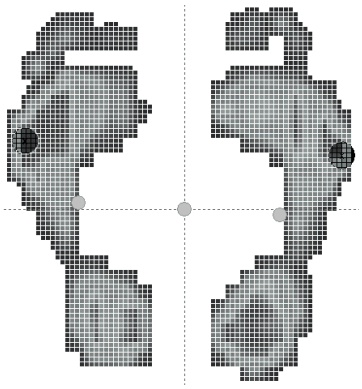

Fig. 4. Distribuţia de presiuni în urma analizei plantare (R. C. 10 ani ♀). Valorile maxime, ale presiunii, se înregistrează la nivelul antepiciorului stâng şi antepiciorului drept (cercurile mari).

(fig. 3). O astfel de diagramă este valabilă în cazul scoliozelor clare.

S-a observat că, cu cât panta baricentrelor este mai mare, cu atât rotaţia în ax şi, implicit, scolioza este mai acentuată.

Pentru cel de al doilea caz (R. C. 10 ani ♀), distribuţia de presiuni plantare este ilustrată în fig. 4. Spre deosebire de cazul anterior, diferenţa presiunilor medii, în procente, dintre piciorul stâng şi cel drept, este destul de mare: 47,3%/52,7%, (fig. 4). Suprafeţele de contact înregistrază, de asemenea, diferenţe semnificative: 97,0 cm^2 (stânga) şi 102,0 cm^2, mai mici decât în primul caz. Presiunile medii, în schimb, au valori sensibil egale (1,2 kgf/cm^2 (stânga) şi 1,3 kgf/cm^2 (dreapta)). Forţele echivalente pentru fiecare picior în parte sunt: 116,4 kgf (1142 N) pentru piciorul stâng şi 132,6 kgf (1301 N) pentru piciorul drept, aceste date ilustrând faptul că glezna dreaptă este mai încărcată decât glezna stângă cu aproximativ 14%, deci, centrul de greutate al organismului este deplasat spre dreapta-anterior, iar coloana este înclinată în acelaşi sens.

Presiunile (în procente din presiunea medie pe întreaga suprafaţă de sprijin) pentru antepicior şi retropicior ilustrează un dezechilibru pentru acelaşi picior, suma pe axul retropicior-antepicior fiind diferită pentru cele două picioare 43,7% pentru stângul şi 52,7% pentru dreptul. Acest dezechilibru este dat de diferenţa mare de presiuni existentă la nivelul călcâielor: 17,8% pentru stângul şi 23,25% pentru dreptul. La nivelul antepiciorului, presiunile sunt sensibil egale: 29,6% pentru stângul şi 29,5% pentru dreptul, astfel încât, se formează, şi în acest caz, două diagonale, o diagonală de presiune maximă retropicior drept – antepicior stâng şi o diagonală de presiune minimă retropicior stâng – antepicior drept (fig. 5).

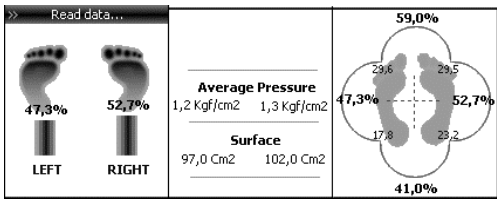

Fig. 5. Distribuţia presiunilor, în valori procentuale şi în valori reale, pentru piciorul stâng şi piciorul drept, şi valorile presiunilor în procente pentru fiecare antepicior şi retropicior în parte, corespunzătoare cadranului suprafeţei de sprijin (R. C. 10 ani ♀).

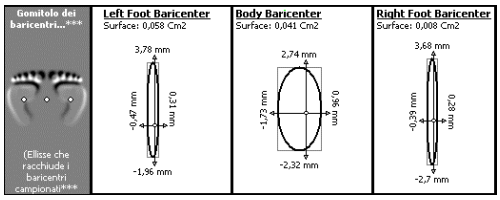

Fig. 6. Poziţia baricentrelor pentru: piciorul stâng, întreaga suprafaţă de sprijin şi piciorul drept (R. C. 10 ani ♀).

De asemenea este semnificativă şi poziţionarea baricentrelor. Se observă că presiunile maxime sunt dispuse anterior, baricentrele din fig. 6 sunt deplasate spre dreapta, dar înclinarea dreptei care este reprezentată de unirea acestora, nu este atât de accentuată precum în cazul anterior.

În acestă situaţie, atitudinea scoliotiocă este cu atât mai evidentă cu cât valorile presiunilor pe antepicior sunt aproape identice, dar diferă cu 4,4% între calcâie.

CONCLUZII

Prezenţa deformaţiilor coloanei vertebrale au influenţă directă asupra distribuţiei presiunilor plantare, fapt confirmat de măsurătorile efectuate pe cei 520 pacienţi şi, implicit, de cele două cazuri prezentate în această lucrare.

Datele culese şi prezentate în această lucrare au condus la concluzia că există două variabile care dau verdictul de „risc de scolioza" : repartiţia pe diagonala a presiunilor maxime şi diferenţa dintre suma presiunilor pe : diagonalele şi/sau pe verticala cadranelor.

Repartiţia baricentrelor pe diagonală, indică o deviaţie în ax a coloanei.

Linia baricentrelor poate fi orizontală, dar cu baricentrele deplasate spre dreapta sau spre stânga (în cazul atitudinilor scoliotice fără deformaţie în C sau S), ceea ce indică o înclinare a coloanei în direcţia deplasării baricentrelor.

Metoda prezentată poate fi aplicată şi în cazul unor alte deformaţii ale coloanei, precum lordoză sau cifoză.

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STUDIU DE ANALIZĂ COMPARATIVĂ SEM A INTERFEŢEI RĂŞINĂ DIACRILICĂ COMPOZITĂ-SMALŢ ÎN CAVITĂŢI PREPARATE MECANIC ŞI KINETIC CU LASER

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SEM COMPARATIVE ANALYSIS OF THE COMPOSITE DIACRYLIC RESIN-ENAMEL INTERFACE IN LASER AND BUR PREPARED CAVITIES (Abstract): The aim of this study was to demonstrate that the size of the hybrid layer (HL) in the prepared enamel mechanic (M) and kinetic (K) with laser (WaterlaseMDBiolase), evaluated by scanning electron microscopy (SEM) restored with two types of resin composite is different. **Material and method**: The study was realized *in vitro* on a sample of 40 extracted human premolar and molars teeth for orthodontic or periodontal reasons. The teeth were divided at random into equal four groups (Gr). There has been Class I cavities with a depth of 2 mm (M) at high and low speed with cylindrical diamond No.1 and globular carbide and (K) peaks MZ6-5, 5W+20 Hz 30% water 60% air. The teeth were restored using 3M™ Schotchbond Etch, 3M™Single Bond Dental Adhesive System: Gr.1: (K)(n=10)-3M™Filtekflow; Gr.2: (K)(n=10)3M™FiltekZ250; Gr.3: (M)(n=10)3M™Filtekflow; Gr.4: (M)(n=10)3M™FiltekZ250. The materials were placed in a single layer and photoactivate source LED SmartLite (Dentsply), termocycling 500cycles (5^0-55^0), stored (48h), cut lengthwise (diamond), polished, conditioned (H3PO4-37% -5s) analyzed by SEM (JEOLJSM 6390), SPSS 13.00 statistically analyzed (ANOVA, p = 0.05). **Results**: Analysis of the (HL) has highlighted differences between groups p = 0.05 average size (HL) being Gr.1; 20.80 (± 0.72)μm, Gr.2; 4.034 (± 0.0076)μm,, Gr.3, 14.13 (± 0.63)μm, Gr.4, 1,43 (± 0.16)μm. Value size (HL) was influenced by the cavity preparation method for samples prepared by laser p=0,005 in terms of size and in advantage of mechanical preparations in terms of quality (HL). Also the value of (HL) size was influenced by the type of material used in advance Gr.2 p= 0.0017. **Conclusions**: The sealing of cavity depends more on the creep of materials and less on the type of preparation. Permission was obtained from a institutional ethical committee of "Gr.T. Popa" University of Medicine and Pharmacy Iaşi and that subjects gave written, informed consent). Founding source: CNCSIS-Project: No. 2669/2008. **Key words**: COMPOSITE RESIN, LASER, SEM.

Scopul acestui studiu a fost analiza comparativă prin microscopie electronică cu baleiaj a stratului hibrid în cavităţi de clasa a I-a preparate mecanic(M) şi kinetic (K) – LASER (WaterlaseMD-Biolase), restaurate cu răşină compozită hibridă 3M™ Filtek Z250 si Filtek flow 3M™.

MATERIAL ŞI METODĂ

Studiul a fost realizat "in vitro" pe 40 de dinţi umani premolari şi molari, extraşi prin obţinerea consimţământului informat al acestora din motive ortodontice sau parodontale.

TABELUL I
Statistica descriptivă a grupurilor studiate

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean		Std.	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
GR.1 FILTEK FLOW-LASER	10	3,38	18,56	21,94	20,8080	,40227	1,27209	1,618
GR.2 FILTEK Z250-LASER	10	,33	3,85	4,18	4,0340	,03899	,12331	,015
GR.3 FILTEK-FLOW-MECHANICAL	10	3,41	12,64	16,05	14,3190	,34511	1,09132	1,191
GR.4 FILTEK Z250-MECHANICA	10	,82	1,19	2,01	1,4340	,08999	,28457	,081
Valid N (listwise)	10							

Dinţii au fost împărţiţi în mod aleatoriu în două grupuri egale la care s-au realizat cavităţi de clasa I de formă dreptunghiulară cu o profunzime de 2mm acţionate în interiorul unui şablon pentru a obţine cavităţi de volum aproximativ egal : Gr. 1,2 - cavităţi preparate mecanic la viteze înalte şi joase cu freze diamantate cilindrice şi freze extradure globulare nr.1 şi Gr. 3,4 – cavităţi preparate kinetic - laser(Waterlase Biolase MD) cu vârfuri de zirconiu MZ6-5, la 5W+20 Hz cu 30% apă si 60% aer acţionate de asemenea în interiorul unui şablon. Materialele de restaurare utilizate au fost : 3M™ Filtek Z250 ESPE), 3M ™Schotchbond etchant, 3M ™ Single Bond Dental Adhesive System. Pentru fotoactivarea materialelor s-a utilizat sursa de fotoactivare luminoasă LED SmartLite (Dentsply). Obturarea dinţilor s-a realizat astfel : Gr.1 : (K) (n=10)-3M™Filtekflow ; Gr.2 : (K)(n=10) 3M™FiltekZ250 ; Gr.3 : (M)(n=10)3M™ Filtekflow ; Gr.4 : (M)(n=10)3M™Filtek Z250. Probele au fost termociclate 500cicli (5^0-55^0) şi păstrate în flacoane cu ser fiziologic până la prepararea probelor pentru analiză (maxim 48 de ore), apoi au fost secţionaţi longitudinal cu discuri diamantate, spălaţi cu alcool de 90^0 pentru a îndepărta stratul de răşină nepolimerizată. Secţiunile rezultate au fost prelucrate şi lustruite cu discuri de hârtie, gume şi paste abrazive cu granulaţie descrescătoare, spălate cu apă distilată timp de 2 minute, demineralizate superficial cu H3PO4 35% timp de 5 secunde pentru a se îndepărta smear-layerul şi au fost apoi spălate timp de 2 minute cu apă distilată. Analiza probelor s-a realizat prin microscopie electronică scanată SEM (JEOLJSM 6390A) iar rezultatele au fost procesate cu ajutorul programului SPSS 14.0 pentru Windows şi a testului statistic ANOVA la un prag de semnificaţie de $p = 0.05$.

REZULTATE

Analiza ultrastructurală a stratului hibrid a pus în evidenţă diferenţe semnificative din punct de vedere statistic între grupuri $p = 0.05$, media dimensiunii stratului hibrid fiind de 20.80 ($\pm$ 0.72)μm pentru Gr.1 de 4.034 ($\pm$ 0.0076)μm pentru Gr. 2, de 14.13 ($\pm$ 0.63)μm pentru Gr. 3 şi de 1,43 ($\pm$ 0.16)μm pentru Gr.4 : (Tab. I, II, III, Fig.1). De asemenea grosimea stratului hibrid a fost influenţată de tipul de material de obturaţie utilizat, Gr. 2 prezentând valorile cele mai bune $p= 0.0017$.

Aspectul interfeţei smalţ - răşină compozită în cavităţi preparate cu LASER : A- GR.1 SEM X 1300–interfaţa smalţ/ compozit Filtek flow (3MESPE). Se observă o intricare optimă a materialului în substructura dentară cu o grosime crescută a stratului hibrid ; B- GR.2 SEM X 3300–inter-

TABELUL II

Multiple comparaţii între grupuri

Dependent Variable: HYBRID LAYER

Bonferroni

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GR.1.FILTEK-FLOW LASER	GR.2 FILTEK-Z250 LASER	16,77400*	,38114	,000	15,7099	17,8381
	GR.3 FILTEK-FLOW - MECHANICAL	6,48900*	,38114	,000	5,4249	7,5531
	GR.4 FILTEK-Z250 - MECHANICAL	19,37400*	,38114	,000	18,3099	20,4381
GR.2 FILTEK-Z250 LASER	GR.1.FILTEK-FLOW LASER	-16,77400*	,38114	,000	-17,8381	-15,7099
	GR.3 FILTEK-FLOW - MECHANICAL	-10,28500*	,38114	,000	-11,3491	-9,2209
	GR.4 FILTEK-Z250 - MECHANICAL	2,60000*	,38114	,000	1,5359	3,6641
GR.3 FILTEK-FLOW - MECHANICAL	GR.1.FILTEK-FLOW LASER	-6,48900*	,38114	,000	-7,5531	-5,4249
	GR.2 FILTEK-Z250 LASER	10,28500*	,38114	,000	9,2209	11,3491
	GR.4 FILTEK-Z250 - MECHANICAL	12,88500*	,38114	,000	11,8209	13,9491
GR.4 FILTEK-Z250 - MECHANICAL	GR.1.FILTEK-FLOW LASER	-19,37400*	,38114	,000	-20,4381	-18,3099
	GR.2 FILTEK-Z250 LASER	-2,60000*	,38114	,000	-3,6641	-1,5359
	GR.3 FILTEK-FLOW - MECHANICAL	-12,88500*	,38114	,000	-13,9491	-11,8209

*. The mean difference is significant at the .05 level.

TABELUL III

Testul de omogenitate a varianţelor

HYBRID LAYER

Levene Statistic	df1	df2	Sig.
11,489	3	36	,000

faţa smalţ/ compozit Filtek Z250 (3MESPE). Se observă o intricare optimă a materialului în substructura dentară dar cu o grosime considerabil mai mică a stratului hibrid comparativ cu răsina Filtek flow (3MESPE).

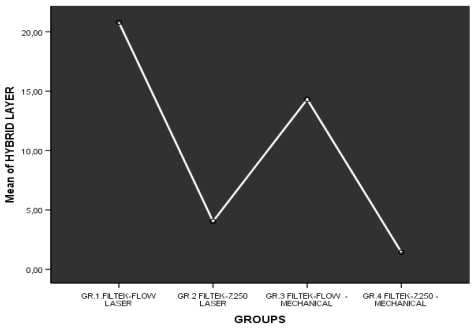


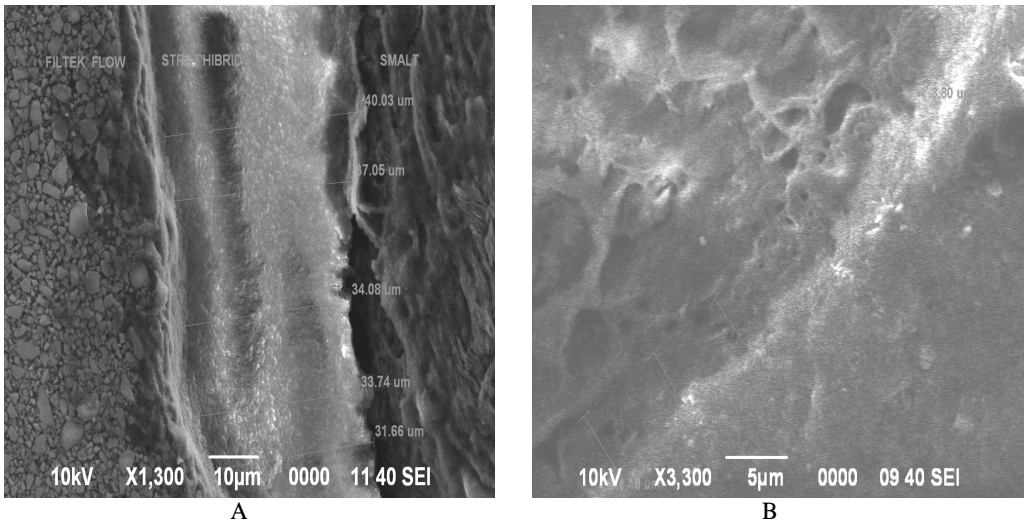
Fig. 1. Reprezentarea grafică a mediei dimensiunii stratului hibrid

TABELUL IV

Testul ANOVA de comparare a datelor

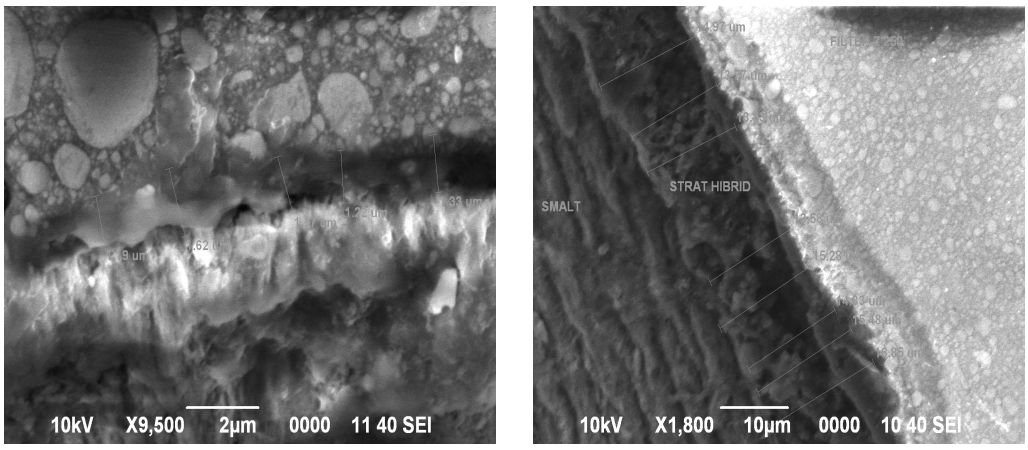
HYBRID LAYER

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2443,476	3	814,492	1121,352	,000
Within Groups	26,149	36	,726		
Total	2469,625	39			



A B

Fig. 2



A B

Fig. 3

Aspectul interfeţei smalţ - răşină compozită în cavităţi preparate meanic A- GR.3 SEM X 5500- interfaţa smalţ/ compozit Filtek flow (3MESPE). Se observă o intricare optimă a materialului în substructura dentară cu o grosime scăzută dar constantă şi un strat hibrid uniform ; B- Gr.4 SEMX1800 – interfaţă smalţ/ compozit Filtek Z250 (3MESPE). SEM X 1800 . Se observă o intricare optimă a materialului în substructura dentară.

DISCUŢII

Adeziunea biomaterialelor la structurile dure dentare este rezultatul combinării mai multor mecanisme realizându-se o adeziune chimică (legături primare între adeziv si aderent), adeziune fizică (legături secundare, eficientă când distanţa dintre materiale este foarte mică) şi adeziune mecanică (penetrarea materialului în structurile dure dentare la nivel micrometric sau chiar nanometric. Evaluând capacitatea de intricare a materialului de obturaţie în substructura

dentară am concluzionat că materialul 3M™ Filtekflow realizează o grosime a stratului hibrid mult mai mare în comparaţie cu 3M™ FiltekZ250 : 20,80 (laser) şi 14,31 (mecanic) faţă de 4,03 (laser) şi 1,43 (mecanic) (Fig.2,3).

Studiul nostru a arătat că dimensiunea stratului hibrid a fost influenţată de metoda de preparare a smalţului, cavităţile preparate cu laser (Waterlase Biolase MD) prezentând o intricare optimă.

Din punct de vedere calitativ am concluzionat faptul că prepararea mecanică spre deosebire de cea laser a determinat obţinerea unui strat hibrid uniform distribuit la nivelul interfeţei RDC-smalţ, cavităţile preparate fiind astfel mai puţin susceptibile la microinfiltraţie. Rezultatele obţinute sunt susţinute de alte studii de specialitate care au evidenţiat că prepararea laser nu creşte adeziunea materialelor de obturaţie la smalţ şi nici nu ajută la scăderea gradului de microinfiltraţie (1,2,3).

În ceea ce priveşte microinfiltraţia diferitor materiale de restaurare cu structură nanometrică aplicate în cavităţi de clasa V preparate prin metode mecanice şi kinetice--laser, studiile internaţionale au demonstrat faptul că aceasta a fost mai mare la nivelul cavităţilor preparate cu laser decât cea de la nivelul cavităţilor preparate mecanic, indiferent de materialul de obturaţie utilizat (4,5).

Rezultatele prezentate mai sus sunt contrazise de studiul realizat de Yazici în 2009 care susţinea că prin ambele metode de preparare, mecanic şi kinetic, s-au realizat cavităţi care s-au comportat similar, cu rezultate excelente (6).

CONCLUZII

Permeabilitatea tisulară şi gradul de difuzibilitate a materialului reprezintă factori esenţiali pentru hibridizarea răşinilor în smalţ. Studiul nostru a evidenţiat faptul că sigilarea cavităţilor depinde mai mult de capacitatea de intricare a materialului de obturaţie în substructura dentară decât de tipul preparaţiei.

Variaţii ale morfologiei stratului hibrid au fost puse în evidenţă între grupuri dar şi în cadrul aceluiaşi grup la nivelul fiecărei mostre.

Indiferent de grupul studiat, adeziunea la smalţ s-a dovedit optimă din punct de vedere calitativ, prezenţa dehiscenţelor şi a stratului hibrid inconstant remarcându-se în special în cazul probelor preparate cu laser.

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CONSIDERAŢII ASUPRA DECESELOR CAUZATE DE VIOLENŢA DOMESTICĂ, ÎN JUDEŢUL NEAMŢ, ÎN PERIOADA 1997-2008

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CONSIDERATIONS ON DEATH BY DOMESTIC VIOLENCE IN NEAMT COUNTY, BETWEEN 1997-2008 (Abstract): **Material and methods**: The study include a series of 132 cases of domestic violence (DV) accounting for 58% of the 228 homicides recorded between 1997 and 2008 in the Neamt county. Data were processed and interpreted based on epidemiological, statistic and mathematical computer-based methods. **Results**: In 1998, in the Neamt county, 0.69‰ homicides were recorded as compared to 2.56 at country level. In 2000, the rate of homicides by DV had significantly increased, reaching 12.3‰, compared to 4.5‰ at the national level. The prevalence of homicides by DV, according to environment, gender, age groups and other features is presented. **Conclusions**: Domestic violence is hard to control, this phenomenon being uncommon to Romanian society, but represents a major social and public health problem. **Key words**: DOMESTIC VIOLENCE, AGGRESSION, PUBLIC HEALTH PROBLEM, FORENSIC MEDICINE

Violenţa domestică (VD) este un fenomen care a însoţit dintotdeauna construirea şi dinamica familiei, dar includerea ei în atenţia ştiinţelor juridice şi sociale este de dată recentă. Doar în ultimii 20 de ani, ştiinţele sociale şi reglementările legale internaţionale fac referinţă la VD (1,2,3,4).

În prezent, se consideră că VD însumează efectele unor factori sociali şi psihologici: sărăcie, deficienţe ale puterii socio-politice, promovarea în mass media a violenţei faţă de femei, ideologia dominanţei şi agresivităţii masculine (5,6,7,8).

MATERIAL ŞI METODE

Studiul nostru a inclus un lot de 132 cazuri de VD, reprezentând 58% din cele 228 omucideri înregistrate în judeţul Neamţ, în perioada 1997-2008. Informaţiile au provenit din evidenţele Serviciului de Medicină Legală (SML) şi ale Institutului Naţional de Medicină Legală „Mina Minovici" (fig. 1). Prelucrarea şi interpretarea rezultatelor s-au realizat prin metode epidemiologice, statistico-matematice şi computerizate.

REZULTATE ŞI DISCUŢII

1. Analiza repartiţiei anuale a omuciderilor prin violenţă familială, în judeţul Neamţ, comparativ cu situaţia la nivel naţional. În anul 1998, în judeţul Neamţ, s-au înregistrat 0,69‰ omucideri, faţă de 2,56 la nivel naţional. Evaluarea pentru perioada 1998-2000 a evidenţiat o creştere semnificativă ($p < 0,05$) în anul 2000, a ratei omuciderilor prin VD, aceasta ajungând la 12,3‰ locuitori în judeţul Neamţ, comparative cu 4,5‰ pe întreaga ţară.

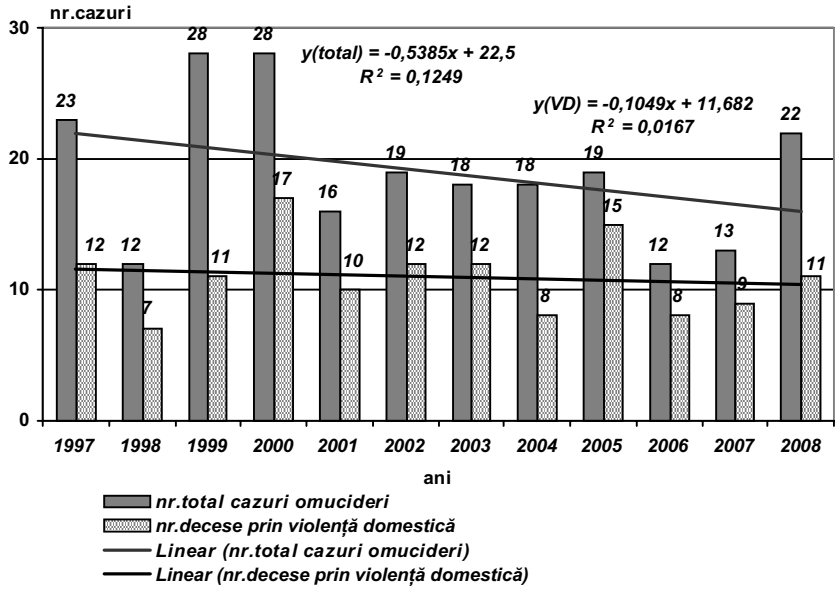


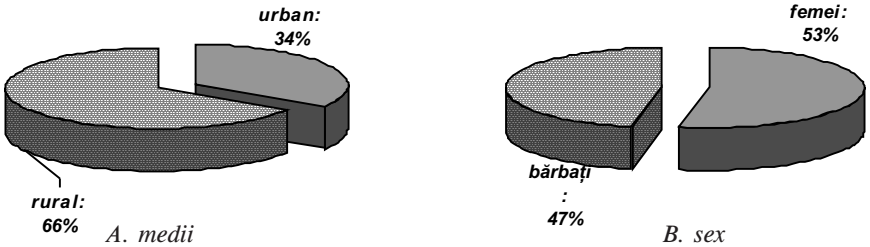
Fig. 1. Repartiţia anuală a deceselor produse prin violenţă familială, înregistrate la SML Neamţ, în perioada 1997-2008

Raportat la numărul total de omucideri, din judeţul Neamţ, în perioada luată în studiu, procentul victimelor a variat între 39% în anul 1999 şi 79% în 2005, cu o medie a intervalului de 58%.

2. Repartiţia pe medii de reşedinţă şi sex. Din totalul omuciderilor prin VD, 34% au provenit din urban şi 66% din rural, 53% femei şi 47% bărbaţi. Forţa fizică mai scăzută a femeilor, implicit, posibilităţilor lor mai reduse de apărare în faţa agresorului fac ca numărul deceselor femeilor victime ale partenerilor de viaţă să fie mai mare. Bărbaţii decedaţi ca urmare a agresiunilor domestice au fost victime atât ale partenerelor de viaţă (soţii, concubine), cât şi ale părinţilor, fiilor, fraţilor, altor rude (cumnaţi, veri) (8,9,10) (fig. 2).

3. Repartiţia pe grupuri de vârstă. Victimele cuprinse în lotul de studiu au avut vârsta între 3 luni şi 83 ani, cele mai multe, respectiv 72 (54%) aparţinând persoanelor active social, cu vârsta între 21 şi 60 ani ($p < 0,05$). Victimele din categoria copii şi tineri au totalizat 7%, iar vârstnicii din propriile familii au reprezentat 22% din totalul omuciderilor prin VD (fig. 3).

4. Distribuţia sezonieră. Numărul cel mai mare a cazurilor de omor urmare a VD s-a înregistrat în lunile ianuarie (15%) şi



A. medii *B. sex*

Fig. 2. Repartiţia pe medii (A.) şi sex (B.) a omuciderilor urmare a violenţei domestice, în judeţul Neamţ, în perioada 1997-2008

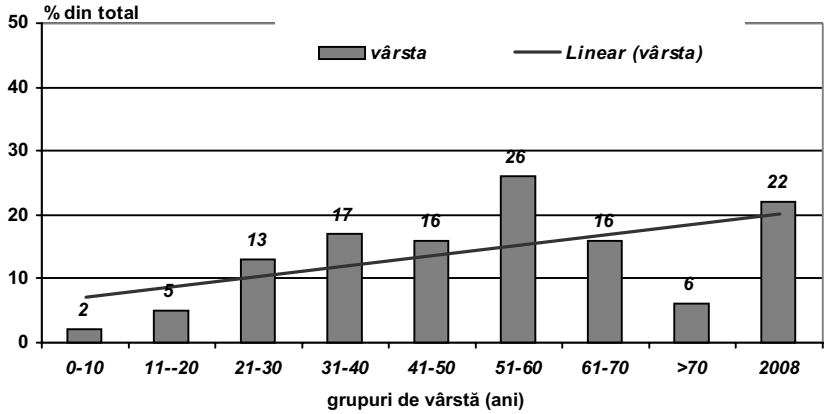


Fig. 3. Repartiţia pe grupuri de vârstă a 132 omucideri prin violenţă domestică, înregistrate în judeţul Neamţ, în perioada 1997-2008

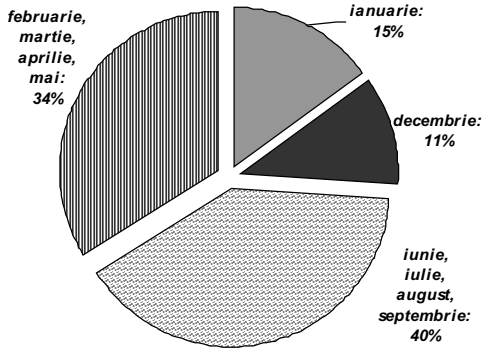


Fig. 4. Distribuţia sezonieră a omuciderilor prin violenţă domestică, înregistrate în judeţul Neamţ, în perioada 1997-2008

decembrie (11%), în principal, asociate consumului de alcool din perioada sărbătorilor de iarnă. În lunile mai, iunie, iulie şi august, au fost raportate 53 cazuri (40% din total) şi 34% în celelalte luni ale anului (fig. 4).

5. Alte caracteristici

5. 1. Examenele toxicologice efectuate pe probele de sânge recoltate de la cadavre au relevat faptul că în 90 de cazuri (68%), victimele consumaseră alcool, având valori ale alcoolemiei de 0,4-3,5%. La acestea, se mai adaugă cazurile la care consumul de alcool s-a stabilit prin prezenţa halenei alcoolice şi cele la care autopsia s-a practicat după 24 ore de la spitalizare.

5. 2. Relaţia victimă-agresor. Ancheta a evidenţiat că 63 (48%) din cazuri au fost victimele soţului sau concubinului, urmate de fiu-părinte (inclusiv ginere-noră) în 45 (34%), de fraţi (inclusiv frate adoptiv) – 16 (12%), alte rude – 8 (6%) şi 5 (3,8%) agresiunea a antrenat mai mulţi membri ai familiei (tab. I).

5. 3. Mijloacele de atac folosite de agresori au inclus: lovire cu pumnii, picioarele, capul, muşcare, comprimare cu propriul corp, strangulare în 34 (26%); lovire cu corpuri dure tăioase (cuţit, ciob de sticlă, topor, secure) – 40 (30%); lovire cu corpuri dure

TABELUL I

Natura relaţiei dintre victimă-agresor în omuciderile incluse în studiu, în judeţul Neamţ, în perioada 1997-2008

Tipul relaţiei	Nr. cazuri	%
soţ / concubin	63	48
părinte / fiu ; bunic / nepot	45	34
frate	16	12
altele	8	6
Total	**132**	**100**

TABELUL II

Mijloacele de atac folosite de agresorii victimelor mortale ale violenţelor domestice, în judeţul Neamţ, în perioada 1997-2008

Mijloace de atac	Nr. cazuri	%*
Lovire cu corpuri dure tăioase	40	30
Lovire cu corpuri dure netăioase	18	14
Substanţe caustice	3	2
Lichide / obiecte fierbinţi	4	3
Mijloace proprii	34	26
Împuşcare	3	2
Mijloace combinate	30	23
Total	**132**	**100**

* pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL III

Cauza medicală a decesului victimelor violenţei domestice, în lotul studiat, în judeţul Neamţ, în perioada 1997-2008

Cauza medicală a morţii	Nr. cazuri	%
Traumatismul cranio-cerebral sau vertebro-medular	33	25
Asfixia	13	10
Hemoragia	36	27
Şocul	23	17
Insuficienţa cardio-respiratorie acută	27	19
Total	**132**	**100**

netăioase – 18 (14%); arsuri cu obiecte sau împuşcarea – 3 (2%); substanţe caustice – 3 (2%); combinarea mai multor mijloace – 30 (23%) (tab. II).

5. 4. Tabloul lezional prezentat de victimele VD a fost deosebit de variat: în 15 (13%) leziunea a fost unică (plagă penetrantă urmată de o hemoragie internă sau externă). În celelalte cazuri, victimele au prezentat multiple leziuni situate pe toată suprafaţa corporală: echimoze, excoriaţii, plăgi, fracturi osoase, contuzii grave cu lezarea unor organe interne, arsuri cu lichide sau obiecte fierbinţi, substanţe chimice etc.; în 4 (3%) din cazuri, victimele au fost şi violate de către agresori.

În 33 (25%) din cazuri, decesul s-a produs urmare a unui traumatism cranio-cerebral sau vertebro-medular, cu leziuni grave ale creierului, măduvei spinării sau meningelor; în 13 (10%) a intervenit asfixia (prin comprimarea gâtului, toracelui, abdomenului sau prin aspirat sangvin în căile respiratorii); hemoragia internă sau externă a produs decesul a 36 (27%) dintre victime; şocul (traumatic, hemoragic, septic) în 25 (17%); insuficienţa cardio-respiratorie acută în 27 (19%) din total cazuri (tab. III).

CONCLUZII

Se poate aprecia că fenomenul VD este unul periculos şi greu de controlat şi, totodată, dificil de surprins în statisticile oficiale. Departe de a fi o particularitate a societăţii româneşti, VD este o problemă socială şi de sănătate populaţională, care cumulează efectele intervenţiei unor factori sociali şi psihologici. Prevalenţa actelor de VD a înregistrat o creştere alarmantă, în contextul unei societăţi generatoare de nemulţumiri şi frustrări.

Violenţa fizică şi consecinţa sa extremă, decesul victimei, şochează prin existenţa unui număr semnificativ de mare a situaţiilor în care un om poate fi ucis de un membru al familiei sale.

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NOUTĂŢI

EROZIUNILE DENTARE LA PACIENŢII CU REFLUX GASTRO-ESOFAGIAN CU SIMPTOME RESPIRATORII

Atât eroziunile dentare cât şi simptomele respiratorii de tipul tusei nocturne, astmului, laringitei, sunt strâns corelate cu refluxul gastro-esofagian. Tutuşi puţine studii au investigat corelaţia dintre cele două tipuri de patologii. Autorii au efectuat studiul pe trei loturi de pacienţi cu reflux gastro-esofagian, selectate în funcţie de frecvenţa simptomelor respiratorii. Eroziunile dentare au fost evaluate utilizând indicele modificat de uzură dentară (Smith şi Knight). Rezultatele studiului au demonstrat că pacienţii cu reflux gastro-esofagian prezintă eroziuni dentare variind de la gradul 1 la 4, frecvenţa acestor leziuni fiind semnificativ mai mare în cazul celor cu simptome respiratorii frecvente (64,52%) comparativ cu cei care prezintă ocazional sau de loc aceste simptome (44,4%, respectiv 36,67%). Gravitatea eroziunilor, evaluată prin valorile indicilor de uzură dentară, s-a corelat de asemenea pozitiv cu frecvenţa simptomatologiei respiratorii. La pacienţii cu simptome respiratorii, refluxul vaporilor şi gazului gastric contribuie alături de refluxul lichidelor şi solidelor, la instalarea patologiei dentare. Fenomenul de microaspirare este mai puternic iar vaporii acizi ajung la nivelul faringelui şi cavităţii orale, determinând efecte importante chiar şi în cazul unor cantităţi mici de acid. Medicamentele utilizate de aceşti pacienţi pot contribui într-o măsură mai mică la agravarea eroziunilor. Aceste rezultate atrag atenţia asupra riscului crescut de eroziune dentară la pacienţii cu reflux gastro-esofagian însoţit de simptome respiratorii şi asupra necesităţii de a introduce măsuri preventive adevate la această grupă de risc (Wang G-R, Zhang H, Wang Z-G, Jiang G-S, Guo C-H. Relationship between dental erosion and respiratory symptoms in patiens with gastro-oesophageal reflux disease. *J Dentist* 2010 ; 38 : 892-898).

Gianina Iovan

EVALUAREA FEEDBACK-ULUI POSTTERAPEUTIC ÎN REABILITAREA ORALĂ ÎN ACORD CU DIFERITELE METODE DE AMPRENTARE

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POSTTHERAPEUTIC FEED-BACK EVALUATION IN ORAL REHABILITATION (Abstract): The goal of this study was to assess the subjective post-therapeutic feedback for a group of patients in order to evaluate the possibility of including the psycho-behavioral parameters into the analysis structure of an expert system. **Material and method:** 135 patients diagnosed with class I and II Kennedy edentation, aged between 40 and 89 years old, where clinically examined. All patients filled in structured questionnaires focused on psycho-behavioral parameters. **Results:** Prosthetic treatment in oral rehabilitation improves subjective perception of facial aesthetic (66,86% total and moderate accord), psychological status (71.14% total and moderate accord) and of the social relations (77.47% total, moderate and low accord) but it is not subjective associated with the improving of general health (55.49% disagreement). **Conclusions:** We can establish statistical support correlation between psycho-behavioral parameters and therapeutic solutions applied in oral rehabilitation, correlation that can be later quantified and used in the development of an expert system. **Key words:** PSYCHO-BEHAVIORAL PARAMETERS, POSTTHERAPEUTIC FEEDBACK, EXPERT SYSTEMS

SYSTEMIC AND PERIODONTAL INFLAMMATORY BURDEN IN CHILDREN AND TEENAGERS WITH DIABETES, AND CLINICAL CORRELATIONS

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SYSTEMIC AND PERIODONTAL INFLAMMATORY BURDEN IN CHILDREN AND TEENAGERS WITH DIABETES, AND CLINICAL CORRELATIONS (Abstract**)** Type 1 diabetes (T1D) remains an eminent global burden, emerging from the impairment of healthy tissues due to an imbalance in the immune system. There is a bidirectional interrelationship between diabetes and periodontal disease (PD), managed through a number of pathways, involving mainly cytokine up- and down regulation. **Objective.** The present study was designed to investigate the possible role of serum and gingival fluid (GCF) levels of interleukin 2 (IL-2) and interferon gamma (IFN-γ) in young patients with systemic alteration in the context of T1D and periodontal injury, and determine its correlation with clinical parameters of the periodontal status. **Material and methods.** Serum and GCF levels of IFN-γ and IL-2 were investigated in 42 patients with T1D with or without periodontal alteration, and 24 systemically healthy controls. Periodontal parameters used in this study were clinical attachment level and papillary bleeding index. GCF was collected using paper strips, while flow cytometric assay was performed to determine the total amount of cytokines. **Results**. The current results revealed that median GCF and serum level of IFN-γ were significantly higher in patients than in systemically healthy controls ($p<0.001$), whereas regarding serum IL-2 levels no significant differences could be noticed ($p>0.05$). Correlation between local and serum cytokines and clinical periodontal parameters revealed significant positive correlation between GCF and serum IFN-γ levels. No association between serum IL-2 levels and clinical parameters of periodontitis was found. **Conclusion**: IFN-γ levels in serum and GCF display a good correlation with clinical parameters and degree of periodontal abuse in young T1D subjects. **Keywords**: TYPE ONE DIABETES, CHILDREN, PERIODONTAL DISEASE

The bidirectional interrelationships between periodontal disease (PD) and type one diabetes (T1D) provide an example of systemic disease predisposing to oral infec-

tion, with subsequent exacerbation of the systemic disease by the oral infection (1).

The inflammatory event that associates periodontal harm is initiated by the accumulation of bacterial plaque and starts in early childhood, reproducing the special significance of the bacterial impact on the host, in a systemic context. Very frequently during children age, the inflammation of the gum remains superficial – at the clinic stage of gingivitis, but there are some cases where the balance between the bacterial assault and the host response is disrupted, leading to destructive action which cause attachment loss, and even the lost of the dental unit.

From the complex interplay between pathogenic bacteria and the host's immune response, a more or less dramatic periodontal injury might result. Several inflammatory mediators, such as IL-2 and IFN-γ have been detected in gingival fluid (GCF) samples in patients with periodontitis, but the results are mostly contradictory due to the lack of homogeneity, collection of sites and protocols for evaluation and analysis.

IL-2 has key roles in significant functions of the immune system primarily via its direct effects on lymphocytes, promoting the differentiation of subsets of immature T cells into regulatory T cells involved in maintaining normal healthy cells in the body (2). Moreover, IFN-γ acts as a positive regulator by selectively prompting Th1 (T helper 1) subset cells differentiation in the coarse of generation of a primary CD4+ (Th1) response.

The present study intended to determine the possible role of Th1-type cells in the disease coarse of T1D children and teenagers with or without periodontal injury, through analysis of serum and GCF levels of IL-2 and IFN-γ, and determine its correlation with different clinical parameters of the periodontal status.

MATERIAL AND METHODS

Subject population and clinical monitoring. Forty-two subjects with diabetes, with (n-20, group 1) or without periodontal changes (n=22, group 2) were recruited from the Metabolic and Nutrition Diseases Department of the University Children Hospital "Sf. Maria" Iasi, and selected based on the following criteria: aged between 6 and 18 years old, diagnosed with T1D. The juvenile individuals with any inflammatory diseases, liver or renal impairment (selected through scan of the serum creatinine levels), periodontal treatment in the previous 6 months prior to the assessment, serious impairment of the dental units or under any medication that could influence the analytes (antibiotics, corticosteroids) were excluded from the study. Twenty-four clinically systemically healthy age matched control group (group 3) were selected among the non-diabetic children and teenagers that followed regular stomatologique cure in the dental unit of the Pediatric Dental Clinic. Informed consent was obtained in all cases, University of Medicine and Pharmacy (Iasi, Romania) Ethics Committee approving the study protocols including the periodontal examination and collection of GCF samples.

Papillary bleeding index (PBI) and clinical attachment loss (CAL) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, mesiolingual and lingual) for all teeth present, by a calibrated examiner.

Gingival crevicular fluid and serum sampling. After isolating the tooth with a cotton roll, supragingival plaque was removed skipping the marginal gingiva. The gingival fluid was collected on paper strips gently placed into the sulcus until mild

resistance was sensed and then left in place for 30 seconds. The volume of each sample was measured using a calibrated device and the reading values were converted to volume (ml) by reference to the standard curve. Following GCF collection, strips were placed in Eppendorf vials and kept at -70°C. Further, the gingival fluid was extracted in 100 ml elution PBS buffer (phosphate saline buffer) by centrifugation at 18.000 g for 5 minutes, at 4°C. From the GCF eluted in PBS, 50 µl specimens were prepared for flow cytometry - FCM analysis.

The degree of metabolic control was considered through glycosylated hemoglobin values (HbA1c) measurements, determined by high performance liquid chromatography; levels of HbA1c $\leq$ 7.5 % were considered for good metabolic control, while poor control was defined as HbA1c > 7.5%, normal values being attributed to HbA1c $\leq$ 6%.

Gingival fluid and serum IL-2 and IFN-γ levels were determined using a high-sensitivity human cytokine multiplex for flow cytometry. Before the assay, GCF samples were eluted using 50 µl of the provided assay buffer, by vortexing for 30 minutes and then centrifuging for 10 minutes at 8000 rpm. The concentrations of the targeted biomarkers in serum and GCF samples were estimated from the standard curve.

Data analysis and statistic evaluation. Periodontal parameters were evaluated from 6 sites per tooth in each subject. Significance of changes in clinical data over time, and serum and CGF cytokine level from the same individual were assessed using the Wilcoxon *t* tests, $p<0.05$ establishing the statistic significance. Interactions between variables were studied using Pearson's correlation, while Mann–Whitney *U* test was used to compare values between groups.

RESULTS

The demographic data including the age of the periodontally affected patients illustrate distributions of more or less severe periodontal impairment: mainly mild affection represented by gingivitis at ages significant lower than previously estimated in the literature (p=0.0048, 95% confidence interval - CI), while form of superficial chronic marginal periodontitis were noticed predominantly in young diabetes subjects close to puberal age. It is to be considered that, very probably, the periodontal injuries recorded around this age interval are modulated by the interdependent metabolic control of the DM disease and hormonal changes registered prior or during puberty (fig. 1).

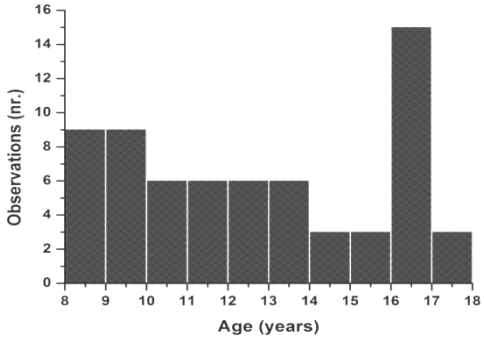

Fig. 1 The histogram of age values in the studied groups

Evaluation of parameters related to distribution, periodontal diagnosis and age that followed the setup of the studied groups, pointed out the highest prevalence of gingivitis (35%), and followed by 20% with chronic superficial marginal periodontitis in the non-diabetes group. Referring to the T1D group, different incidences of periodontal impairment have been recorded in the two subgroups divided by HbA1c levels into: children and adolescents with

good metabolic control (n=10) were diagnosed with bacterial gingivitis in a proportion of 80% (n=8) and 20% aggressive periodontitis (n=2), the poorly controlled T1D, displaying only 60% gingivitis (n=6), chronic superficial marginal periodontitis in 30% (n=3), with 10% aggressive periodontitis (n=1). Therefore, our data support the concept of higher incidence and severity of periodontal breakdown in juvenile individuals with less controlled diabetes at young ages, although there are data that claim no significant correlation between levels of sugar metabolic control and periodontal alter (3).

Correlated to age, systemic disease (T1D) time coarse and metabolic control (reflected by HbA1c levels), analysis of the clinical index that reflect the degree of inflammation on the periodontal area, such as PBI or CAL highlight a significant higher level of the PBI in T1D young subjects (p=0.00, 90%CI) – fig. 2, with significant differences between the mean values of the diabetes duration related to bleeding index, (p=0.0008). PBI correlated well with duration of T1D (r=0.64) but not with the age of the young patients (r=-0.21, p=0.79) in the studied groups.

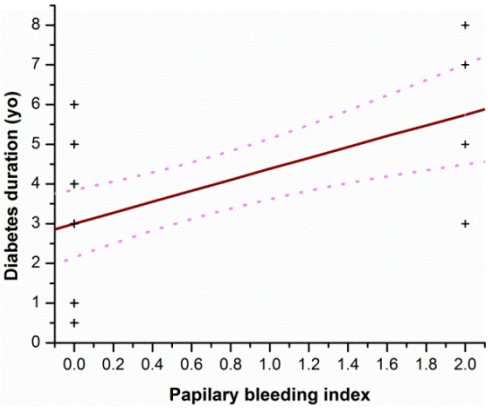

Fig. 2 Regression curve correlating T1D duration *vs.* PBI

The average levels of glycated hemoglobin for our subjects are presented in fig. 3.

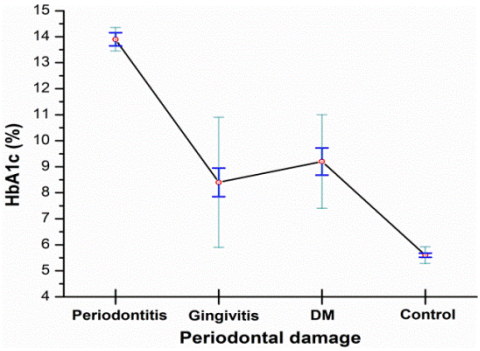

Fig. 3 Mean values of HbA1c *vs.* studied groups

These values have been correlated to clinical index of periodontal modification and revealed that moderate or poor controlled T1D associates higher levels of the bleeding index, the levels of glycated hemoglobin registering significant upper values in groups of diabetic patients attended by periodontal injury, compared to control group (fig. 4). Positive relationships were found between HbA1c and clinical parameters such as attachment loss, correlation coefficient being rather high (r=0,76), with a p value close to null and 95% confidence interval (fig. 5).

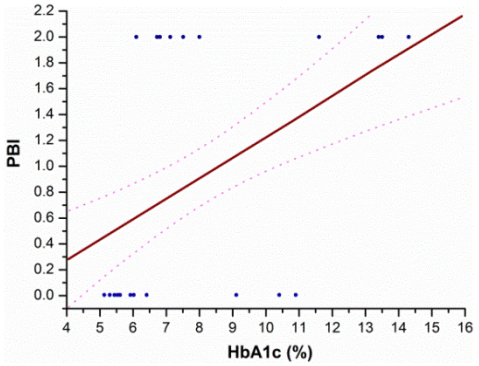

Fig. 4. Regression curve correlating PBI to HbA1c

When the level of periodontal impairment (reflected in clinical index such as PBI and CAL) was correlated with the onset age of the systemic alteration (T1D), a significant positive correlation was seen among all the groups (p=0.03684, 95%CI).

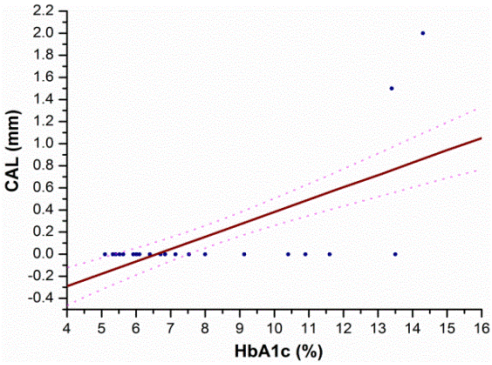

Fig. 5. Regression curve in correlation CAL vs. HbA1c

Slight and moderate elevated levels of serum IFN-γ in T1D subjects compared to control, whereas significant higher GCF amounts within group one were noticed. Both serum and gingival fluid IL-2 levels are very small in patients in group 1, suggesting the existence of a local factor that blocks secretion especially in diabetic patients with periodontal alteration (fig. 6,7).

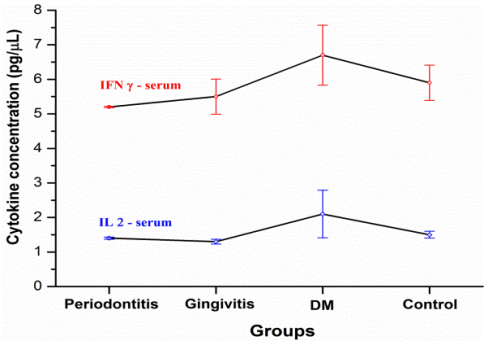

Fig. 6. Mean values of serum IL-2 and IFN-γ in the studied groups

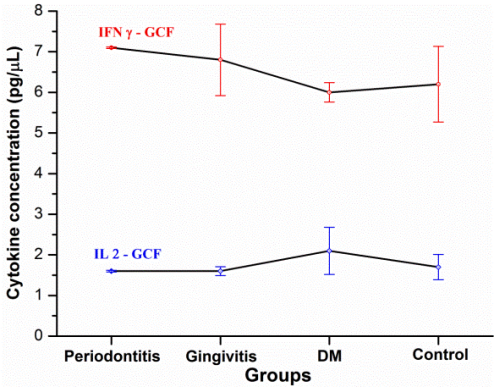

Fig. 7. Mean values of gingival fluid IL-2 and IFN-γ in the studied groups

DISCUSSION

The pro-inflammatory cytokines such as interferon gamma, Il-1 beta, IL-2 as well as eicosanoids like prostaglandin E2, reach elevated tissue levels during PD, the periodontium serving therefore as a pool for spillover of these mediators that can determine extension of systemic outcomes (4,5). More recently, some clinical and laboratory studies have provided irrefutable evidence that periodontal breakdown may affect the host's susceptibility to systemic disease (6).

IFN-γ and IL-2 are cytokines released by the Th1 subset of CD4+ T cells. Moreover, due to the consistent pro-inflammatory events mediated through IFN-γ, there is a significant activity attributed to Th1-type cells for the elicited periodontal injury (7). To date, the literature concerning the behavior of these two mediators is conflicting; therefore, despite that most of the researchers claim the association of elevated local IL-2 and IFN-γ levels with progressing periodontal injury during chronic periodontal harm (7), with recent investigations reporting statistically important decreases in gingival fluid IFN-γ and IL-2 values consequently mechanical periodontal treatment (8), a tendency in augmenting local IFN-γ

levels after periodontal cure have been also reported (9). There is mount literature data that claim cytokines as key factors mediating inflammatory events (10, 11).

Our results recorded significant correlations of the clinical periodontal status, both with the biological parameters of the diabetic disease and also with the host immune-inflammatory reaction. Previous studies revealed that serum IL-2 levels were diminished in patients with chronic periodontal injury as compared to controls (12), while other study pointed out that serum IL-2 recorded higher values in adult individuals with chronic periodontitis compared to control group, although no statistically significance could be noticed (13).

T1D subjects presented significant PBI and CAL levels when compared to control group, in agreement with a number of studies (14, 15), the significant positive correlation registered between the onset of the diabetic disorder and clinical periodontal index pointing out that biochemical changes occurring at systemic level during the diabetic disease occur simultaneously at the local level. Hence, our data sustain the concept that the duration of T1D disease and metabolic control of the systemic impairment could act as contributing elements in convicting DM as risk factor within PD. Moreover, as a future perspective, GCF can be valued as a non-invasive diagnostic fluid over blood analysis in malady diagnosis.

Only IFN-γ registered moderate augmented serum levels within T1D children and adolescents compared to control, whereas significant higher amounts of GCF interferon gamma have been recorded in group one. Measurements of IFN-γ in GCF may be regarded as a valuable noninvasive and rapid approach for monitoring periodontal disease status.

CONCLUSIONS

In our study, serum and gingival fluid IFN-γ correlated well with clinical parameters and degree of periodontal abuse. Our data support the idea that gingival fluid IFN-γ levels could be a suggestive parameter to distinguish the patients with risk of diabetic disease expansion toward periodontal inflammatory or destructive episodes.

AKNOWLEDGMENTS

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EFFECTS OF *ZINGIBER OFFICINALE* ROSCOE FRESH EXTRACT ON AMELANOTIC MELANOMA AND NORMAL SKIN FIBROBLASTS

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EFFECTS OF *ZINGIBER OFFICINALE* ROSCOE FRESH EXTRACT ON AMELANOTIC MELANOMA AND NORMAL SKIN FIBROBLASTS (Abstract): *Zingiber officinale* Roscoe is a very important medicinal plant, with a long history of therapeutic uses, especially in oriental traditional medicine. **Aim**: To investigate the cytotoxicity of a fresh ginger extract on some skin tumor cells compared to normal cells. **Material and methods**: C32 amelanotic melanoma cell line and CCD human skin fibroblasts were used. The fresh extract obtained by crushing ginger rhizome was examined for phenolic content. The *in vitro* cytotoxicity was examined using phase contrast microscopy and MTT assay for the concentrations of 2 and 4 mg% total phenols. **Results**: Both concentrations used for treatment induced no changes in normal the morphology and viability of fibroblasts compared to control cells. Amelanotic melanoma cells displayed profound changes in cell morphology such as cell shrinkage, rounding up and membrane blebbing and a decrease in cell viability in a dose-dependent manner. **Conclusions**: Fresh ginger extract induced no changes in normal skin fibroblast viability, but caused profound cytotoxic effects on amelanotic melanoma. These results could encourage further studies regarding the intimate mechanisms of the antitumor action displayed by the fresh ginger extract. **Keywords**: *ZINGIBER OFFICINALE* ROSCOE, NORMAL SKIN FIBROBLASTS, AMELANOTIC MELANOMA, CELL VIABILITY

Numerous scientific studies linked the biologically active compounds extracted from plants with the antitumor activities against different types of cancer (1).

Ginger (*Zingiber officinale* Roscoe) is a perennial plant used since ancient times for spicing food and beverages, but also in treating different types of ailments, especially in oriental traditional medicine. Digestive disorders, inflammation, cold and hypertension are among the most known diseases against which ginger extracts can be used. The phenolic compounds present in it (especially gingerols and shogaols) have been proposed as important antioxidants with notable anticancer and antimutagenic effects (2). Promising results were obtained after *in vitro* and *in vivo* studies on lymphoma, ovary, gastrointestinal, skin, breast and lung cancers, promyelocytic leukemia, oral squamous carcinoma and others. In these cases, the anticancer

action consists in the inhibition of angiogenesis and apoptosis triggering through caspase-3-dependent mechanisms, down-regulation of anti-apoptotic Bcl-2 protein, up-regulation of pro-apoptotic Bax protein and inhibition of DNA synthesis (3).

Amelanotic melanoma is a type of skin melanoma generally characterized by lack of pigmentation and is associated with very poor prognosis when diagnosed in late stages (4). Promising effects were obtained after exposure to plant essential oils (5) and extracts of *Curcuma longa*, *Bouea burmanica* and *Iris pseudopumila* (6, 7).

The aim of this study was to investigate the cytotoxicity of a fresh ginger extract used in two different total phenols concentrations on some amelanotic melanoma cells in comparison with normal skin fibroblasts.

MATERIAL AND METHODS

Biological material and reagents. The human amelanotic melanoma cells (C32 cell line) and normal human fibroblasts (CCD cell line) were purchased from American Type Culture Collection (ATCC®CRL-1585™ and ATCC®CRL-1459™).

Minimum essential medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic mix and phosphate buffer saline (PBS) were purchased from Gibco-Invitrogen (Grand Island, NY, USA). Trypsin, ethylenediaminetetraacetic acid (EDTA), 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and absolute isopropyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade.

Plant material. *Zingiber officinale* Roscoe rhizome was purchased from the local market and the species was taxonomically identified by specialists from "Alexandru Ioan Cuza" University of Iasi, Romania, Faculty of Biology. A voucher herbarium specimen is deposited in the Laboratory of Biochemistry, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Romania.

Preparation of Zingiber officinale* Roscoe *fresh extract. Ginger rhizome was properly washed with distilled water and the external layer was detached. The material was then crushed using a glass laboratory blender and centrifuged for 30 minutes at 10 000 x g and 4°C. The supernatant was filtered twice using 0.22 □m sterile filters and used for polyphenol content determination and cell viability tests.

Cell culture. The C32 cells were first seeded in culture flasks at a density of 6 x 10^4 cells/mL and grown in *Minimum Essential Medium* (MEM) supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL).

The CCD cells, seeded at a density of 6 x 10^4 cells/mL were grown in DMEM supplemented with 10% fetal bovine serum and an antibiotic mix (100 U/mL penicillin, 10 µg/mL streptomycin and 25 µg/mL amphotericin).

The cell lines were maintained in a humidified atmosphere of 5% CO_2 at 37°C. The culture medium was changed every 2 days until 80% confluence.

Total phenolic content. The total phenolic content was determined by Folin-Ciocâlteu method, modified by Singleton *et al*., 1999 (8). The method consisted in the reduction of Folin-Ciocalteu reagent in alkaline medium. The intensity of the blue compound obtained was proportional with the phenolic content and was determined using a Perkin Elmer Lambda 25 spectrophotometer. The concentration was expressed as mg of gallic acid equivalents (GAE) per 100 ml extract (9, 10).

Cell viability assay. Cell viability was

measured by the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay (11). The C32 and CCD cells were seeded in 12 well plates and cultivated until they reached 80% confluence. Then, they were synchronized in the same phase of cell cycle for 18 hours. A treatment with fresh ginger extract containing 2 and 4 mg% GAE was administered. After 24 hours of exposure, the cells were observed by phase contrast microscopy on an inverted Olympus IX71 microscope in order to establish the morphological changes. The cells were subsequently incubated with 1 mg/mL MTT for 3 h at 37°C. The medium was then removed and 100 µL of absolute isopropyl alcohol were added to each well for the solubilization of formazan crystals. The optical density was read at 595 nm using a TECAN GENios plate reader and was directly correlated with the number of viable cells.

Statistics. All determinations were carried out in triplicate. The results were analyzed using Student t test and Microsoft Excel software. The values were expressed as means ± standard deviation and represented graphically as percentage of control. $p<0.05$ values were considered significant (*), $p<0.01$ distinctly significant (**) and $p<0.001$ very significant (***).

RESULTS AND DISCUSSION

Total phenolic content. The total phenolic content in ginger fresh extract was 203.55 ± 6.27 mg GAE/100 ml. The extract was then diluted in cell culture medium in order to obtain a concentration of 2 and 4 mg% GAE for the treatment of CCD and C32 cells.

Cell morphology. After the 24 hour treatment with 2 and 4 mg% GAE, the treated normal human skin fibroblasts, amelanotic melanoma cells and control cells were observed by phase contrast microscopy. Untreated CCD cells are presented in figure 1a. Figures 1b and 1c show the human fibroblasts after exposure to fresh ginger extract. C32 cells (control and treated ones) can be seen in figure 2.

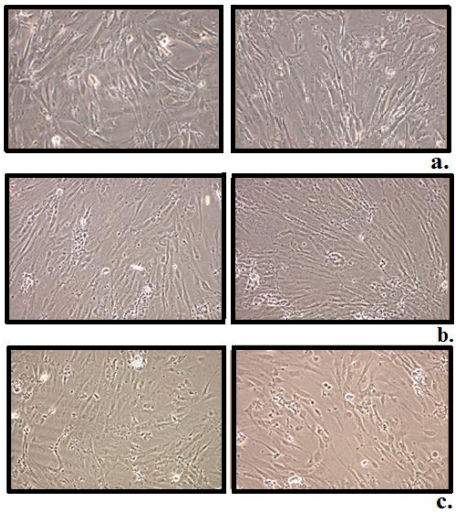


Fig. 1. Fields of control CCD cells (a) and CCD cells treated with 2 mg% GAE (b) and 4 mg% GAE (c)

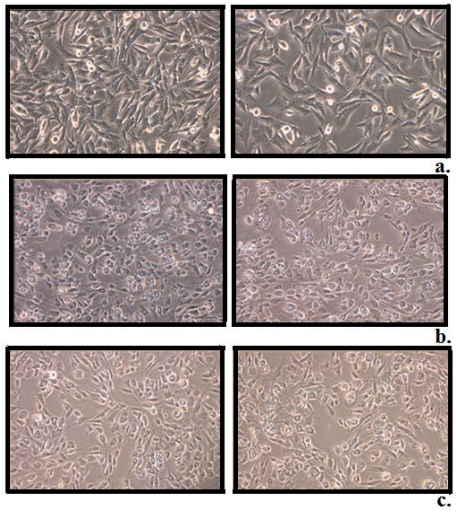
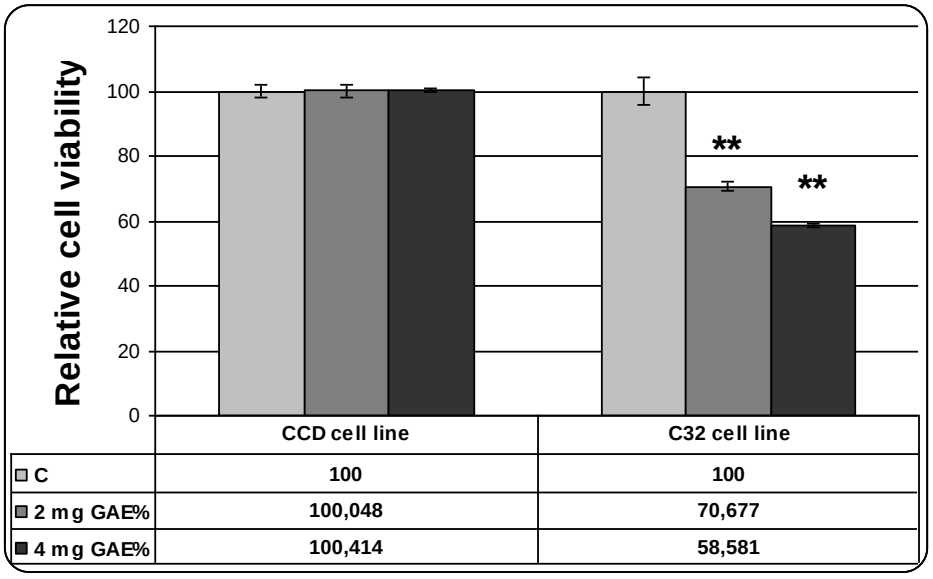


Fig. 2. Fields of untreated C32 cells (a) and C32 cells treated with 2 mg% GAE (b) and 4 mg% GAE (c)

Cell viability. Figure 3 shows the results obtained after MTT cytotoxicity test on normal and tumor cells. Both phenolic concentrations used for treatment induced no changes in the viability of normal fibroblasts. In the case of C32 cell line, with the first concentration (2 mg% GAE), cell viability decreased by almost 30% compared to control cells. The concentration of 4 mg% GAE induced a higher decrease in cells viability, by about 41% compared to controls.



	CCD cell line	C32 cell line
C	100	100
2 mg GAE%	100,048	70,677
4 mg GAE%	100,414	58,581

Fig. 3. Relative CCD and C32 cell viability after 24 hours of treatment

Previous studies showed an important inhibitory activity induced by some ginger varieties, also revealed by MTT assay on some human breast cancer cell lines (MCF-7 and MDA-MB-231) (12). The 6-shogaol, one of the main biologically active compounds of ginger, was reported as a significant dose-dependent inhibitor of HeLa cells proliferation (13).

In order to establish the efficacy of fresh ginger extract in the induction of cell death, we first observed the amelanotic melanoma cells by phase contrast microscopy. The effect was compared to the one obtained in normal skin fibroblasts.

Treated CCD cells shown in figure 1 revealed no significant changes in cell morphology. The cells remained oblong and no detachment from the growing surface was observed. The higher level of viable normal cells was confirmed by MTT test.

On the other hand, fresh ginger extract was able to induce a decrease in vitality of C32 cells after 24 hours of incubation (fig. 2). The melanoma cells treated with fresh extract containing 2 and 4 mg% phenolic compounds displayed evident morphological changes typical for cells undergoing cell death, such as: cell shrinkage, rounding up and membrane blebbing. Furthermore, the results obtained by MTT assay (fig. 3) revealed a dose-dependent decrease in cell viability after exposure to fresh plant extract. It is possible that at polyphenols con-

centrations higher than those used by us tumor cell viability to decrease even more.

CONCLUSIONS

Our comparative analysis on the effects induced by a fresh ginger extract on C32 amelanotic melanoma cell line and CCD normal human fibroblasts revealed a down regulation of tumor cell viability in a dose-dependent manner and no change in normal cell viability at the same treatment concentrations. Further studies are needed in order to understand the intimate mechanisms of inhibition of melanoma development due to the fact that the extract used by us seems to be effective in this type of cancer.

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ANALYSE OF SOCKET-PROSTHESIS-BLUNT COMPLEX FOR LOWER LIMB AMPUTEE USING OBJECTIVE MEASURE OF PATIENT'S GAIT CYCLE

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ANALYSE OF SOCKET-PROSTHESIS-BLUNT COMPLEX FOR LOWER LIMB AMPUTEE USING OBJECTIVE MEASURE OF PATIENT'S GAIT CYCLE (Abstract): The prosthetic application is a highly complex process. Modeling and simulation of biomechanics processes in orthopedics is a certainly field of interest in current medical research .Optimization of socket in order to improve the quality of patient's life is a major objective in prosthetic rehabilitation. A variety of numerical methods for prosthetic application have been developed and studied. **Material and methods**: An objective method is proposed to evaluate the performance of a prosthetic patient according to surface pressure map over the residual limb.The friction coefficient due to various liners used in transtibial and transfemoral prosthesis is taken into account also. **Results**: Creation of a bio-based modeling and mathematical simulation allows the design, construction and optimization of contact between the prosthesis cup and lack of functionality of the patient amputated considering the data collected and processed in real time and non-invasively. The von Mises stress distribution in muscle flap tissue at the bone ends shows a larger region subjected to elevated von Mises stresses in the muscle tissue underlying longer truncated bones. **Conclusions:** Finite element method was used to conduct a stress analysis and show the force distribution along the device. The results contribute to a better understanding the design of an optimized prosthesis that increase the patient's performance along with a god choice of liner, made by an appropriate material that fit better to a particular blunt. The study of prosthetic application is an exciting and important topic in research and will profit considerably from theoretical input. Interpret these results to be a permanent collaboration between math's and medical orthopedics. **Keywords:** PROSTHETIC, SOCKET, BLUNT, SOLID WORKS, NUMERICAL MODELING.

Lower limbs prosthetic have many particular features that are taken into account in design and adjustment of prosthesis to make less discomfort as possible for patient. There are many papers related usage of finite-element method applied to transfemoral amputation but there are relatively less papers related to trans-tibial amputation.

The liner used as interface between skin and socket alleviates the satisfaction and patient's comfort improving considerably the quality of life. The material of liner and consequently the coefficient of friction

between skin and liner has a major influence in performance of prosthetic amputee. The dynamic level of pressure between stump and prosthetic socket has a very high influence on patient's comfort (1). Two new materials (Dermo and the Seal-In X5 liner) are tested in (1). The points of measurement for interface pressure were chosen according to information from literature were the most probable location of the pressure peaks: anterior, posterior, medial and lateral regions. The patient' satisfaction was measured qualitatively using a questioner (Prosthetic Evaluation Questionnaire) and the conclusion was that Dermo liner provide more comfort that the other liner, Sean in (1).

An investigation related to interface stress between stump and trans-tibial prosthetic socket using 3D nonlinear finite element based on geometry of limb was done in (2). Automated surface-to-surface contact available in ABAQUS was used to model boundaries conditions (friction/slip)(2). The effect of load during gait cycle internal tissue of residual limb was studied in (3,4). The authors proved that the internal stress in are dependent on walking terrain, the more uneven or difficult is the terrain (e.g. stairs), the higher are the peaks of internal mechanical stress in the muscle flaps (4). Turning plays an important role in all the daily activities of patients with amputation of lower limb. In (5), compensatory strategy mechanisms developed by transtibial amputee were studied in comparison with non-amputee. The authors studied the evolution of main forces involved in turning: radial ground reaction and anterior–posterior forces (5). Modelling by finite element method (FEM) is often used in analysis of prosthetics for lower limb. FEM is used in (6) in donning procedure using patient-specific geometry given by CT and laser scan data. In distal-end socket type, the friction role is analyzed in (7).

Most of the studies in prosthetic application use the elastic, homogenous and isotropic models for bones soft tissues, flap muscles, fat, liner, and socket. Based on theory of large elastic deformation (8), other approaches more realistic studies that tackle with hyperelastic tissues are published by different authors (6,7,9). The models are more realistic ones, and in condition that constants for most common materials used in prosthetic models are available in publication we will use in this paper the hyperelastic Mooney-Rivlin model (7).

MATERIALS AND METHODS

We propose to use a fused adapted gait deviation index (faGDI) as objective measure of performance for lower limb amputee for pairs liner-socket materials.Gait deviation index (GDI) is proposed to be used as index of gait pathology for non-amputee persons in very recent papers (10,11). About our best knowledge, only one paper that tackle with lower limb amputee using GDI is published until now.There are few proposals for gait deviation index: Gillette Gait Index (GGI), Gillette Functional Assessment Questionnaire Walking Scale (GFAQW), and topographic classifications (diagnosis of Cerebral Palsy -CP). GDI include a balanced analysis of univariate parameters, SVD (Singular Value Decompositions) and singular values for each parameter are identified (10).The moments and joint power in different location are correlates with the body mass of subject.

Instead to use the GDI for pathology identification, we propose to use infor-

mation given by pressure sensors in order to evaluate the faGDI as objective measure for quality of prosthesis. The moment $M_{sensorx}$ is used for rotation (turning gait).

$$G_f = \begin{bmatrix} F_{sensor1}, F_{sensor2}, F_{sensor3}, \\ F_{sensor4}, F_{sensor5}, F_{sensor6} \\ M_{sensorx} \end{bmatrix}$$

Fig. 1. Six pressure sensors mounted on stump

Data were collected from two amputee (fig. 1) with set of 32 repetition of cycle (8 cycles for normal walking, 8 cycles for up the stairs, 8 cycles for down the stairs and 8 cycles normal walking with two rotation on each cycle. The vectors collected from each subject were concatenated in a faGDI matrix:

$$G_f = \begin{bmatrix} \begin{pmatrix} g_{1`}^1 \\ g_2^1 \\ \vdots \\ g_{32}^1 \end{pmatrix} \begin{pmatrix} g_{1`}^2 \\ g_2^2 \\ \vdots \\ g_{32}^2 \end{pmatrix} \cdots \begin{pmatrix} g_{1`}^m \\ g_2^m \\ \vdots \\ g_{32}^m \end{pmatrix} \end{bmatrix}$$

Practically, faGDI is a scaled measure of distance between a patient' gait ant the other from the same group.

RESULTS AND DISCUSSION

In the first stage we considered the materials homogenous. The material properties are taken from literature (13) and are summarized in tab. 1. The materials used in the analysis, with the characteristic parameters, are listed in a library of materials, from where are accessed.

TABLE I

Material Properties for homogeneous FE model

	Young's Modulus	**Poison's ratio**
Bone	15 GPa	0.3
Soft tissue	200 KPa	0.45
Socket	15 GPa	0.3
Liner	0.38 MPa	0.49

All soft tissues were assumed to be hyperelastic, homogeneous, and isotropic, and were modeled using the Generalized Mooney-Rivlin Solid strain energy function (7):

$$E = C_{10}(I_1 - 3) + C_{11}(I_1 - 3)(I_2 - 3) + D_1(J - 1)^2$$

The constants which depend on material are taken from literature (7). It is clear that on wearing trans-femoral prosthesis higher strain occurs at the brim of socket. Equivalent Von-Mises stresses acting on different parts of above knee prosthesis is as shown in fig. 2-4. The von Mises stress distribution in muscle flap tissue at the bone ends shows a larger region subjected to elevated von Mises stresses in the muscle tissue underlying longer truncated bones

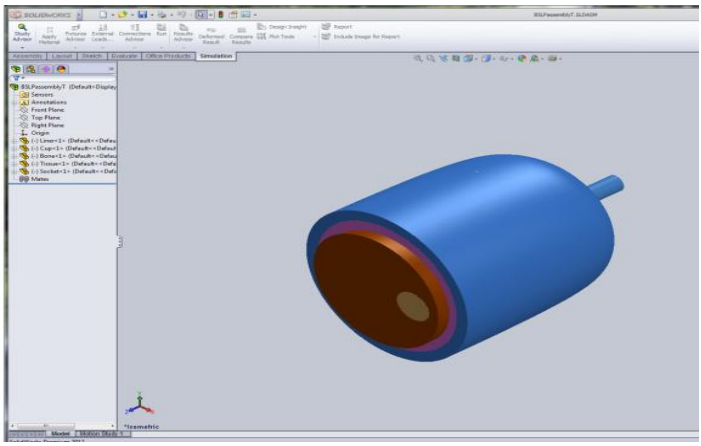

Fig. 2. SolidWorks approximation for assembly femur-stump-liner-socket.

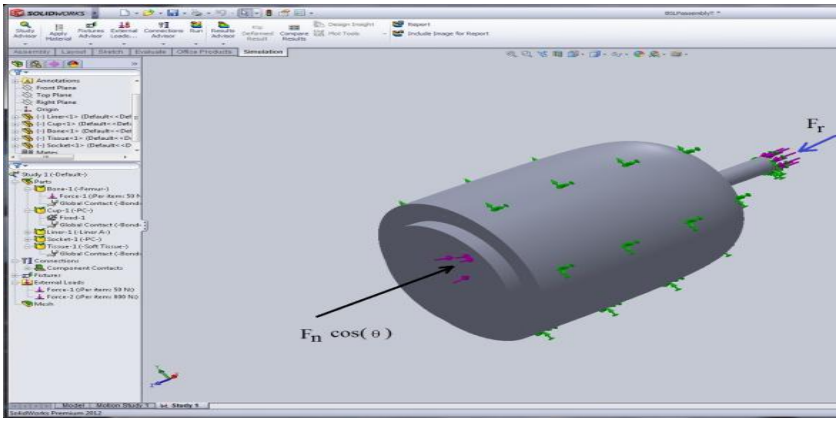

Fig. 3. Force applied to assembly femur-stump-liner-socket during gait cycle

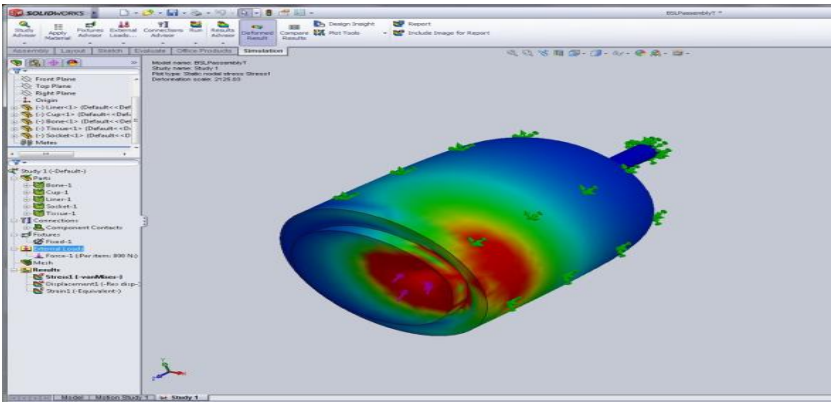

Fig. 4. Von Misses force in internal tissues for assembly femur-stump-liner-socket during gait cycle. The von Mises strain wasfound maximum at the bottom line of the socket

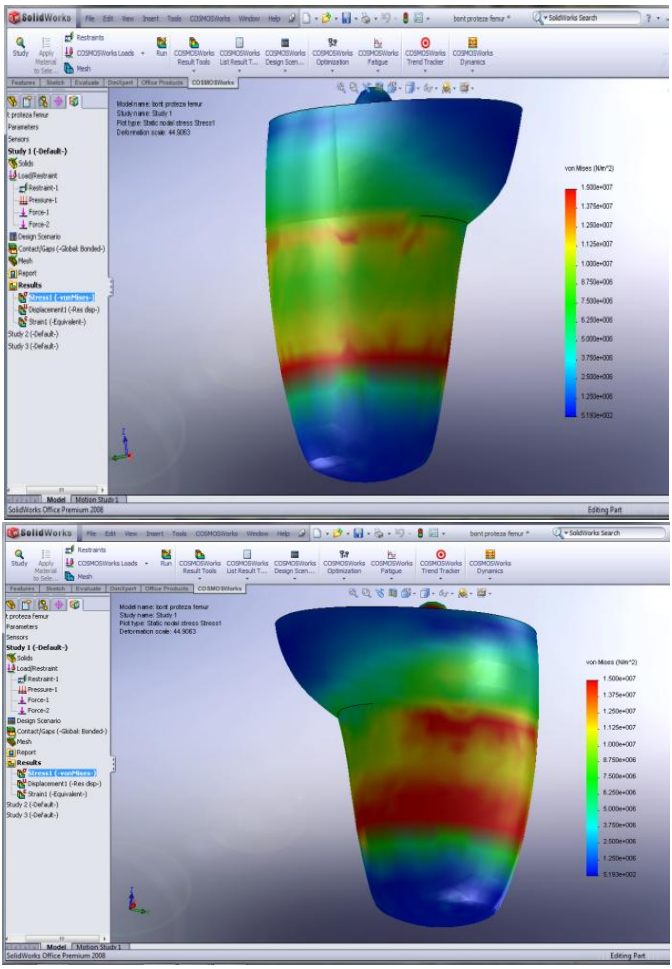

Fig.5. Von- Mises result, at the level of internal side of the thigh and Von- Mises result at the level of external side of the thigh.

CONCLUSIONS

The use of Solid Works in this article illustrates the important role of informatics in research in biomechanical modeling .An objective method is proposed to evaluate the performance of a prosthetic patient according to surface pressure map over the residual limb is proposed in this paper. Two liners are investigated during simulations using FEM. The distal end boundary conditions are marked by using large deformations permitted by SolidWorks Premium 2012 and kinetic records. The experimental results showed an average of faGDI ≈ 121,47 for Dermo liner and faGDI ≈ 105,62 for Sean-in. Based on this results, we can conclude that the combination polyurethane socket-Dermo liner is more performant that polyurethane socket- Sean-in liner. The design solution obtained from the results can be used as a reference to choose material for fabrication of socket in developing countries like Romania, depending on the cost and availability.

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EFFECTS OF TWO SEROTONINERGIC AGENTS ON THE BEHAVIORAL MANIFESTATIONS IN RATS

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EFFECTS OF TWO SEROTONINERGIC AGENTS ON THE BEHAVIORAL MANIFESTATIONS IN RATS (Abstract) **Aim:** To investigate the effects of two serotonin receptor antagonists on spontaneous behavior in rats. **Material and method:** The experiment was carried out on white male Wistar rats (150-200g) divided into 3 groups of 6 animals each, treated intraperitoneally with the same volume of solution as follows: Group I (Control): saline solution 0.1ml/10g weight; Group II **(**SB-269970**):** SB-269970 1mg/kbw; Group III (NAN-190): NAN-190 1mg/kbw. The effects of serotonin receptor antagonists on the spontaneous psychomotor skills of rats were tested in Actimeter LE-8811 (PanLab). The data were presented as mean +/- standard deviation and significance was tested by SPSS Statistics for Windows version 17.0 and ANOVA method. The experimental protocol was implemented according to the guidelines for handling and use of experimental animals of the Research Ethics Committee of the Iasi "Grigore T. Popa" University and ethical standards of the European Community. **Results:** The 5HT1 serotonin receptor antagonist NAN-190 determined a statistically significant reduction ($p<0.01$) in both horizontal and vertical movements as compared with the control group, whereas the 5HT7 serotonin receptor antagonist SB269970 had no influence on rat behavioral manifestations. **Conclusions:** In our experimental conditions 1 mg/kbw NAN-190 decreased the total escape attempts, corresponding to a significant diminution of exploratory and self-maintenance spontaneous behavior in this experimental animal model. These manifestations may be correlated with the anxiolytic effect of 5HT1 serotonin receptor antagonist NAN-190 in rats. The administration of 5HT7 serotonin receptor antagonist SB-269970 did not alter the spontaneous activity in this behavioral experimental model in rats. **Keywords:** NAN-190, SB-269970, BEHAVIOR, RATS.

The monoamine neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) has been implicated in different types of behavior, such as emotional, motor, cognitive processes. It is also implicated in the mediation of autonomic neural processes, neuroendocrine functions, circadian rhythm, and feeding behavior (1). Serotonin has important physiological effects being interconnected with many mediators that participate in the hypothalamic control of pituitary secretion, predominantly in the modulation

of adrenocorticotropin, growth hormone, and prolactin secretion (1, 2). The main source of 5-HT release in the brain is represented by the neurons of raphe nuclei (1).

Various disturbances in central tryptaminergic pathways are suggested to be linked with the manifestation of the most important behavioral disorders. This fact may explain the effectiveness of serotonin receptor antagonists in different behavioral diseases (3).

Serotonin has an intense influence on mood. High levels of serotonin lead to a positive disposition and coping more effectively with daily stressful situations. On the other hand low serotonin levels are responsible for the development of different degrees of depression (4).

5-HT has an inhibitory effect on loco motor activity, as a result of a complex action mediated through modulation of the serotoninergic system, but this activity is interconnected with both dopaminergic and glutamatergic pathways (5).

The perturbation of serotoninergic system has been shown to influence a variety of behavioral disorders, including obsessive compulsive disorder, panic disorder, body dysmorphic disorder, phobias, posttraumatic stress disorder, anorexia, bulimia, attention-deficit hyperactivity disorder, and insomnia, and thus a first therapeutic possibility of defense consists of drugs that influence serotonin levels in the brain (3, 6).

Literature data show that active substances affecting serotonergic neurons and their receptors are used to treat diseases such as depression, anxiety disorders, and schizophrenia (2, 4).

NAN-190 is a (1-(2-methoxyphenyl)-4-[4-(2-phtalimido)butyl]piperazine) derivative, with a selective antagonistic activity on $5HT_{1A}$ serotonin receptor subtype. It has been demonstrated that this pharmacologically active substance also has an adrenergic stimulation activity and a presynaptic $5HT_{1A}$ serotonin receptor agonistic effect (7, 8, 9).

(R)-3-[2-[2-(4-Methylpiperidin-1-yl) ethyl] pyrrolidine-1-sulfonyl]phenol hydrochloride compound, SB269970 is a 5HT7 serotonin receptor antagonist and also an alpha 2 adrenergic receptor blocker (10, 11, 12).

This research is part of a large study aimed to investigate the effects of various serotoninergic and dopaminergic agents on spontaneous motor activity, behavior, and cognitive processes in laboratory animals (13, 14).

MATERIAL AND METHODS

Male white Wistar rats (150–200g) were used in the study. The animals were housed in plastic cages for 10 days prior to initiation of experiments under standard conditions of room temperature (23 ± 1°C) and 12:12 h light/dark cycle (light on at 07:00).

Water was available *ad libitum* and animals were maintained at 85% of their free feeding weight by controlling the amount of their single daily meal 45 minutes after the experimental session. Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

The animals were divided into 3 groups of 6 rats each, and treated intraperitoneally with the same volume of solution as follows: Group I (Control) saline solution 0.1ml/10g weight; Group II (NAN-190) NAN-190 1mg/kbw; Group III (SB-269970) SB-269970 1mg/kbw.

Actimeter test was performed to determine the effect of serotonin receptor antagonists on spontaneous loco motor activity and exploration in rats. This apparatus consists of three transparent cages each with eight infrared lights located in a frame

around the cage and connected to silent electronic counters. The device is composed by a two dimensional (X and Y axes) square frame, a frame support, and a control unit. Animal activity is measured by light beams connected to a photoelectric cell. The number of light beam interruptions due to the animal movements inside the cage was automatically recorded. The system is completely modular: each frame may be used for evaluation of general activity, locomotor, stereotypic movements, rearing or exploration (15). The frames are connected directly through SeDaCom computer Software, which allows easy exportation of data in a format compatible with Excel program for Windows.

The animals were kept in the test room for 1 hour before the test for habituation. After the vehicle or tested substances were injected, the animals were placed on the cage device and each movement produced a signal caused due to variation in inductance and capacity of the apparatus resonance circuit. These signals were automatically converted to numbers.

Horizontal or vertical activity is defined as the total number of beam interruptions over a certain period. In this experiment the total number of horizontal cage crossing, vertical and stereotypic movements were recorded automatically over a period of eight minutes (16).

Each animal in the study groups was subjected to 2 minute successive tests, the animal being placed on the work surface and repeatedly replaced in its cage.

The values were recorded electronically and displayed according to prior programming of an electronic system for data processing.

Data for each measure were evaluated separately by *analysis of variance* for repeated determinations. Results were expressed as *arithmetic mean* ± SD and represented graphically.The analyses of the effect of serotonin receptor antagonist on rat behavior in the Actimeter test were conducted by *SPSS version 17.0* for Windows and by 1-way analysis of variance (ANOVA). P-values less than 0.05 were considered statistically significant compared to controls.

Experimental protocol was conducted according to the guidelines for handling and use of experimental animals of the Research Ethics Committee of the Iasi ”Grigore T. Popa”University,in compliance with the ethical standards of the European Community.

Each animal was used only once and the duration of the experiments was kept as short as possible. For ethical reasons, all animals were sacrificed at the end of the experiment (17, 18).

RESULTS AND DISCUSSION

Using the behavioral experimental model, we obtained four types of information regarding the animal spontaneous motor activity: total, horizontal, vertical and stereotypic movements.

The number of total movements represents a feature that reflects the overall intensity of animal behavior over a period of time and is also a good way of corroboration and validation of data biological significance (15).

Actimeter test exhibited sensitivity for detecting changes in total movement counts, as was seen after NAN-190 and SB-269970 treatment.

The change in spontaneous loco motor activity of laboratory animals (as assessed by the number of infrared beam breaks along horizontal or vertical planes) represents a versatile preclinical analysis procedure regarding the central neurobehavioral

effects of tested substances. Spontaneous loco motor activity assessment may be a suitable method for the screening and comparison of the effects of different pharmacologically active substances (19, 20).

The administration of NAN-190 (1 mmol/kbw) resulted in a statistically significant reduction of total movement (147.17±4.36), ($p<0.05$) as compared to the control group (179.67±7.03).

In the same experimental conditions, intraperitoneal injection of SB-269970 1mg/kbw was not associated with a significant change in rat total movements (176.67±8.91) compared to saline solution group (fig. 1).

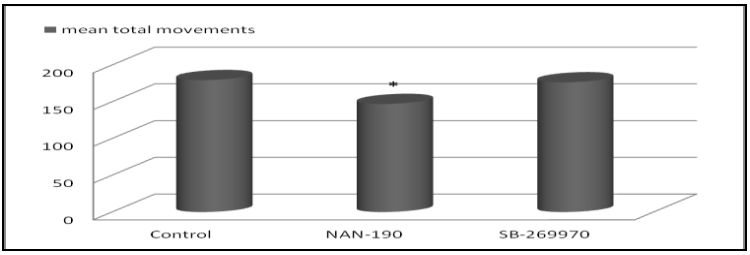

Fig 1. The number of total movements induced by NAN-190 and SB-269970 in Actimeter test. Each point is the mean ± SEM of latency time (seconds) for six rats. *p<0.05, **p<0.01 *vs.* control.

The number of movements in the horizontal plane provides data on the general behavior exploration of the environment, but also about the self-maintenance of animal personal hygiene (paw-licking behavior, hair combing and nose cleaning, by and large, self-directed, possibly recuperative, behaviors – auto-grooming) (16, 19).

The Actimeter test offer the possibility to detect changes in horizontal activity counts, as was observed after NAN-190 and SB-269970 treatment.

Intraperitoneal administration of NAN-190 (1 mmol/kbw) produced a reduction of horizontal movements (131.83±5.19), statistically significant ($p<0.05$), comparing with control group (160.17±8.64) in this behavioral manifestations in rats.

Using SB-269970 (1mg/kbw) we did not obtained a significant modification of animals horizontal movements (157.50± 8.55) compared to control group (fig. 2).

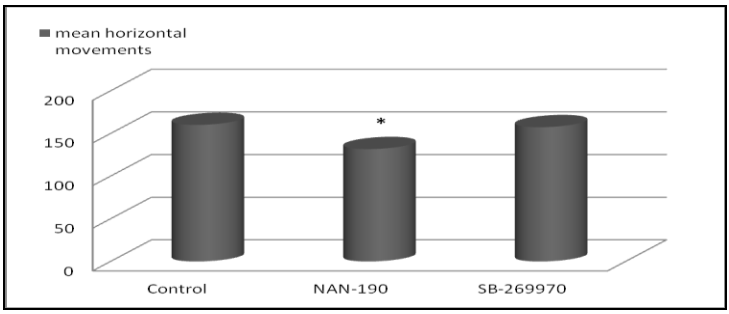

Fig 2. The number of horizontal movements induced by NAN-190 and SB-269970, in Actimeter test. Each point is the mean ± SEM of latency time (seconds) for six rats. *p<0.05, **p<0.01 *vs.* control.

The vertical movements (rearing behavior) reflect that animals are exploring, trying to climb on the transparent walls of registration cage, and are suggestive for attempt behavior, a correspondent for the indirect fear and anxiety states (15, 20).

The Actimeter method gave us the opportunity to detect changes in horizontal activity counts after NAN-190 and SB-269970 administration. NAN-190 produced a statistically significant decrease in vertical movements (15.33±3.33), compared to control group (19.5±1.87) in Actimeter test in rats.

The administration of SB-269970 did not statistically influence the rat vertical movements (19.17±1.94) in this experimental behavioral model (fig. 3).

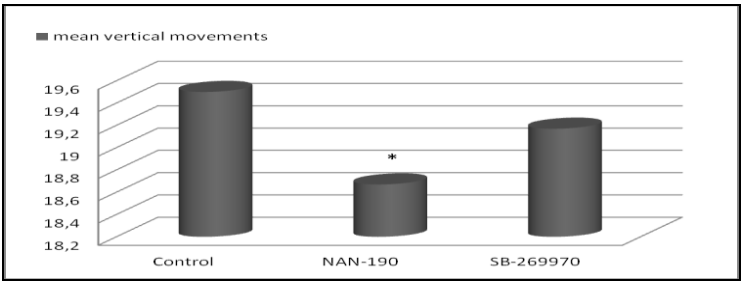

Fig 3. The number of vertical movements induced by NAN-190 and SB-269970, in Actimeter test. Each point is the mean ± SEM of latency time (seconds) for six rats. *p<0.05, **p<0.01 *vs.* control.

CONCLUSIONS

Using a behavioral model to evaluate the exploratory activity and motor coordination in rats, we determined that in our experimental conditions the intraperitoneal administration of 1 mg/kbw NAN-190 resulted in a statistically significant reduction of movements in horizontal and total movement counts compared to controls.

These results reflect the decrease in the number of escape attempts and a significant diminution of exploratory and self-maintenance spontaneous behavior. Corroboration of the two observed changes reflects a type of behavior that in humans corresponds to a decrease of anxiety.

Our results show that in the used mouse model of behavioral manifestations, magnesium soft matter vesicles carrier diminished both global motor behavior and the number of escape attempts, which could correspond somehow to general behavioral inhibition or sedation in humans.

Extrapolation of these results is however difficult, because of the huge differences in behavior, cognitive function, and motor coordination skills between laboratory animal and humans.

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SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BISOPROLOL FUMARATE IN TABLETS

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SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF BISOPROLOL FUMARATE IN TABLETS (Abstract): Bisoprolol fumarate is prescribed for the treatment of hypertension and angina pectoris. **Aim:** The purpose of this study was to develop a simple, sensitive, accurate, and reproducible method for estimation of bisoprolol fumarate in tablets. **Material and methods:** The proposed method was based on a yellow colored complex formed with tropaeolin 00, extractable in dichloromethane with maximum absorbance at 412 nm. The method was validated statistically. **Results:** The linearity domain was observed in the concentration of 5-30 µg/ml. The recovery studies confirmed the accuracy of the proposed method. **Conclusions:** The proposed method can be applied for the routine analysis of bisoprolol from formulations. **Keywords:** BISOPROLOL FUMARATE, SPECTROPHOTOMETRIC METHOD, TROPAEOLIN 00.

Bisoprolol is a β blocker cardiovascular drug. It is structurally similar to metoprolol, acebutolol and atenolol in that it has two substituents in the para position of the benzene ring (fig.1) which might be the reason for its β1-selectivity.

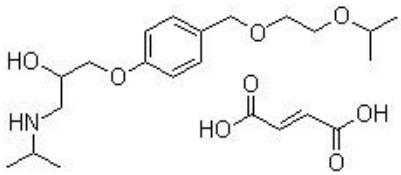

Fig. 1. The structural formula of bisoprolol fumarate

Bisoprolol is used for the treatment of hypertension, cardiac ischemia and congestive heart failure. This is also indicated for preventative treatment before and primary treatment after heart attacks thus reducing the chances of recurrence (1). Bisoprolol reduces the high blood pressure in hypertension, while in cardiac ischemia this drug is used to reduce the activity of the heart muscle and therefore reduce oxygen and nutrient demand, so reduced blood supply can still transport sufficient amounts of oxygen and nutrients (2-6).

Various methods such as UV-Vis spectrophotometry (7-10), high-performance liquid chromatography (11-13), and thin layer chromatography (14-16) are available for determination of bisoprolol in biological fluids and in pharmaceutical formulations.

The aim of the present work was to de-

velop a new rapid and reproducible spectrophotometric method for the quantitative determination of bisoprolol fumarate in pharmaceutical products.

MATERIAL AND METHODS

A Hewlett Packard 8453 double beam UV–Vis spectrophotometer equipped with 10 mm matched quartz cells was used in the present investigation. A Kern 770 analytical balance, an ultrasonic bath and a vibration shaker IKA-Werke type VX2 were also used.

The reagent used were: bisoprolol fumarate – 100.07% pure reference substance (Unichem Laboratories LTD, India), hydrochloric acid (Tunic Prod, Romania), dichloromethane (Fluka, Germany); tropaeolin 00 (Tunic Prod, Romania).

The pharmaceutical products Concor® (Merck), Bisotens® (Antibiotice Iasi) and Bisoblock® (Keri Pharma Generics Ltd) have been purchased from the local pharmacies.

Preparation of the solutions:

- stock solutions of bisoprolol fumarate was prepared by dissolving accurately weighed 10 mg of standard substance in a 100 mL volumetric flask with distilled water;
- standard solutions of various concentrations of bisoprolol were prepared by suitable dilution from stock solutions;
- 0.01% (m/v) aqueous solution of tropaeolin 00: 0.01 g of tropaeolin 00 was dissolve on ultrasonic bath in 100 mL volumetric flask with distilled water;
- 2M aqueous solution of hydrochloric acid was prepared by transferring 3.8 mL hydrochloric acid (96%) in 50 mL volumetric flasks and bringing up to the mark with distilled water;
- sample solution: twenty tablets of each formulation were accurately weighed and the average weight was calculated for each. These tablets were grounded to a fine powder. An accurately weighed tablet powder equivalent to 5 mg of bisoprolol fumarate was mixed with 30 mL of methanol and sonicated for 30 minutes. Insoluble excipients were separated through filtration. Then the solution was evaporated to dryness. The residue was dissolved into a 100 mL volumetric flask using distilled water. The obtained solution was diluted with distilled water to obtain working standard solution corresponding to 20 μg/ml.

Assay procedure: 1 mL 0.05 M hydrochloric acid and 1 mL 0.01% (w/v) tropaeolin 00 aqueous solution were added to 1 mL of bisoprolol fumarate solution with a concentration in between 5-30 μg/mL. The complex was then extracted using dichloromethane. Fifteen minutes later the absorbance was measured at 412 nm, using as reference a blank sample prepared in the same conditions.

Validation: The method was validated with respect to linearity, accuracy, precision and LOD and LOQ (17).

RESULTS AND DISCUSSION

The analysis of the absorption spectra (fig. 2) revealed a maximum absorbance of the reaction product at 412 nm. This value was used for all determinations.

As can be notice in fig. 3, bisoprolol in dichloromethane solution presented a maximum absorbance peak at 385 nm and tropaeolin 00 had no absorption peaks in the interest range.

Comparing the spectra from fig. 3 and 4, it was observed that the proposed method was selective because it had the ability to separate the signal of bisoprolol from the one corresponding to the reaction product.

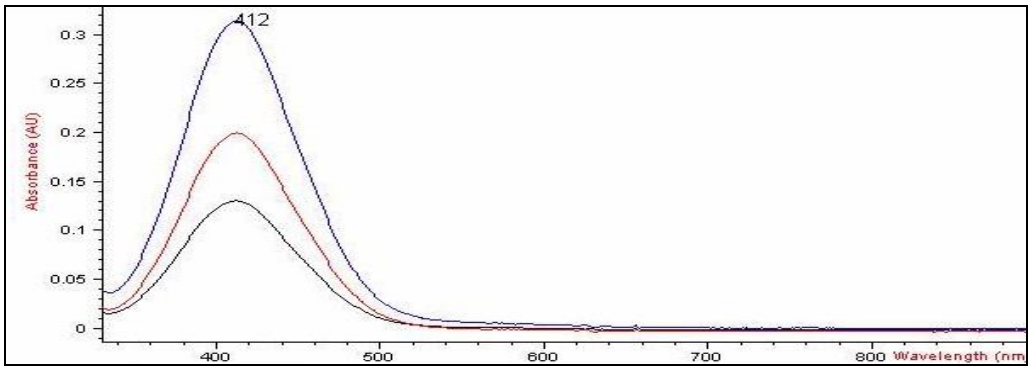

Fig. 2. Absorption spectra of the reaction product

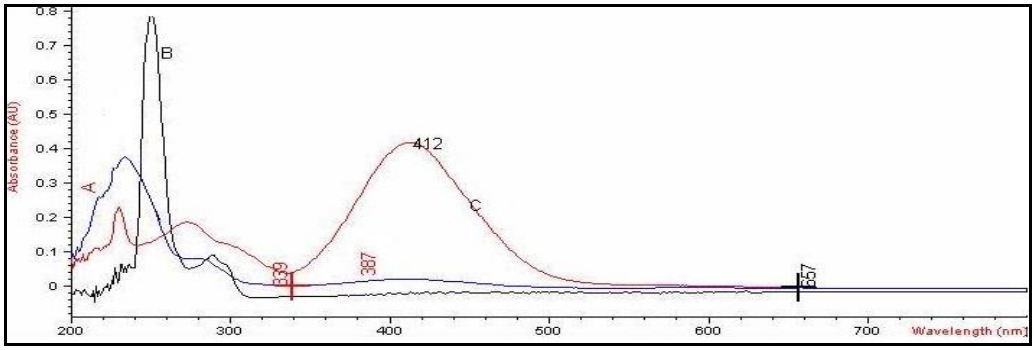

Fig. 3. Absorption spectra of bisoprolol (A), tropaeolin 00 (B) and reaction product (C)

The excipients used in the pharmaceutical formulation did not interfere the maximum absorption of bisoprolol complex.

While choosing the best solvent of the extraction, experiments were done using ethanol, methanol, chloroform, dichloromethane, acetonitrile (fig. 5). It has been observed that the best results were obtained when the extraction was performed using dichloromethane.

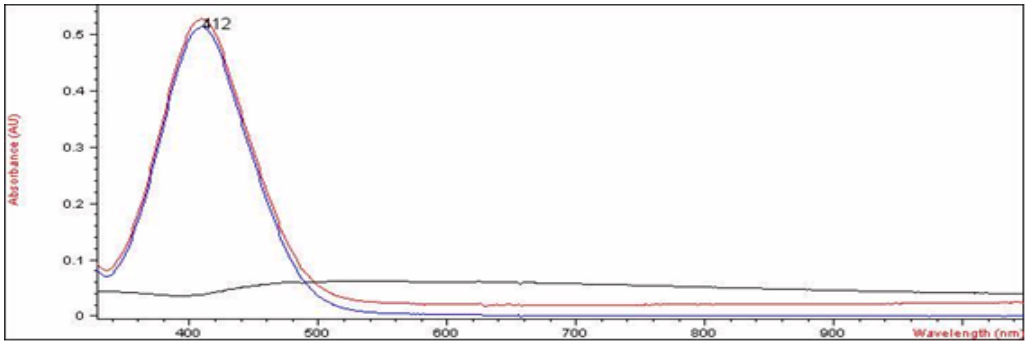

Fig. 4. Absorption spectra of placebo (1), sample solution (2) and standard (3)

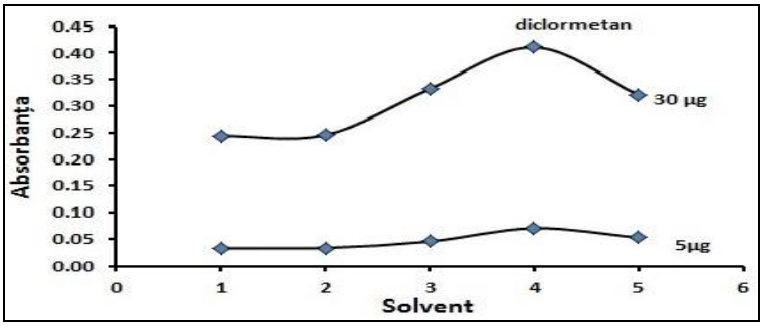

Fig. 5. Choice of solvent for the extraction

The calibration curves for bisoprolol fumarate was founded to be linear in the concentration range of 5-30 µg/mL. The optical and regression characteristics of the drugs are show below (tab. I).

The absorbance of each tablet formulations was measured and the results of analysis were shown in tab. II.

Accuracy: To study the accuracy of the proposed methods, recovery studies were carried out by adding a known amount of drug to the pre analyzed tablet powder and percentage recoveries were calculated. The result of recovery studies are presented in tab. III.

Precision: The reproducibility of the proposed method was determined by performing the analysis at different time moments in the same day (intra-day assay precision) and three different days (inter-day assay precision). The results of intra-day and inter-day precisions expressed in relative standard deviation percentages were found to be 0.8 and 0.9, respectively.

Limit of detection and limit of quantification: The LOD and LOQ were determined based on the standard deviation of the intercept and the slope of the calibration curves. LOD and LOQ for bisoprolol were found to be 0.67 µg/mL and 2.23 µg/mL respectively.

TABLE I

Optical and regression characteristics

Wavelength (nm)	412
Linearity range, µg/mL (µg/ml)	5-30
Molar Absorptivity (1/mol·cm)	$1.03 \cdot 10^4$
Sandell's Sensitivity (mg/cm^2/0.001 absorbance unit)	0.0134
Standard error	0.0029
Correlation coefficient (r)	0.9997

TABLE II

Result of tablet analysis

Brand	Label Claim (mg)	Amount of Estimated Drug (mg)	Label Claim Percentage (%)	Standard Deviation
Concor®	2.5	2.49	99.33	0.59
Bisotens®	5	4.96	99.86	0.67
Bisoblock®	10	10.03	100.31	0.13

TABLE III

Result of recovery studies

Brand	Bisoprolol in tablet (µg/mL)	Pure Drug Added (µg/mL)	Recovery* Percentage (%)	Standard Deviation
Concor®	12	8	99.15	0.42
Bisotens®	15	5	98.87	0.96
Bisoblock®	17	3	99.23	0.26

*Mean for 3 determinations

CONCLUSIONS

The presented assay provides a simple, accurate, economical and convenient method for the analysis of bisoprolol using VIS spectrophotometry. Results of percentage recovery showed that the method was not influenced by excipients.

The method was validated and its sensitivity and precision proved the suitability of the proposed method for the routine estimation of bisoprolol in pharmaceutical formulations.

ACKNOWLEDGEMENTS

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NEWS

NEW PROPERTIES OF SOME SUGARCANE CULTIVARS

The phenolic composition, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*) were investigated by Abbas *et al*. Sugarcane is a dietary source of flavonoids and it is used as functional food in many countries. In addition, it is an important cash crop of Pakistan. The sugarcane leaves are the main raw material for the sugar industry. The authors studied the juices and the water extracts obtained from the leaves of thirteen varieties of sugarcane. The antioxidant activity of sugarcane extracts and juices was evaluated using DPPH radical scavenging assay. In this assay, the extracts showed better antioxidant activity than the juices. The IC_{50} values ranged from 20.82 to 27.47 µg/mL for the leaves extracts and from 63.95 to values higher than 200 µg/mL for the juices. In the phosphomolybdenum assay, the juices showed a better electron-donating activity than the extracts. In this assay, the IC_{50} values ranged between 62.3 and 80.26 µg/mL (extracts) and 70.43 and 99.9 µg/mL (juices). The leaves extracts and juices showed strong ability to protect against DNA damage induced by hydroxyl radical generated in Fenton reaction. The high performance liquid chromatography (HPLC) analysis of extracts was also performed. Ferulic acid (14.63 ± 0.03 mg/g), cumaric acid (11.65 ± 0.03 mg/g), quercitrin (10.96 ± 0.02 mg/g), caffeic acid (9.16 ± 0.01 mg/g) and ellagic acid (9.03 ± 0.02 mg/g) were detected as major compounds into CPF-246 sugarcane leaves extract. The results of the study showed that these cultivars might be used not only as an accessible source of natural antioxidants but also as an ingredient in functional foods (Abbas SR, Sabir SM, Ahmad SD, Boligon AA, Athayde ML. Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). *Food Chem* 2014; 147: 10-16)

Alexandru Vasincu

BOOK REVIEW

NEUROFIZIOLOGIE. ROLUL UNOR NEUROTRANSMIȚĂTORI ŞI ZONE NERVOASE ÎN MODULAREA PROCESELOR COGNITIVE ŞI IMUNITARE

by Lucian Hrițcu,

Publishing House of the University "Alexandru Ioan Cuza" from Iaşi, 2011, 231 pages

Reviewed by Prof. Ursula Stănescu, Ph.D.

"*Neurophysiology. The role of neurotransmitters and nervous areas in cognitive and immune modulation*" by lecturer Lucian Hritcu, Ph.D. was published at the end of 2011 by the prestigious academic publisher "Alexandru Ioan Cuza" University Press. The monograph is a thorough and complex study of the highly topical issues of the implications of nervous areas, certain neurotransmitters in cognitive processes and defense function of the animal organism, being a model for the study of such issues for all those interested in similar researches.

Innovative research methods, many experimental variations of studied parameters, the richness of obtained original results and their interpretation in accordance with literature data highlight the scientific value of this work and reflect author's hard work, perseverance, scientific competence and passion.

Based on these considerations, the author conducted a complex work with a comprehensive content covering 231 pages, including numerous current theoretical and a wide range of personal results. The monograph is divided into six chapters. It includes 78 tables, of which 73 contain original data and 5 data in the literature, 91 clear and suggestive figures, and an extensive bibliography of 370 titles, of which 12 are personal papers of which some were published in prestigious foreign journals. Of these 12 scientific papers three were published in ISI journals, two in the European Journal of Pharmacology (IF = 2.516) and one in the Turkish Journal of Hematology (IF = 0.341).

The first chapter, entitled **The current state of research on neuroimmunomodulation**, presents some theoretical aspects of the innervation of primary lymphoid organs such as: bone marrow, thymus, and bursa of Fabricius, and secondary lymphoid organs: spleen, lymph nodes and gut-and bronchus-associated lymphoid tissue. Also presented in this chapter are hormone and neuropeptide receptors, the main characteristics of the specific receptors in the lymphoid organs, and other receptors, such as those for arginine vasopressin, vasoactive intestinal polipeptid and cytokines.

Chapter 2 deals with the **Correlations of the central nervous system neurotransmitters and the immune system** and highlights the key issues on three neurotransmitters: acetylcholine, norepinephrine and dopamine. Thus, the author presents some basic characteristics of acetylcholine chemical structure, biosynthesis, specific receptors and its implications in cognitive processes. As to adrenaline and noradrenaline, a series of features, source, receptors, role and implications of these catecholamines in immune and cognitive processes

are detailed. The effects of dopamine on cells involved in immune processes, such as thymocytes and lymphocytes, particularly neutrophils and macrophages are well documented.

In Chapter 3 **a number of genetic aspects about degradation of the neurotransmitter system** involved in some severe neurodegenerative diseases like Parkinson's disease, Alzheimer's disease and schizophrenia are discussed. The features of these diseases are presented with particular emphasis on the genetic changes associated with neuronal alteration, disruption of neurotransmitter system and immune capacity, drawing our attention on important issues unsolved so far.

In Chapter 4 **Materials and working methods** are detailed in order to facilitate the understanding and assessment of experimental results. Experimental results are a model and an important guide for all researchers in this field. Techniques, laboratory materials and experimental animals for the four categories of operations: neurosurgical interventions, cognitive performance assessment, calculation of the immune indices and assessment of the effects of acute and chronic stress are presented.

In Chapter 5, the author discusses a significant number of results of experiments **on ways to modulate learning and memory processes via cholinergic and dopaminergic receptors**. To highlight the role of these receptors in cognitive processes, the author used a logical approach managing specific agonists or antagonists of these receptors then observing the test results on cognitive performance.

Following the correlation between nervous structures involved in cognitive processes and those involved in animal body defense function, the author studied **the ways to modulate immune function under the influence of nervous and neurotransmitter areas under stress**, a large number of original results being presented in Chapter 6 of this monograph.

The monograph ends with **general conclusions**, concise and enlightening, highlighting the originality and value of the results.

The present monograph is addressed to undergraduate, postgraduate and doctoral students, also, academics and researchers in the fields of animal physiology, medicine, neurophysiology, neuroendocrinology, neuropsychiatry and neuroimmunology.

Professor Ursula Stănescu, Ph.D.

DENTAL MEDICINE **ORIGINAL PAPER**

PREVENTIVE ATTITUDES TOWARDS INFECTION TRANSMISSION IN DENTAL OFFICES IN NORTH-EAST ROMANIA

Lucia Bârlean[1], I. Dănilă[1], Carina Balcoş[1], Iulia Săveanu[1], Adriana Balan[2]
University of Medicine and Pharmacy "Grigore T. Popa" - Iaşi
Faculty of Dental Medicine
1. Discipline of Preventive Dentistry
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PREVENTIVE ATTITUDES TOWARDS INFECTION TRANSMISSION IN DENTAL OFFICES IN NORTHEAST ROMANIA (Abstract) **Aim**: The aim of this study was to evaluate the level of knowledge and the current attitudes towards infection control in the dental offices in the Northeast Romania. **Material and methods**: Questionnaire-based study conducted on 152 dentists aged between 25-65 years practicing in 6 Northeast Romania counties. The questionnaire included questions related to current infection control procedures and attitudes. Data were statistically analyzed using *SPSS 14.0* and chi-square test ($p<0.05$). **Results:** The majority of the dentists (83.6%) consider that universities should provide a substantial education regarding infection control through postgraduate courses, but 7.2% of the respondents are not sure about their usefulness. The clinical decision related to patient's treatment is influenced by his infectious status for 67.8% of the dentists. Of these, 19.1% have over 20 years of practice and 48.0% are females. Almost all dentists (93.4%), regardless of gender (96.4% females and 87.8% males) undergo periodic testing for blood-borne viral infections (hepatitis B, C and HIV). Full protective equipment is used for all the patients by 86.2% of the dentists, while 12.5% use it only for the infectious ones. 65.2% of the dentists use steam sterilization equipment (autoclave), and 80,8% use dry heat. The majority of the dentists (82.6%) believe that the patient must always be informed about the adopted infection control measures, but 21.7% declare to do so only in some particular cases. **Conclusions** Dentists knowledge and attitudes towards infection control must be improved by educational interventions in order to adhere to the European standards. **Keywords**: DENTAL OFFICE, INFECTION CONTROL, EDUCATION

Occupational health of oral health care personnel and patient safety are of particular importance warranting imposition of rigorous rules of professional conduct in view of meeting the European safety standards of healthcare in the dental office (1).

MATERIAL AND METHODS

In order to assess the attitudes towards infection prevention and control in dental offices in North-east Romania, in the interval January-February 2011 we conducted a questionnaire-based study involving 152 dentists, 62.4% women and 37.6% men, aged between 25 and 65 years, working at private dental offices in 6 counties of Moldova: Iasi, Botosani, Neamt, Suceava, Bacau and Vaslui.

This study was part of a market research survey conducted in 5 development regions

of Romania in order to identify the current situation and current needs in the field of prevention, ergonomics and management. (European Project "ergonomics, prevention, performance management in dentistry by adopting European Standards, Contract: POSDRU/81/3.2/S/55651).

The questionnaire included 21 questions with one or more correct answers on the attitude of physicians towards infection control procedures established in dental offices.

The results were interpreted according to gender and years of practice and compared with those obtained by a study covering all Romanian regions. Data were processed using *SPSS 14.0* system, and compared using Chi-square test ($p < 0.05$). The approval of the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi was obtained.

RESULTS

Analysis of responses showed that the majority of dentists (83.6%) considered necessary a substantial education in infection control both during university studies and postgraduate education, but 7.2% of respondents were unsure about their usefulness. Comparing these results with those of the nationwide study, it came out that the percentage of those not giving great importance to this knowledge was significantly higher (7.2% versus 1.9%).

Therapeutic approach in clinical practice was influenced by patient's infectious status in 67.8% of the dentists. Of these, 19.1% had over 20 years of practice and 48.0% were women. These values are close to those reported by the nationwide study (57.0 %, 19.2% and 23.7%, respectively) (fig. 1).

Most dentists (82.6%) believe that patients should be constantly informed on infection control measures taken in the dental office, but 13.0% say they do so only in special cases.

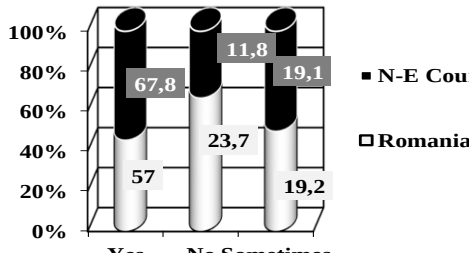

Fig. 1. Influence of patient's infectious status on therapeutic approach

Full protective equipment (gown, gloves, mask, goggles) is used for all patients by 86.2% of dentists, while 12.5% of them use it only for patients considered infected. Slightly more women (72.9%) use full protective equipment than men (68.4%). Nationwide data were: 90.2% and 9.8%, respectively (fig. 2).

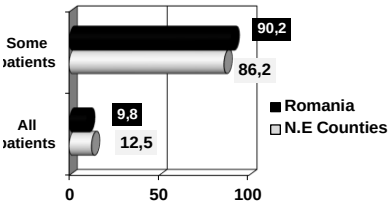

Fig. 2. Use of full protective equipment

In North-east Romania 98.9% of dentists use rubber gloves, 98.6% masks, and 53.9% goggles.

62.2% of doctors are immunized against hepatitis B. The majority of physicians (93.4%), with no significant gender difference (96.4% women and 87.8% of men) say they carry out periodic testing for blood-borne diseases (hepatitis B, hepatitis

C, HIV infection).

As to sterilization methods, 80.8% of the dentists use dry heat sterilization (Poupinel), 65.2% moist heat (autoclave), and 8.7% resort to chemical sterilization (fig. 3). Data obtained for Romania were 63.5%, 75.5% and 8.9%, respectively.

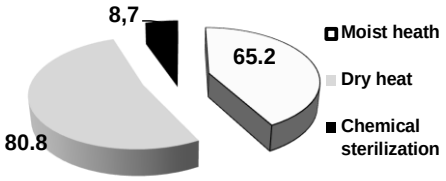

Fig. 3. Sterilization methods used in Northeast Romania dental offices

Hand hygiene, essential for infection control in healthcare facilities, is performed before applying protective gloves (22.5% of dentists), before each patient (60.5%) after each patient (56.3%), whenever necessary (66.3%).

Ultraviolet light devices are used to control air contamination in 28.1% of dental offices.

The methods of decontamination of dental unit waterlines were: water circulation in the water systems at the beginning of the working day and between patients (27.5%), use of distilled water (20.7%), water sterilization (3.4%), and germicidal agents (12.4%) (fig. 4).

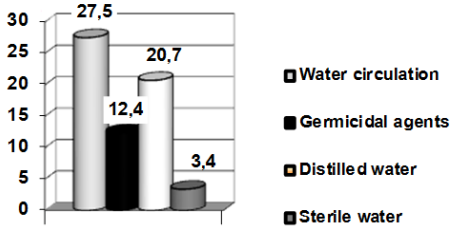

Fig. 4. Methods of decontamination of dental unit waterlines

DISCUSSION

Assessment of adherence to infection control recommendations is essential for estimating the safety and predictive risk factors for patients and medical staff in dental offices.

Immunization is an important measure in the dental office occupational protection, as the oral healthcare personnel belongs to a risk category due to direct contact with blood or other body fluids. The study results show a small percentage of dentists immunized against hepatitis B (62.2%) compared with data reported in the literature: 93% - USA, 91% - Canada, 86% - England (2).

Hand hygiene is considered the most effective measure to reduce the risk of transmission of pathogens in healthcare facilities (3). Our results show that only 25% of the dentists wash their hands or use hand sanitizers before applying protective gloves although these do not provide complete protection against pathogen (4).

Protection provided by the use of protective equipment is scientifically substantiated and is one of the basic rules of Universal Precautions (5). Goggles and masks significantly reduce mucosal exposure, and gloves are remarkably effective in reducing occupational exposure to diseases transmitted via blood at hand skin level (6, 7). The evaluation of doctors' attitudes demonstrated a good compliance (86.2%) in wearing full protective equipment (gown, mask, goggles, gloves) used with all patients regardless of infectious status or clinical procedure. The results show an increase in the compliance level compared with than reported by Bârlean et al. (full equipment: 68.4%) (8). A lower proportion of physicians (53.9%) use goggles ignoring the risk of infection transmission to the eyes.

Devices used to sterilize dental instru-

ments are mainly represented by dry-heat autoclave (80.8%), but the number of dentists using moist heat autoclave is increasing (65.2% compared to 40.2% in our 2007 study) (9). Although guidelines and recommendations of the European organizations restrict chemical sterilization in dentistry, our study shows that 8.7% of the dentists in Northeast Romania and 8.9% in all Romania use of this method (10).

Control of dental unit waterlines contamination is not a major concern for dentists as only 12.3% of them use germicidal products for decontamination (11).

The comparative assessment of the results showed that the level of knowledge and current practices of infection control of the dental practitioners in Northeast Romania corresponds to the levels revealed by the study in 5 development regions of Romania.

CONCLUSIONS

Assessing current attitudes and practices regarding infection control procedures in dental offices in the Northeast Romania has shown the need to promote a realistic perception of the risk of infection transmission and to implement specific measures for the protection of oral healthcare personnel and patients in view of adhering to the European standards.

ACKNOWLEDGEMENTS

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CLINICAL-RADIOLOGICAL STUDY ON THE ROLE OF BIOSTIMULATING MATERIALS IN IATROGENIC FURCATION LESIONS

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CLINICAL-RADIOLOGICAL STUDY ON THE ROLE OF BIOSTIMULATING MATERIALS IN IATROGENIC FURCATION LESIONS (Abstract): Root perforation is an unwanted incident which may occur at any stage of endodontic treatment and can adversely affect tooth prognosis. **Aim**: To compare the recovery rate after treatment of root perforations in the interradicular area of the molars, using two different materials: MTA and ceramic nanoparticles mineral cement DiaRoot® BioAggregate, by a clinical-radiological and statistical analysis over a period of up to 24 months. **Material and methods:** The study was conducted on 28 molars from patients of both sexes, mean age 33.29 ± 6.2DS, with iatrogenic perforation of pulp chamber floor. The teeth were divided into 2 groups according to the applied material: group 1 - gray MTA (ProRoot ® MTA, Dentsply, Tulsa Dental), and group 2 - BioAggregate (Diadent ® Group International, Vancouver). Patients included in the study were monitored and assessed by radiological examinations at 6, 12 and 18 months. **Results:** Pulp chamber floor perforations are significantly associated with tooth location ($\chi 2 = 35.60$, $r = 0.67$, $p = 0.00359$, 95% CI). Both when the perforation was repaired with MTA and BioAggregate, the clinical improvement was significant ($\chi 2 = 17.608$, $r = 0.58$, $p = 0.0035$, 95% CI). **Conclusions:** Based on the results of this study, both MTA and BioAggregate are excellent materials for root perforation repair**. Key words:** ENDODONTIC TREATMENT, ROOT PERFORATION, REPAIR MATERIALS.

Endodontic treatment is a delicate clinical procedure, unfortunately resulting sometimes in failures. Root perforation is an unwanted incident which may occur at any stage of endodontic treatment and can adversely affect tooth prognosis. Although caries or resorption processes may also cause this type of tissue destruction, most root perforations are iatrogenically induced. Perforations usually produce an artificial communication between the radicular canal and the periodontal space. They can be located at different levels of the root, from the accidental perforation of the pulp chamber floor to the side walls of the canal, continuing to the root apex, with inflammatory and resorptive implications. Numerous research papers show the possi-

bility of applying modern techniques and materials to restore damaged structures, based on animal experiments and histological studies (1, 2).

In addition to providing a good seal, the material of choice for root perforation repair must be biocompatible, nontoxic, insoluble in the presence of tissue fluids, and able to promote periradicular tissue regeneration. Untreated, perforation results in a chronic inflammatory reaction of the periodontium, sometimes with serious complications, such as suppuration, abscess, fistula or bone resorptive processes that can lead to irreversible loss of tooth attachment. Seltzer et al. (4) have shown that successful root perforation repair is conditioned by their location, shape, size, and the time before sealing.

This study aimed at comparing the recovery rate after root perforation treatment with two different materials: MTA (ProRoot ® MTA, Dentsply, Tulsa Dental) and DiaRoot ® BioAggregate (Diadent ® Group International, Vancouver), by a clinical-radiological and statistical analysis conducted over a period of up to 24 months.

MATERIAL AND METHODS

The study was conducted on 28 molars (12 maxillary and 16 mandibular) from 28 patients (13 female and 15 male) aged between 25 and 46 years with an average of 33.29 ± 6.2 DS. The patients presented to our office for a root canal treatment due to a symptomatic tooth or iatrogenic maneuvers performed in the past. All cases were evaluated before and after treatment by a double blind study to determine the presence or absence of pathological changes surrounding the perforation site. The 28 molar were randomly divided into 2 equal groups: in group 1 we used gray MTA (ProRoot ® MTA, Dentsply, Tulsa Dental), and in group 2 BioAggregate (Diadent ® group International, Vancouver), both mixed to a paste consistency according to manufacturer's instructions.

After tooth isolation and cavity cleaning, the communication between pulp chamber and periodontal space was detected and in case of bleeding hemostasis was achieved using cotton pellets soaked in 5% sodium hypochlorite. The material was applied with a special carrier, (MTA- gun) and then easily condensed with a plugger. Over it a pellet soaked in distilled water was placed, because both materials are setting in a wet environment and finally provisional cement (Citodur, DoriDent, Austria) for 2-3 days. At the next visit, the provisional cement was removed, MTA strength and the degree of perforation coverage were checked with a palpation probe, followed by the standard mechanical, chemical and drug treatment of root canals.

RESULTS

After physical examination, history taking, and interpretation of radiographic images, the data were recorded in case-record forms including all relevant radiographs. The analysis showed that of the studied 28 teeth, 10 presented untreated pre-existing perforations with associated chronic periapical pathology, 7 presented perforations previously incorrectly and ineffectively treated with persistent interradicular radiolucency, and 11 perforations made during endodontic treatment or retreatment (fig. 1).

Of all study patients, 16 (6 upper molars and 10 lower molars) had a radiolucency at the furcal area on the initial radiograph, accompanied by painful symptoms, confirming the presence of chronic inflammation in that area (fig. 2)

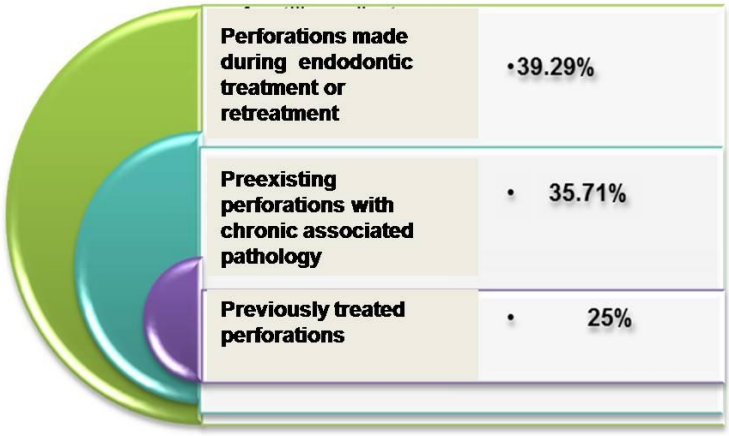

Fig.1. Case distribution according to the cause of root perforation

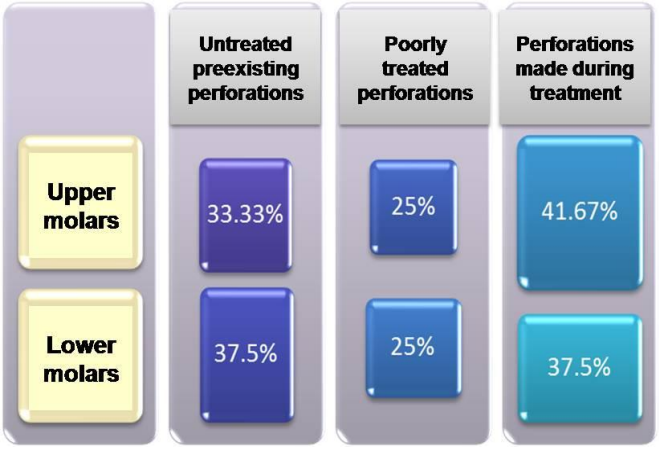

Fig. 2. Initial situation of perforations in molars

Of the 12 upper molars studied, 6 presented perforation of the pulp chamber floor located in the disto-buccal area, 2 perforations located in the mesio-buccal area, and 4 central perforations. In the mandible, of the 16 studied molars, 7 perforations were located medially, 5 distally, and 4 centrally. The statistical analysis showed that there is a significant association between pulp chamber floor perforation and tooth location (χ^2 = 35.60, r = 0.67, p = 0.00359, 95% CI); thus, in the upper molars perforations are more frequent in the disto-buccal area,(21.43%), while in the lower molars in the mesial area (25%) (fig. 3).

The cause of pulp chamber floor perforations was significantly correlated with tooth type, as demonstrated by the higher frequency of untreated pre-existing perforations in the lower molars (37.5%), and perforations made during endodontic treatment especially in upper molars (χ^2 = 26.505, r = 0.59 , p = 0.00547, 95% CI) (fig. 4).

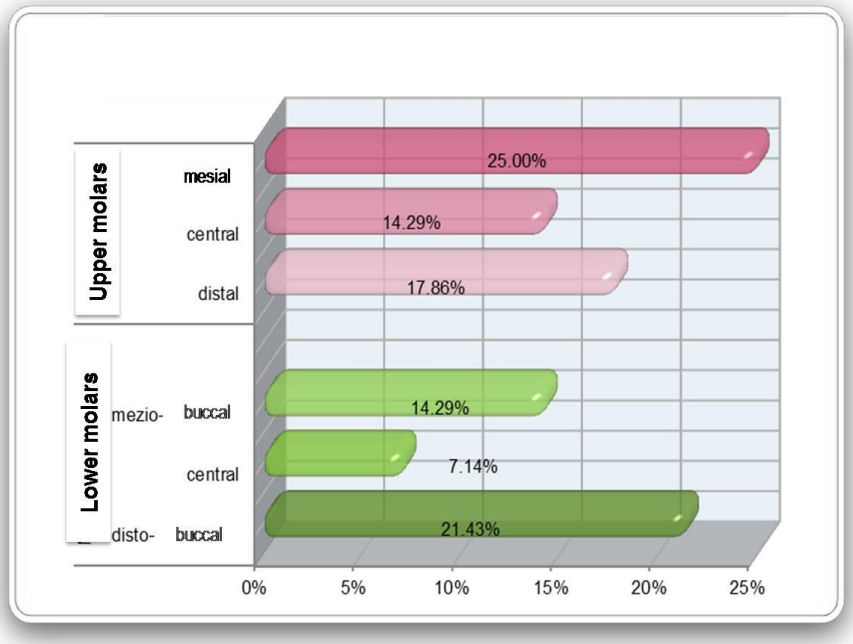

Fig. 3. Case distribution according to the location of pulp chamber floor perforation
La lower molars - mesio-

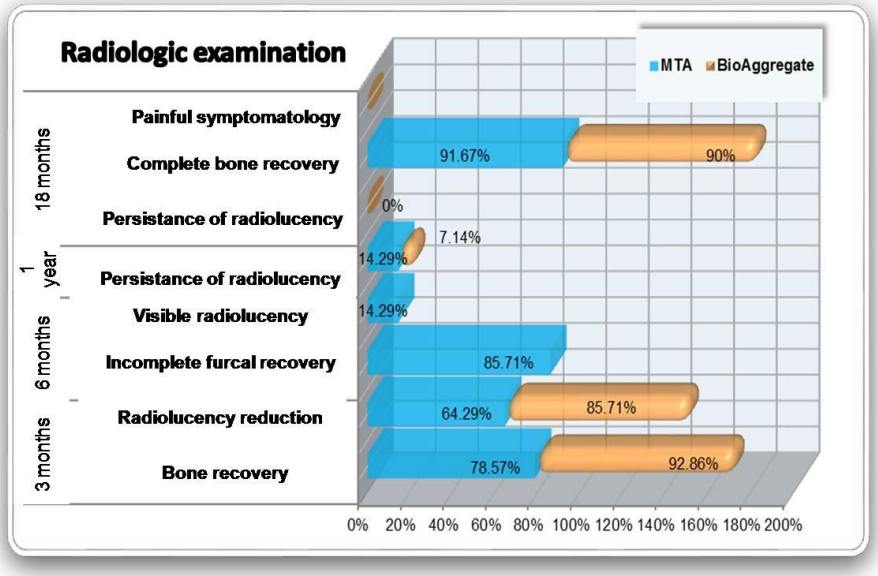

Fig. 4. Radiological examination of pulp chamber floor perforations

One-year postoperatively, all molars in group 1 were asymptomatic, had no attachment loss or fistula. Radiological examination revealed, however, in two cases, the persistence of a radiolucent furcal area in molars with larger initial lesions (one lower and one upper). The same was seen in group 2 molars, except for one case, with a larger initial lesion, that still had an area of osteolysis, lesser than the initial one, accompanied by mild pain when chewing (fig. 5).

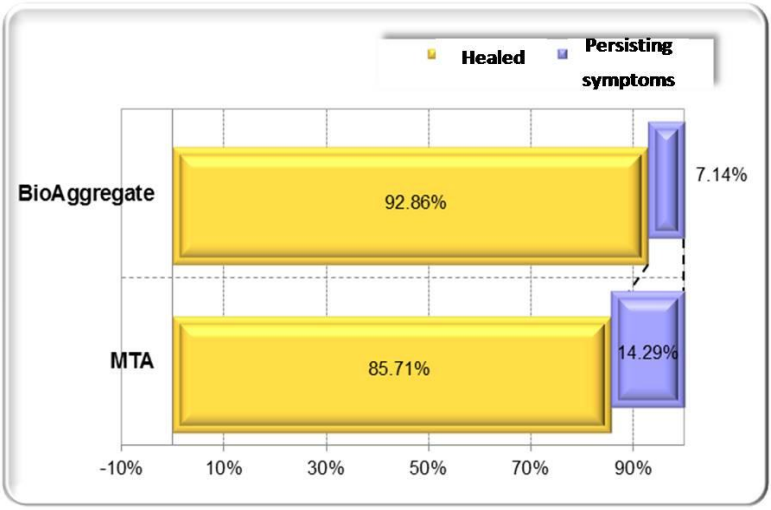

Fig. 5. Association of the type of repair material with postoperative course

Radiological images showed better results especially in group 2, where the used material was BioAggregate. Thus, the type of repar material significantly influenced the postoperative course (χ^2 = 6.15, r = 0.46, p = 0.03, 95% CI).

To illustrate the ability of the above mentioned materials to induce periradicular tissue recovery and healing, we here mention one of the cases included in this study. A 42-year-old female patient was referred to the clinic for a mild pain when chewing in the lower left molar. After the removal of the massive crown restoration a 1.5-2 mm communication area between the pulp chamber and the furcal periodontium was seen. Radiologically, a large interradicular radiolucent area was revealed, implying the presence of a local irritation factor. The tooth was treated with BioAggregate cement, according to the protocol used in all group 2 teeth (fig. 6)

DISCUSSION

The main goal in the treatment of root perforations is stopping further loss of periodontal attachment by preserving healthy tissue at the perforation site. Several studies (5, 6, 7) have shown that if the lesion is already present, it is important to restore the attachment tissue. It was found that important factors in determining the success of a perforation repair procedures are perforation location, time between the occurrence of perforation and its repair. The results of this study are consistent with those obtained by other authors in their research on similar subject (8, 9).

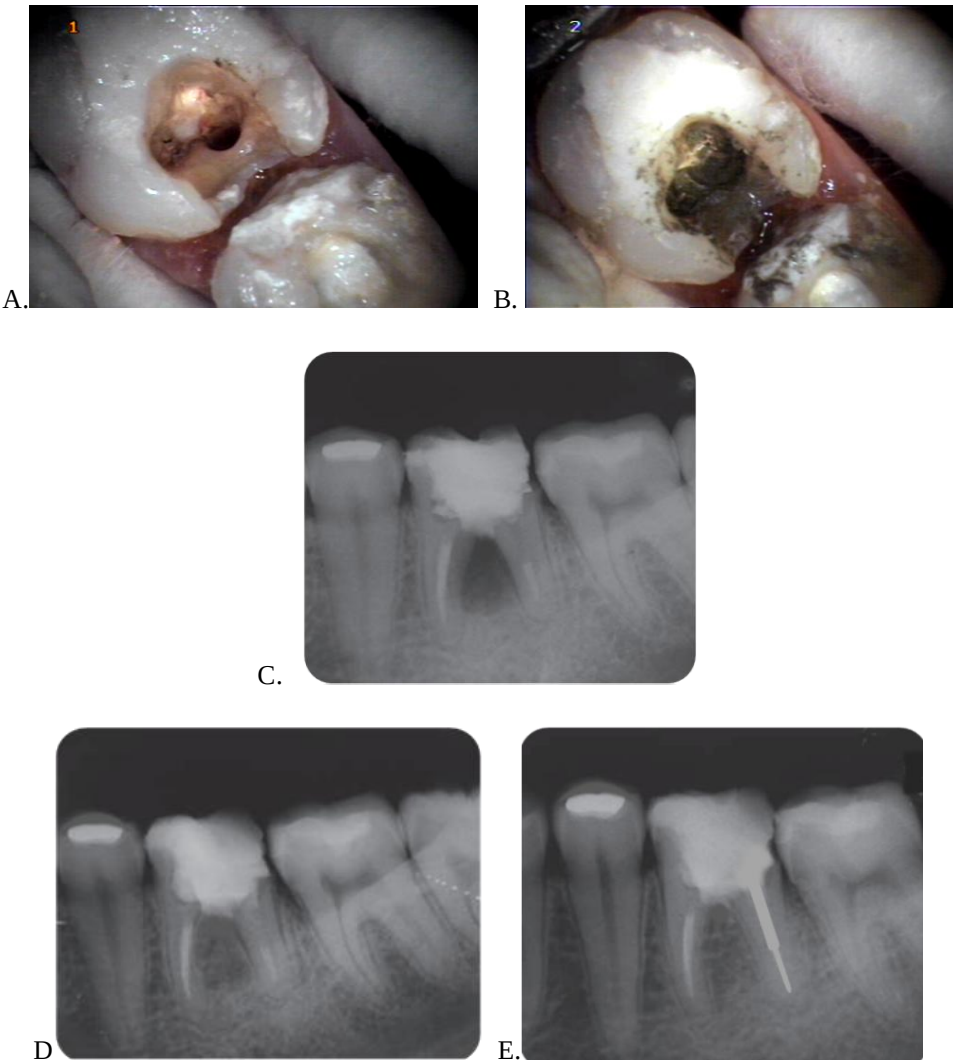

Fig. 6. A. Lower left molar with restorative material and pulp chamber floor perforation. **B.** MTA application over the communication. **C.** Preoperative radiograph shows an incomplete endodontic treatment and a well defined interradicular radiolucency. **D.** The 6 months radiograph indicates a reorganization of demineralized trabecular bone with furcal recovery. **E.** The radiographic exam after two years shows complete healing of interradicular and periradicular bone with complete restoration of the periodontal space.

CONCLUSIONS

Researches have shown that treatment plan for perforation repair depends on the visibility and accessibility of perforation, its size, periodontal status, and strategic importance of the tooth. Successful repair of root perforations is conditioned by its the location, shape, size, and the time be-

fore it is sealed. One of the most important parameters affecting treatment outcome have proved to be the location on the root surface and the type of repair material, which appears to significantly influence the postoperative course.

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MODULATORII INCRETINELOR, O NOUĂ PERSPECTIVĂ ÎN TRATAMENTUL DIABETULUI ZAHARAT

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INCRETIN MODULATORS, A NEW PERSPECTIVE IN DIABETES MELLITUS TREATMENT (Abstract): **Aim:** The study was designed to present the modern therapy used for the treatment of type 2 diabetes mellitus (T2DM). **Material and methods**: Diabetes mellitus and the pharmacological and the pharmacokinetic characteristics of the incretins and of the incretin modulators are presented. **Results**: Diabetes mellitus is a chronic metabolic disorder, which is considered to be a major health issue all over the world. World Health Organization indicates that world-wide almost 3 million deaths per year are caused by diabetes and 1,5 million new cases are diagnosed annually, T2DM representing approximately 90-95% of the diagnosed cases. This form of diabetes mellitus is characterized by several path physiological defects including insulin resistance at peripheral target tissues, excess hepatic glucose production and progressive pancreatic beta cell dysfunction. The newest direction in the treatment of T2DM mellitus is the incretin modulators. The incretins (GLP-1 - glucagon-like-peptide-1 and GIP - glucose-dependent insulin tropic peptide), are natural hormones that contribute to glucose homeostasis by acting on the pancreas, gastrointestinal tract, muscle and brain tissue. After secretion, GLP-1 and GIP are immediately metabolized by dipeptidyl peptidase IV enzyme (DPP IV), which limits their therapeutic use. **Conclusions:** In order to achieve optimal glycemic control, by using the therapeutic effects of incretins, research has been directed to the development of synthetic analogues of GLP-1 resistant to DPP IV enzyme inactivation and DPP IV inhibitors. **Key word**: DIABETES MELLITUS, INCRETINS, DIPEPTIDYL PEPTIDASE

Diabetul zaharat (DZ) este definit ca fiind un grup de boli metabolice caracterizate prin hiperglicemie, datorată deficienţelor în procesul de insulino-secreţie controlat de pancreas, sau perturbărilor în a utiliza insulina endogenă, sau a ambelor (1).

Ţinând seama de factorii etiologici implicaţi, sunt recunoscute două forme de DZ, cel de tip 1 (insulino-dependent, DZ 1) caracterizat prin distrugerea celulelor beta pancreatice producătoare de insulină din insulele Langerhans şi cel de tip 2 (diabetul non - insulino dependent, DZ 2), rezultând o perturbare cronică a metabolismului, în principal, asociată cu rezistenţa la insulină în ţesuturile ţintă, producerea în exces de glucoză în ficat şi rezistenţa la insulină în muşchii scheletici, ficat şi în ţesutul adipos (1).

Terapia clasică a DZ 2 dispune de patru

categorii de agenți farmacologici: sulfoniluree şi glinide, care stimulează secreția de insulină în celule beta pancreatice; biguanide, care întârzie absorbția glucozei; tiazolidindione, care cresc efectele insulinei prin reducerea rezistenței în țesuturile periferice (ficat, muşchi); inhibitori ai alfa-glucozidazei, care întârzie absorbția glucozei şi reduc secreția postprandială de glucoză prin inhibarea glucozidazei, prezentă în celulele mucoasei intestinale.

Deşi eficient, tratamentul DZ tip 2 poate fi asociat cu efecte secundare (hipoglicemie, creştere în greutate, edeme, afectare renală, hepatică şi gastrointestinală, acidoză lactică, anemie, etc.) care deseori pun în pericol viața pacientului.

NOI ȚINTE ÎN TERAPIA DIABETULUI ZAHARAT

Cea mai nouă şi modernă opțiune terapeutică în tratamentul DZ tip 2, o constituie modulatorii *incretinelor* – analogii sintetici ai GLP-1 (glucagon-like-peptide-1) şi inhibitorii dipeptidil peptidazei IV (DPP-IV) (2).

Incretine. Sub denumirea de incretine, se cunosc doi hormoni endogeni cu structură peptidică - GLP-1 (glucagon-like-peptid-1) şi GIP (glucose-dependent insulinotropic peptide), care contribuie la homeostazia glucozei acționând asupra pancreasului, tractului gastro-intestinal, țesutului muscular şi creier.

Mecanismul, care stă la baza efectului incretinic, este reprezentat de eliberarea imediată din celulele intestinale, ca răspuns la ingestia alimentelor, a celor doi hormoni, care la rândul lor stimulează secreția de insulină în celulele beta pancreatice. Se apreciază că 50-60% din insulina totală secretată în timpul mesei se datorează celor doi hormoni (2).

Pe lângă stimularea secreției de insulină, GIP şi GLP-1 au şi alte efecte fiziologice: inhibă secreția de glucagon (hormon secretat de către pancreas care creşte concentrația glucozei în sânge) la nivelul celulelor alfa (GLP-1), creşte proliferarea şi reduce apoptoza celulelor beta (GLP-1, GIP), creşte saţietatea şi reduce greutatea corporală (GLP-1) şi întârzie golirea stomacului (GLP-1) (2).

Dipeptidil peptidazele (DPP) reprezintă o familie de serin proteaze, care scindează diferite peptide în proteine, la nivelul aminoacidului serină. Din această familie fac parte, DPP I-IV, FAP (fibroblast activation protein-α), DPP VIII şi DPP IX (3).

După secreție, GIP şi GLP-1 sunt rapid metabolizați de către DPP IV, timpul de injumătățire al acestora fiind de 5 minute (GIP) şi respectiv de 2 minute (GLP-1). Inactivarea se produce în proporție de peste 50% la nivelul capilarelor intestinale, aproape de locul de eliberare a celor doi hormoni, fapt care limitează utilizarea acestora în scop terapeutic (4).

În scopul obținerii unui control glicemic optim, folosind efectele benefice ale incretinelor, s-a reuşit dezvoltarea a două noi clase de agenți terapeutici (2): analogi sintetici ai GLP-1, rezistenți la inactivarea enzimatică produsă de DPP IV şi inhibitori ai DPP IV, care blocând enzima, prelungesc timpul de acțiune al peptidului, acționând fiziologic asupra secreției glucozo-dependentă de insulină şi glucagon.

Analogi sintetici ai GLP-1. Cercetările derulate în grupul analogilor sintetici ai GLP-1, au fost încununate de succes prin introducerea în terapeutică în aprilie 2005 a Exenatidei, un polipeptid constituit din 39 de aminoacizi (Byetta, sol. inj. 5μg, stilou injector). Acest medicament suprimă secreția de glucagon, creşte secreția de insulină într-o manieră glucozo-dependentă, întârzie

golirea stomacului şi reduce pofta de mâncare. Exenatida restabileşte secreţia de insulină şi activează proliferarea celulelor beta (2) (fig. 1).

Un alt analog sintetic al GLP-1, introdus în terapeutică în 2009, este Liraglutida (Victoza, sol. inj. 6 mg/ml, stilou injector). Acesta este derivatul palmitoil al GLP 1, cu acţiune de lungă durată, având un timp de înjumătăţire de 11-15 ore. Asemănător Exenatidei, Liraglutida reduce concentraţia plasmatică de glucoză prin stimularea activităţii celulelor beta pancreatice cu creşterea concentraţiei de insulină şi reduce activitatea celulelor alfa pancreatice cu scăderea concentraţiei de glucagon (2) (fig. 2).

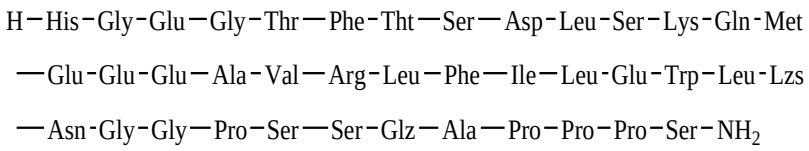

Fig. 1. Structura chimica a Exenatidei

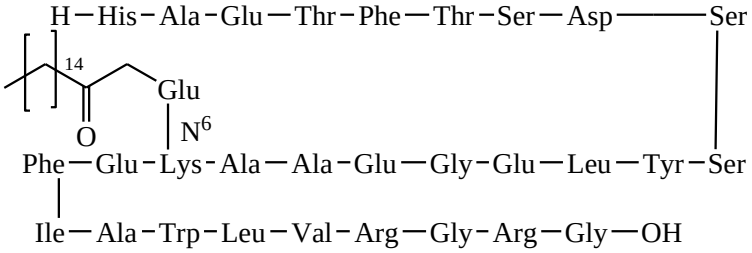

Fig. 2. Structura chimica a Liraglutidei

Inhibitori ai DPP IV. Primele cercetări în direcţia inhibitorilor dipeptidil peptidazei IV datează din 1996, când s-a confirmat *in vivo* acţiunea acestora de reducere a concentraţei de glucoză; studiile suscitând interese considerabile în industria farmaceutică (3).

Inhibitorii DPP IV suprimă degradarea unor peptide bioactive, incluzând GLP-1 şi GIP, ceea ce are drept rezultat creşterea duratei lor de acţiune (T1/2). În experimentele pe animale de laborator, inhibitorii DPP IV au redus activitatea enzimei cu până la 90%. Studiile clinice, au arătat că inhibitorii DPP IV reduc atât nivelul glucozei „*à jeun*" cât şi a glucozei postprandiale şi stimulează activitatea celulelor beta pancreatice (4).

Din punct de vedere structural inhibitorii DPP IV pot fi cu structură peptidică şi non-peptidică, iar la rândul lor inhibitorii peptidici, pot fi încadraţi în trei clase: inhibitori analogi reversibili (pirolidine, tiazolidine – P32/98, PSN 9301 (Prosidion) şi MK-432; inhibitori analogi acţionând covalent (cianopirolidine - NVP DPP 728, LAF 237 -Vildagliptina, BMS-477118 - Saxagliptina, ABT-279); şi inhibitori heterociclici non-peptidici reversibili (xantine, aminometilpirimidine) (4).

Dintre aceştia, terapeutica beneficiază deja de patru reprezentanţi, Sitagliptina fosfat aprobată de FDA (*Food and Drug Administration*) în octombrie 2006, Vilda-

gliptina ce a primit aprobarea EMA (*European Medicines Agency*) în septembrie 2006, Saxagliptina ce a primit aprobarea de punere pe piață pe 1 octombrie 2009 şi Linagliptina ce a primit aprobarea FDA în mai 2011 (fig. 3).

Alți inhibitori ai DPP IV se află în studii clinice avansate, printre aceştia numărându-se Alogliptina (aflată în faza III a trialului clinic), PF-00734200, LAF237 (Novartis Pharmaceuticals), P32/98 (Probiodrug şi Merk Company) aflat în faza I a trialului clinic, MK-0431 (Merck Company şi Bristol-Myers Squibb) intrat în faza III a trialului clinic în 2004 (3, 4) (fig. 3).

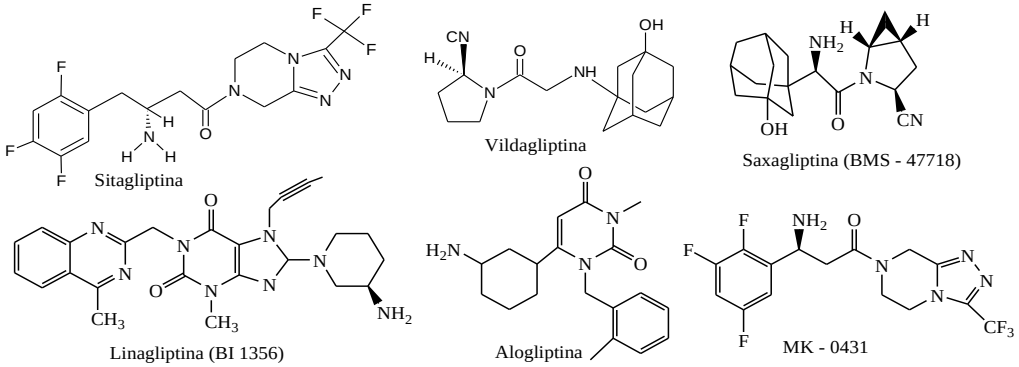

Fig. 3. Structura chimică a unor inhibitori ai DPP IV

Vildagliptina (Galvus, Novartis) este un inhibitor potent, selectiv şi competitiv al DPP IV, activ la administrare orală. Administrat pacienților cu DZ tip 2, în doză de 100 mg/mg, timp de 4 săptămîni, reduce semnificativ concentrația glucozei în sânge (cu 1.4 mmol/L postprandial) (5). Sitagliptina a fost aprobată de FDA ca monoterapie (Januvia, Merck™ & Co) şi în asociere cu Metformin (Janumet, Merck™ & Co) (6). Multe studii au arătat că atât sitagliptina cât şi vildagliptina au o siguranță ridicată şi sunt bine tolerate ca terapie combinată sau ca monoterapie, având o incidență scăzută a efectelor adverse incluzând: hipoglicemie, creştere în greutate, afecțiuni gastro intestinale.

Saxagliptina (Onglyza, Bristol Myers Squibb, Astra Zeneca) este un cianopirolidin inhibitor, cu acțiune de lungă durată şi cu stabilitate crescută comparativ cu Vildagliptina. Se administrează în doză de 2,5-100 mg/mg ca monoterapie sau ca terapie combinată în asociere cu metformin (7).

Cel mai recent inhibitor al DPP IV introdus în terapeutică este Linagliptina (Ondero, Boehringer Ingelheim International) ce este rezultatul unor intense cercetări, ce au avut drept obiectiv modulări structurale pe nucleul xantinic şi evaluarea acestora asupra capacității de inhibarea a activității enzimei DPP-IV. Linagliptina este un inhibitor nonpeptidomimetic cu acțiune de lungă durată, bine tolerat, sigur şi cu risc redus de hipoglicemie. La doze terapeutice (5 mg/zi), produce inhibarea activității DPP IV în proporție de 50%, fiind mai activă decât vildagliptina, sitagliptina şi saxagliptina (8).

CONCLUZII

Terapia cu antidiabetice orale cuprinde sulfoniluree, glinide, biguanide, tiazolidin-

dione şi inhibitori de alpha-glucozidază. În ciuda eficienței lor terapeutice, controlul glicemic al pacienților cu DZ tip 2, se deteriorează progresiv. Speranța bolnavilor de DZ tip 2 vine din partea modulatorilor incretinelor, şi îndeosebi a analogilor sintetici ai GLP-1 şi a inhibitorilor de DPP IV, ce au dovedit un profil farmacologic şi o siguranță terapeutică mai bună comparativ cu antidiabeticele orale existente.

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DISTRIBUȚIA GEOGRAFICĂ A VIRUSURILOR *PAPILLOMA* UMANE ŞI CORELAȚIILE ACESTEIA CU ATIPIILE CITOLOGICE DIN LEZIUNILE COLULUI UTERIN ŞI CU VÂRSTA LA O POPULAȚIE FEMININĂ DIN TRANSILVANIA

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GEOGRAPHICAL DISTRIBUTION OF HUMAN PAPILLOMAVIRUS AND THEIR CORRELATION WITH CYTOLOGIC ABNORMALITIES IN CERVICAL LESIONS AND WITH THE AGE IN A FEMALE POPULATION FROM TRANSILVANIA (Abstract): Cervical samples from 182 females aged 16-63 years were tested between 2008-2011 for human papillomaviruses (HPV) using PCR methods through the reverse hybridization and Roche genotyping. In 99 (54.4%) cases, different types of HPV were detected in 53 (53.5%) single infections and in 46 (46.5%) multiple infections, frequent in urban (94 cases: 50 single infections and 44 multiple infections). 32 different types of HPV were identified, 15 types being with high-risk. There were only seven rural patients, having single infections with the types 51, 58, 84; multiple infections with three or more than three associated HPV were not found. The most frequently identified was type 16 with high-risk, both in urban and rural areas, prevalent at 20-30 years of age. The types 35, 39, 59, 68 with high-risk were isolated only from multiple infections. Of the 9 females of rural origin investigated through cytological examination Babeş-Papanicolaou, the types 16, 31, 58 (with high-risk) were detected in five cases. 42 (42.4%) cases with HPV infections were found in females aged 20 to 25 years. The frequency of HPV infection decreases with the age. The mean of age is significantly increased in females without infection (p=0.00007) and it is significantly decreased in females with multiple infections, as compared with those with single infections or without infections. The females with single infections have the average of age significantly lower, as compared with the females free of HPV infections. There is a significant correlation between the age and the total risk of HPV infection (the age increase and the total risk decrease). **Key words:** HPV, PCR, AGE, CYTOLOGICAL EXAMINATION, URBAN, RURAL.

Virusurile Papilloma umane (HPV; familia *Papovaviridae*) constituie un grup foarte heterogen de virusuri ADN de talie mică (circa 55 nm). Se cunosc peste 130 de genotipuri de HPV. Rolul lor în patologie este recunoscut pentru câteva genotipuri (peste 40 identificate în tractul genital feminin) (1, 2). La om, HPV infectează celulele epiteliale ale pielii şi mucoaselor, determinând o gamă variată de leziuni proli-

ferative, nu şi litice (3, 4). După aspectul clinico-patologic şi structura genetică, HPV sunt împărţite în tipuri cu risc scăzut (HPV 6 şi 11 sunt cele mai comune), care produc condiloame acuminate, respectiv tipuri cu risc crescut (oncogene), HPV16 şi HPV18 fiind cele mai cunoscute ca asociate cu cancerul de col uterin, cauzând aproximativ 70% din cancerele cervicale în întrega lume (2, 4). Unele din virusurile specifice pentru mucoasa genitală, în special HPV 6 şi 11, pot infecta şi alte mucoase (tractul respirator, cavitatea bucală, conjunctiva) (2). Răspunsul imun faţă de HPV este specific de tip (5). Infecţiile cu mai multe tipuri de HPV par să acţioneze sinergic în carcinogeneza cervicală (6). Infecţia cu HPV este considerată cea mai frecventă infecţie cu transmitere sexuală (ITS) (2, 7), prevalenţa infecţiei cu HPV la adulţii tineri fiind de 20-50% (6). Cancerul cervical este a doua cauză frecventă de deces prin cancer la femeile din întreaga lume (8). Stabilirea prevalenţei infecţiei cu HPV în diferite tipuri de leziuni cervicale este deosebit de importantă, ca şi evidenţierea relaţiei dintre HPV şi gravitatea leziunilor cervicale (6). Vaccinarea poate reduce apariţia cancerului cervical cu 70% şi a neoplaziei intraepiteliale cervicale cu 50% (4).

MATERIAL ŞI METODE

În perioada 2008-2011 am examinat secreţii cervicale recoltate de către medici ginecologi de la paciente în vârstă de 16-63 de ani din mediul urban şi rural, efectuând genotiparea HPV şi examenul citologic Babeş-Papanicolaou (frotiuri colorate) la 39 de femei în vârstă de 16-60 de ani în perioada 2008-2009 şi 143 de femei cu vârsta de 19-63 de ani în perioada 2010-2011.

Pentru a identifica genotipul de HPV prezent în probe şi a ierarhiza riscul oncogen al tulpinilor HPV a fost utilizată tehnica PCR:

1. Tehnica PCR de hibridizare inversă (testul INNO-LiPA HPV Genotyping *Extra*– de la INNOGENETICS), utilizată în anul 2008. Testul permite decelarea a 28 de genotipuri de HPV (9);

2. Tehnica PCR (metoda de genotipare Roche – kitul Linear array HPV genotyping test), utilizată în perioada 2010-2011, care poate detecta 37 de tipuri de HPV (10).

Examenul citologic Babeş-Papanicolaou a fost interpretat în conformitate cu criteriile Sistemului Bethesda 2001 pentru evaluarea frotiurilor cervico-vaginale.

La prelucrarea statistică a rezultatelor am utilizat testul *t-Student* pentru compararea a două medii cu distribuţie de probabilitate normală, iar în cazul în care nu a existat o distribuţie normală a fost utilizat testul Mann-Whitney. Pentru a compara mai mult de două medii cu distribuţie de probabilitate normală a fost utilizat testul ANOVA. Pentru testarea distribuţiei normale am folosit testul Kolmogorov-Smirnov. În cazul în care nu a existat o distribuţie normală a fost utilizat testul Kruskal-Wallis. Pentru compararea a două frecvenţe a fost utilizat testul χ-pătrat sau Fisher-exact în cazul în care frecvenţele teoretice sunt sub 5. Analiza de corelaţie între variabile continue a fost efectuată prin calcularea coeficienţilor de determinare şi ecuaţia dreptei de regresie. Pragul de semnificaţie pentru testele folosite a fost luat p = 0,05. Calculele statistice au fost efectuate cu ajutorul aplicaţiilor *SPSS 13.0*.

REZULTATE

Examenul citologic Babeş-Papanicolaou a fost efectuat la nouă paciente; la patru (din mediul urban) nu a fost identificat nici

un tip de HPV, însă s-au constatat: modificări reactive (inflamație) la două femei de 34 şi 36 ani, la o femeie de 32 ani a fost evidențiat ASC-US (*Atypical Squamous Cells of Undetermined Significance*), atipii ale celulelor pavimentoase intermediare, iar la o femeie de 41 ani NILM, inflamație, foarte numeroase hematii. În celelalte cinci cazuri investigate prin examen citologic (patru din mediul urban şi unul din rural) au fost identificate: HPV tipul 16 la o femeie de 41 ani (care prezenta ASC-US) şi respectiv la o femeie de 23 ani (care prezenta ASC-H: *Atypical Squamous Cells "cannot exclude high-grade squamous intraepithelial lesion* (HSIL)", plus *Gardnerella vaginalis*), precum şi tipul 31 (asociat cu NILM, inflamație, la o femeie de 22 ani), tipul 6 (asociat cu tipul 31 la o femeie de 22 ani care prezenta NILM, inflamație marcată, numeroase hematii), precum şi tipul 58 (la o femeie de 63 ani din mediul rural cu NILM, atrofie, inflamație).

Din cele 182 de testări, 99 (54,4%) cazuri au fost pozitive pentru HPV, 53 (53,5%) fiind infecții unice şi 46 (46,5%) infecții multiple, detectate predominant în mediul urban (94 cazuri: 50 de infecții unice şi 44 de infecții multple). Numărul de testări la femeile din mediul rural a fost foarte mic (şapte cazuri): trei cazuri cu infecții unice cu HPV, două cazuri cu infecții multiple şi două cazuri fără HPV. Au fost identificate 32 tipuri de HPV: 15 tipuri cu risc crescut (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82), 14 tipuri cu risc scăzut (6, 11, 42, 54, 55, 61, 62, 70, 72, 74, 81, 83, 84, CP6108) şi 3 tipuri cu risc probabil crescut (26, 53, 66). Grupul filogenetic $\alpha 9$ este implicat în 64 cazuri cu HPV, $\alpha 3$ în 25 cazuri şi $\alpha 10$ în 24 cazuri.

Din cele 94 de infecții cu HPV detectate prin PCR *în mediul urban, 50 de infecții au fost unice* cauzate fiecare de un singur tip de HPV: 30 de infecții cauzate de HPV cu risc crescut (16, 52, 31, 33, 58, 45, 18, 82, 51, 56), 14 infecții produse de HPV cu risc scăzut (42, 6, 55, 70, 62, 83, 84, 81, CP6108), 4 infecții cu HPV 66 cu risc probabil crescut; pentru 2 infecții nu s-a putut preciza tipul de HPV. *În mediul rural* au fost detectate 5 cazuri cu HPV, din care 3 cazuri cu *infecții unice* (detectate în anul 2010: HPV tipul 51 la o pacientă de 39 ani, tipul 58 la o pacientă de 63 de ani şi tipul 84 la o pacientă de 24 de ani).

Din cele *46 de infecții multiple* cu HPV, *44 de cazuri* identificate *în mediul urban* au diverse asocieri de tipuri de HPV: 6 tipuri (1 caz la 36 ani cu tipurile 16, 26, 39, 42, 51, 59), 5 tipuri (4 cazuri; dintre HPV cu risc crescut, predomină tipurile 73, 31, 33), 4 tipuri (7 cazuri; dintre HPV cu risc crescut predomină tipurile 52, 31) sau 3 tipuri de HPV (8 cazuri; HPV cu risc crescut predominate sunt 52, 51, 16, 73), 2 tipuri de HPV (24 cazuri; predomină tipurile 16, 52 dintre HPV cu risc crescut). De la pacientele tinere au fost depistate în mediul urban asocieri de 5 tipuri de HPV la vârsta de 19, 21, 22, 23 de ani şi respectiv de 4 tipuri la vârsta de 20, 24, 26, 27 de ani. În mediul urban, au fost izolate tipurile 35, 39, 59, 68, 73 de HPV cu risc crescut, numai din infecții multiple.

Infecțiile multiple cu HPV detectate *în mediul rural* în anul 2010 au fost produse de tipul 16 asociat cu tipul 61 la o pacientă de 23 de ani şi de tipul 18 asociat cu CP 6108 la o pacientă de 20 de ani. În mediul rural nu s-au constatat infecții multiple cu trei sau mai mult de trei HPV asociate. Nu au fost depistate HPV care să fie prezente numai în mediul rural şi să nu fie depistate în mediul urban.

Numărul de infecţii cu HPV detectate a fost: două sub 20 ani (la 16 şi 19 ani), 55 între 20-30 de ani, 25 între 30-40 de ani, 15 între 40-50 de ani, 1 între 50-60 de ani, 1 > 60 de ani. Intervalul de vârstă cel mai frecvent afectat a fost cel cuprins între 20 şi 25 de ani (42 de cazuri pozitive = 42,4%; 10 cazuri la vârsta de 22 de ani şi numai în mediul urban). La 16-20 de ani au fost detectate doar 9 (9%) infecţii (2 unice şi 7 multiple). Tipurile de HPV frecvent implicate, mai ales în infecţii multiple, între 20 şi 30 de ani, se observă în tabelul I.

Numărul de virusuri izolate pe intervale de vârstă este prezentat în figura 1. În corelaţia dintre numărul de virusuri şi vârstă, coeficientul de corelaţie Spearman r = -0,34 şi p = 0,000002 (figura 1).

TABELUL I.

Tipuri de HPV identificate

Riscul HPV izolate de la cele 182 paciente	Tipuri de HPV izolate	Cazuri cu tipul de HPV izolat		Număr de cazuri cu HPV izolat între 20 şi 30 de ani
		Număr	Procent (%)	
HPV cu risc crescut	16 (α9)	25	13,7	16
	52 (α9)	16	8,7	10
	31 (α9)	11	6	8
	51 (α5)	10	5,4	6
HPV cu risc scăzut	6 (α10)	16	8,7	11
HPV cu risc probabil crescut	66 (α6)	10	5,4	7
	53 (α6)	9	4,9	4

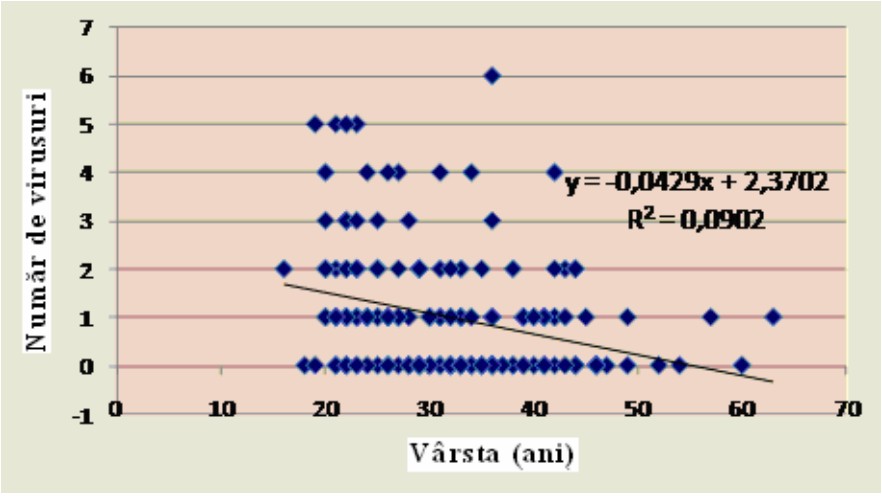

Fig. 1. Corelaţia dintre numărul de virusuri şi vârstă

La cele 182 de femei, media vârstei a fost de 31,84 de ani. Media vârstei este crescută semnificativ la femeile fără infecţie cu HPV (p = 0,00007) comparativ cu cele care au infecţie, dar este scăzută semnificativ la femeile cu infecţie multiplă comparativ cu cele care au infecţie unică şi comparativ cu cele fără infecţie (fig. 2).

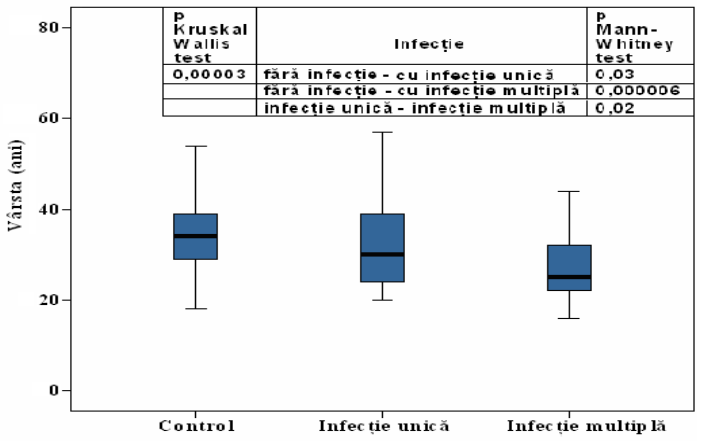

Fig. 2. Tipul de infecție pe intervale de vârstă

Se constată scăderea frecvenței infecției cu HPV odată cu înaintarea în vârstă. Scade frecvența HPV cu risc crescut odată cu înaintarea în vârstă, aspect observat mai ales la tipurile 16, 52, 31, 51, care – comparativ cu celelalte HPV cu risc crescut – sunt izolate în număr mai mare, din infecții unice şi multiple, în mediul urban, frecvent între 20-30 de ani.

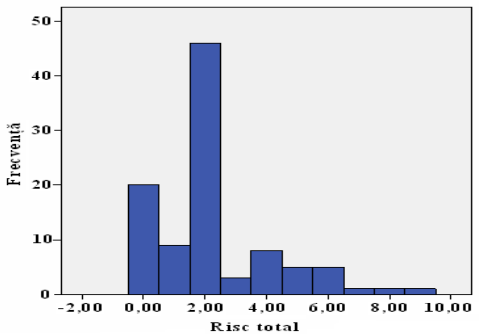

Fig. 3. Corelația dintre vârstă şi riscul total

Pentru a calcula **riscul total** se consideră: riscul scăzut (S) = 0, probabil crescut (PC) = 1 şi crescut (C) = 2 şi apoi se adună (se adună riscurile HPV din fiecare infecție multiplă). Corelând vârsta cu riscul total, coeficientul de corelație Spearman r = -0,26, p = 0,0005. Rezultă că există o corelație semnificativă între vârstă şi riscul total. Cu cât creşte vârsta, cu atât scade riscul total (fig. 3):

DISCUȚII

Pozitivitatea testărilor pentru HPV şi proporția infecțiilor unice şi multiple concordă cu alte cercetări din diverse zone ale lumii (11, 12, 13, 14, 15, 16). Tipul 16 de HPV cu risc crescut a fost cel mai frecvent depistat, prevalența mare a cazurilor din care se izolează acesta fiind semnalată şi de alți autori (13, 14, 15, 17, 18, 19, 20, 21). La fel ca şi în alte studii (18, 19, 20, 22), HPV 16 a fost implicat în infecții unice şi multiple, fiind izolat predominant între 20 şi 30 ani, din infecții multiple, în mediile urban şi rural, iar prevalența tipului 52 a fost de asemenea remarcată după tipul 16 (23,24).

Valori similare cu cea obținută pentru media vârstei celor 182 de paciente testate au fost constatate şi în alte cercetări (11, 25, 26), intervalul de vârstă cel mai frecvent afectat de infecția cu HPV fiind între 20 şi 25 de ani (10 cazuri la vârsta de 22 de ani, numai în mediul urban), aşa cum reiese şi din alte studii (27, 28). Scăderea semnificativă a mediei vârstei la femeile care prezintă infecție cu HPV față de cele fără

infecție, a fost evidențiată şi de alți autori (29, 30), chiar dacă unii nu găsesc o astfel de scădere semnificativă (31). S-a constatat scăderea frecvenței infecției cu HPV odată cu înaintarea în vârstă, la fel ca şi în alte determinări (19, 23, 32), mai ales scăderea cu vârsta a frecvenței infecției cu HPV cu risc crescut (13, 17, 20). Există studii care evidențiază o corelație pozitivă semnificativă a prevalenței HPV cu vârsta (33) sau care arată că prevalența HPV a fost foarte crescută la toate grupele de vârstă, fiind de 54,4% la 18-20 de ani (34) față de 9% în studiul prezent la 16-20 de ani. Şi alte cercetări (16, 17) arată că infecția cu HPV la femeile tinere este mai frecventă şi adesea sunt depistate mai multe tipuri de HPV asociate, aşa cum am constat şi noi (asocieri de 5 sau 4 tipuri, între 20 şi 30 de ani, în mediul urban). În mod cert şi semnificativ, frecvența mare a infecțiilor cu serotipuri multiple de HPV la populația feminină de vârstă tânără din mediul urban semnifică procese comportamentale simultane în cerc vicios: activitatea sexuală începută de la vârstă tânără, în paralel cu lipsa atât a unui program sistematic de educație sexuală oferit de la vârsta şcolară, cât şi a accesibilității la vaccinarea HPV oportună. Fără a fi vorba neapărat de prostituție, ITS denotă nu numai un nivel educativ şi comportamental deficitar, ci sunt markerul epidemiologic de alarmă pentru o populație confruntată cu carențe educaționale în context economic precar şi fără asistență familială şi medicală adecvate (35).

Chiar dacă în această lucrare examenul citologic Babeş-Papanicolaou a fost efectuat numai în 9 cazuri, în 5 cazuri au fost detectate HPV 16, 31, 58 cu risc crescut şi HPV 6 cu risc scăzut, unii autori (36) demonstrând că tipurile 16, 31, 33 sunt asociate cu un examen citologic cu anormalitate. Datele din literatură arată că sensibilitatea şi specificitatea screeningului de cancer cervical creşte prin combinarea citologiei cu hibridizarea (37), iar genotiparea HPV permite o clasificare mai bună a femeilor cu anormalități cervicale în diferite grupe de risc şi poate fi utilă în tratament (38). Se impune o aprofundare a acestui aspect.

CONCLUZII

Nu se cunoaşte suficient statusul infecției cu HPV la populația feminină activă sexual din mediul rural.

Frecvența mai mare a infecției cu HPV (unică sau multiplă) la vârsta tânără trădează lipsa educației sexuale la această grupă de populație şi a impactului ei asupra comportamentului sexual – mai probabil unul promiscuu.

Circulația tulpinilor HPV oncogene impune programul de imunoprofilaxie specifică prin vaccinare HPV.

Carențele de depistare a infecției cu HPV şi a entităților clinice specifice, mai ales în mediul rural, precum şi a accesibilității la imunizarea anti-HPV, au cel mai probabil motivație financiară, ceea ce trebuie să alarmeze decidenții din sistemul sanitar.

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ACTUALITĂȚI PRIVIND PREVALENȚA OBICEIURILOR VICIOASE LA COPII

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NEWS ON PREVALENCE OF VICIOUS HABITS IN CHILDREN (Abstract): **Aim:** The purpose of this study was to determine the prevalence of oral habits among children according to sex and location. **Material and methods:** This study was conducted on 416 children aged 6-11 years old. The children were selected from urban and rural schools of Bacau and Iasi, Romania. Sex and location differences were calculated by using Fisher's exact test. **Results :** The results showed that the prevalence of oral habits was 38,70%; girls (61,5%) were more affected than boys and also the children from urban schools 65,8%. Mouth breathing was the commenest habit (59,5%), followed by thumb thrust (21,5%) and finger sucking (14,1%). The presence or absence of a habit was recording by interview and clinical examination. **Conclusions:** The prevalence of vicious habits in children in our study was 38.70% - representative of the population prevalence of children aged 6-11 years in Romania **Key words:** ORAL HABITS, MOUTH BREATHING, DIGIT SUCKING

Aparatul dentomaxilar îndeplineşte funcţii esenţiale vieţii, în modul acesta se formează, se modelează şi se definitivează, în procesul complex al creşterii şi dezvoltării masivul maxilo- facial. Funcţiile principale ale aparatului dento-maxilar (respiraţia, masticaţia, deglutiţia, fonaţia) sunt asigurate prin mişcările ritmice ale mandibulei, limbii, buzelor, muşchilor mimicii, deprinse în cursul ontogenezei şi coordonate neuro-muscular şi în parte automatizate. Aceste funcţii la rândul lor stimulează dezvoltarea normală a maxilarelor şi modelează forma lor în vederea adaptării la condiţiile de mediu. Mişcările rezultate din funcţionalitatea aparatului dento-maxilar condiţionează în mod egal poziţia dinţiilor (1). Se consideră că direcţia de creştere a arcadelor dentare şi apofizelor alveolare se face într-un culoar muscular din care forţele izvorâte din acţiunea celor două grupuri musculare antagoniste (muşchii jugali şi muşchii orbiculari ai buzelor, situaţi extraoral şi muşchii limbii, situaţi intraoral), se echilibrează. Modificarea ritmului, secvenţei şi intensităţii contracţiei unor fascicule musculare în timpul exercitării funcţiilor aparatului dento-maxilar, creează un dezechilibru funcţional cu interferarea dezvoltării arcadelor dento-alveolare cu apariţia unor rezultante spre vestibular sau oral faţă de această linie a arcadelor. Respiraţia orală, tulburările masticatorii, deglutiţia atipică şi celelalte tulburări funcţionale sunt conside-

rate obiceiuri vicioase. Activităţi reflexe condiţionate patologice, generate şi întreţinute de cauze externe ce se exercită pe un teren dezechilibrat ereditar sau câştigat în cursul vieţii, obiceiurile vicioase sunt acte, gesturi însuşite spontan de subiect, practicate în mod consecvent, uneori pe o durată îndelungată a existenţei, cu o anumită frecvenţă, ritmicitate şi intensitate şi care, în perioada dezvoltării aparatului dento-maxilar – în special în corelaţie cu alţi factori pot genera sau agrava anomaliile dento-maxilare (2,3). Apariţia lor are loc la naştere, evoluând lent pe durata copilăriei, se menţin pe durate foarte variabile, dispărând în scurt timp sau persistând până la 16 ani, chiar mai mult iar când intervine voinţa, dispariţia lor se produce în mod conştient.

MATERIAL ŞI METODE

Studiul de faţă reprezintă o parte dintr-unul mai larg cu privire la modificările sistemului stomatognat, consecinţă a prezenţei obiceiurilor vicioase la copil. Studiul s-a realizat în judeţul Iaşi şi Bacău, incluzând un lot de 416 copii cu vârste de 6-11 ani, de ambele sexe, din mediul urban şi rural. Datele asupra subiecţilor au fost înregistrate de 3 examinatori, special instruiţi cu privire la culegerea datelor. Prezenţa sau absenţa obiceiului vicios s-a înregistrat prin anamneză şi examen clinic. Codurile folosite au fost: 1 - fără obicei vicios; 2 - respiraţie orală; 3 - deglutiţie atipică; 4 - sugerea degetului; 5 - alt obicei vicios. Pentru analiza informaţiilor s-a realizat un program de înregistrări în baza de date SQL, Microsoft Office Excel. S-a stabilit semnificaţia statistică pentru obiceiurile vicioase în relaţie cu sexul, (tab. I, fig. 1) mediul de proveniență (tab. II, fig. 2) şi de importanţa lor (tab. III., fig. 3).

TABELUL I

Distribuţia subiecţilor pe sex

Sexul	Nr.	%
Masculin	62	38.5
Feminin	99	61.5
Total	**161**	**100**

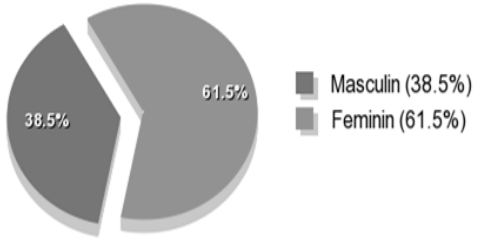

Fig. 1. Distribuţia pe sex a subiecţilor incluşi în studiu

TABELUL II

Distribuţia subiecţilor în relaţie cu mediul de proveniență

Mediul	Nr.	%
Urban	106	65.8
Rural	55	34.2
Total	**161**	**100**

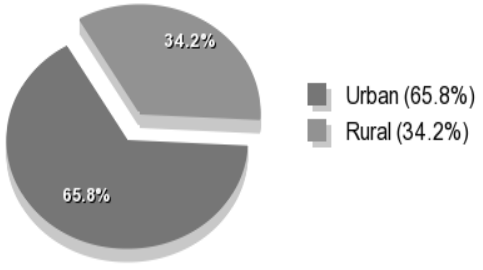

Fig. 2. Distribuţia subiecţilor în relaţie cu mediul de proveniență

TABELUL III

Prevalența cazurilor cu obiceiuri vicioase

Obiceiuri	Nr.	%
Respirație orală	122	59.5
Deglutiție atipică	44	21.5
Sugerea degetului	29	14.1
Alt obicei vicios	10	4.9
Total	**205**	**100**

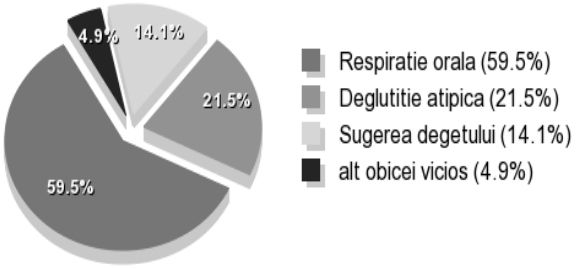

Fig. 3. Prevalența cazurilor cu obiceiuri vicioase

REZULTATE ŞI DISCUȚII

Valorile prevalenței cazueilor cu obiceiuri vicioase la copiii incluşi în studiul nostru au fost de 38,70% fiind reprezentative pentru copiii cu vârste între 6-11 ani din România (tab. IV). Astfel lotul de studiu a fost alcătuit din 161 de subiecți, fete şi băieți, ce prezentau unul sau mai multe obiceiuri vicioase. Fetele, atât din mediul urban cât şi rural au fost mai afectate decât băieții (tab. V). Obiceiul de a respira pe cale orală a fost cel mai frecvent întâlnit, atât în mediul rural cât şi urban. Respirația orală şi deglutiția atipică au fost semnalate mai frecvent la fete, sugerea degetului neavând o diferență semnificativă din punctul de vedere al sexului (tab. VI).

Rezultatele cercetărilor noastre sunt similare altor studii, realizate în Romania şi alte arii geografice (2,5,6). Unii autori (5,7) au constatat valori de prevalențe mai mari la băieți decât la fete în ceea ce priveşte respirația orală. Obiceiurile vicioase, găsindu-le în etiologia anomaliilor dento-maxilare într-o proporție foarte ridicată (54,5%), putem spune, deci, că sunt un factor important în producerea acestora. Combaterea lor poate determina o scădere de 30-40% a anomaliilor dento-maxilare. Obiceiurile vicioase rup echilibrul muscular şi tulbură creşterea oaselor, dând o nouă direcție de creştere acestora, în sensul tracțiunii şi presiunii, producând modificări de poziție a dinților, arcadelor şi oaselor maxilare. Acestea sunt însoțite însă, şi de tulburări fizionomice, cu consecințe în dezvoltarea psiho-intelectuală, creând copilului un complex de inferioritate, ce duce la schimbarea comportării în colectivitate (5, 6, 7).

TABELUL IV

Prevalenţa cazurilor cu obiceiuri vicioase în relaţie cu sexul

Total cazuri				Repartiţia pe sex							
Examinate		Cu obiceiuri vicioase		Examinate				Cu obiceiuri vicioase			
Nr.	%	Nr.	%	M		F		M		F	
				Nr.	%	Nr.	%	Nr.	%	Nr.	%
416	100	161	38,70	177	42,5	239	57,5	62	38,5	99	61,5

TABELUL V

Prevalenţa cazurilor cu obiceiuri vicioase repartizate pe sex şi mediul de proveniență

Subiecţi cu obiceiuri vicioase	Masculin		Feminin	
	Nr.	%	Nr.	%
Urban	38	35,8	68	64,2
Rural	24	43,6	31	56,4

TABELUL VI

Distribuţia pe sex a cazurilor cu obiceiuri vicioase

Obicei vicios	Masculin		Feminin	
	Nr.	%	Nr.	%
Respiraţie orală	49	40,2	73	59,8
Deglutiţie atipică	11	25	33	75
Sugerea degetului	14	48.3	15	51,7
Alt obicei vicios	6	60	4	40

CONCLUZII

Cercetările noastre au evidenţiat faptul că prevalenţa obiceiurilor vicioase la copii din lotul de studiu a fost de 38,70%, fiind reprezentativă pentru populaţia cu vârste între 6-11 ani din România. Fetele, atât din mediul urban cât şi din cel rural au fost mai afectate decât băieţii iar frecvenţa respiraţiei orale s-a situat pe primul loc între obiceiurile vicioase, atât în mediul rural cât şi urban. Respiraţia orală şi deglutiţia atipică au fost înregistrate mai frecvent la fete dar sugerea degetului nu a prezentat o diferenţă semnificativă a repartiţiei pe sex.

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STUDIU ASUPRA CONSUMULUI ASOCIAT AL DIFERITELOR TIPURI DE DROGURI DE CĂTRE ELEVII DE LICEU

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STUDY ABOUT THE ASSOCIATED USE OF DIFFERENT TYPES OF DRUGS BY HIGH SCHOOL STUDENTS (Abstract): Socially acceptable drugs and illegal substances consumed by high school students affect their nervous system, which is under development and anticipate an extensive drug use later. **Material and method**: In our study we have assessed the associate drug consumption at a group of high school teenagers by applying a questionnaire with 23 items. **Results and discussion:** Tobacco use is the lowest in ninth grade students and it is significantly better represented in higher grade students. Regardless of the grade, there are no significant differences in the proportion of boys and girls who smoke, even the health risks posed by smoking are known to over 80% of respondents. Among the reasons that could cause the student smoking cessation predominates, at the smallest students, parents' threat (significantly stronger than at those of senior grades), unlike the senior students, which give a higher value to a possible alteration of their own health status. The temptation for beer consumption appears before the age of 11 years and it is mentioned about a quarter of all respondents. Drinking a glass of wine for the first time is placed around the age of 14 years for boys and 15 years for girls. Marijuana is the drug exclusively consumed in the ninth grade and holds supremacy and other classes. Barbiturates and amphetamines are known about two thirds of the surveyed students. Risks involved by the association of alcohol consumption, tobacco and drugs are represented differently in this group of adolescents, and evolve differently as the students grow, mature and evolve, in a positive manner for their existence or not. **Conclusions:** during the high school years teenagers are tempted to diversify their drug consumption and to associate them, even they know very well how serious are the health consequences of this attitude. **Key words:** SMOKING, ALCOHOL, DRUGS, TEEN-AGERS, HIGH SCHOOL

Drogurile licite sau acceptate social (tutunul sau alcoolul) sau cele ilicite (substanţe sedative, stimulante, narcotice sau halucinogene) consumate de către elevii de liceu le afectează sistemul nervos, care este în proces de dezvoltare, şi anticipează o utilizare extensivă a drogurilor mai târziu (1, 2, 3).

Stilul de viaţă ce implică un consum regulat de droguri include nonconformismul faţă de valorile tradiţionale, trăsătură întâlnită în rândul majorităţii adolescenţilor din

ziua de azi. Relaţiile familiale dificile, lipsa intereselor educaţionale sau stările de alienare şi revoltă, precum şi experienţele emoţionale negative sunt factori ce determină consumul de droguri în rândul adolescenţilor (2, 3).

Studiile realizate până în prezent nu indică un anumit tip de personalitate asociat cu consumul de droguri. Adolescenţi sunt tentaţi să încerce drogurile dintr-o multitudine de motive, cum ar fi curiozitatea, dorinţa de a trăi o altă stare de conştiinţă, de a se integra într-un nou grup, de a evita durerea fizică sau orice altă problemă gravă, ori, pur şi simplu, pentru a "condimenta" un stil de viaţă devenit plictisitor (4, 5).

OBIECTIVE

În această lucrare ne-am propus să evaluăm comparativ, la diferite vârste (clase de liceu) cum percep elevii adolescenţi drogurile, atât cele acceptate social (tutunul şi alcoolul) cât şi substanţele psihotrope ilicite, dacă le consumă, pe care le consumă şi cu ce frecvenţă, cum le asociază, respectiv dacă sunt conştienţi de riscurile care le incumbă consumul pentru sănătatea lor.

MATERIAL ŞI METODĂ

În realizarea studiului am elaborat şi aplicat un chestionar cu 23 de itemi unui număr de 200 de elevi dintr-un liceu din zona urbană II (adolescenţi cu vârste cuprinse între 15 şi 19 ani, înmatriculaţi în clasele a IX-a, a X-a, a XI-a şi a XII-a).

Din fiecare an de studiu au fost selectate aleatoriu la începutul semestrului II din anul şcolar 2009-2010 câte două clase, însumând 50 de elevi, care au răspuns întrebărilor din chestionar în cadrul orelor de dirigenţie, în prezenţa unor persoane calificate.

Datele obţinute (care au un caracter strict de confidenţialitate, pentru a creşte acurateţea analizei) au fost prelucrate statistic utilizând programului *EpiInfo 3.5.1*, prin analiză univariată, folosind indicatorii statistici descriptivi.

Pentru determinarea semnificaţiei statistice între diferite frecvenţe au fost aplicate variantele testului χ^2 *Mantel-Haenszel* şi *Yates* (formula corectată).

REZULTATE ŞI DISCUŢII

Subiecţii incluşi în studiu sunt predominant băieţi comparativ cu fetele (la nivelul întregului lot raportul M:F fiind de 1,70:1). Cei mai mulţi dintre elevii incluşi în studiu (59%) provin din mediul rural şi doar 41% provin din mediul urban, cu o dezvoltare staturo-ponderală în limitele normale pentru vârsta lor (normoponderali, dezvoltaţi armonic).

Consumul de tutun

Consumul de tutun, sau fumatul, este cel mai redus la elevii de clasa a IX-a şi este semnificativ mai bine reprezentat în clasele a X-a (χ^2=4,54 şi p=0,0329), a XI-a (χ^2=6,3529 şi p=0,00117) şi a XII-a (χ^2=14,77 şi p=0,0001).

Indiferent de clasa în care se află 55% dintre elevi au fumat prima ţigară înainte de 15 ani (momentul intrării la liceu), respectiv sunt diferenţe semnificative între proporţia băieţilor şi cea a fetelor care fumează.

Dintre elevii chestionaţi, 16% dintre cei din clasa a IX-a fumează zilnic, respectiv sunt semnificativ mai mulţi cei care au acelaşi obicei, dar sunt în clasa a XI-a (χ^2=7,071 şi p=0,007) respectiv în clasa a XII-a (χ^2=20,33 şi p=0,000006).

În ceea ce priveşte motivaţia fumatului 32% dintre toţi elevii este, indiferent de clasă, menţionează curiozitatea sau lipsa unui motiv anume.

Referitor la riscurile pe care le presupune fumatul peste 80% dintre cei chestionaţi, indiferent de clasă, ştiu că duce la schimbarea aspectului/culorii dinţilor şi la diferite forme de cancer (laringian, esofagian, pulmonar).

Motivaţia care ar determina renunţarea la fumat, prezentată în figura 1, dobândeşte alte valenţe în timp, pe măsură ce adolescentul se maturizează. Astfel, dacă la elevul de clasa a IX-a ameninţarea părinţilor este semnificativ mai puternică (χ^2=7,04 şi p=0,0079) decât la cei de clasa a XI-a şi a XII-a, posibila alterare a propriei stări de sănătate este semnificativ mai importantă pentru cei din clasele mari (a XI-a şi a XII-a) (χ^2=6,35 şi p=0,001 respectiv χ^2=5,26 şi p=0,0217). De remarcat că preţul de cost al ţigărilor nu este un motiv de renunţare la acest obicei suficient de percutant la nici o vârstă.

Majoritatea elevilor chestionaţi asupra fumatului (între 88 şi 96%, cu o medie de 91,5%) admit că fumatul nu are nici un beneficiu real pentru consumatorul adolescent.

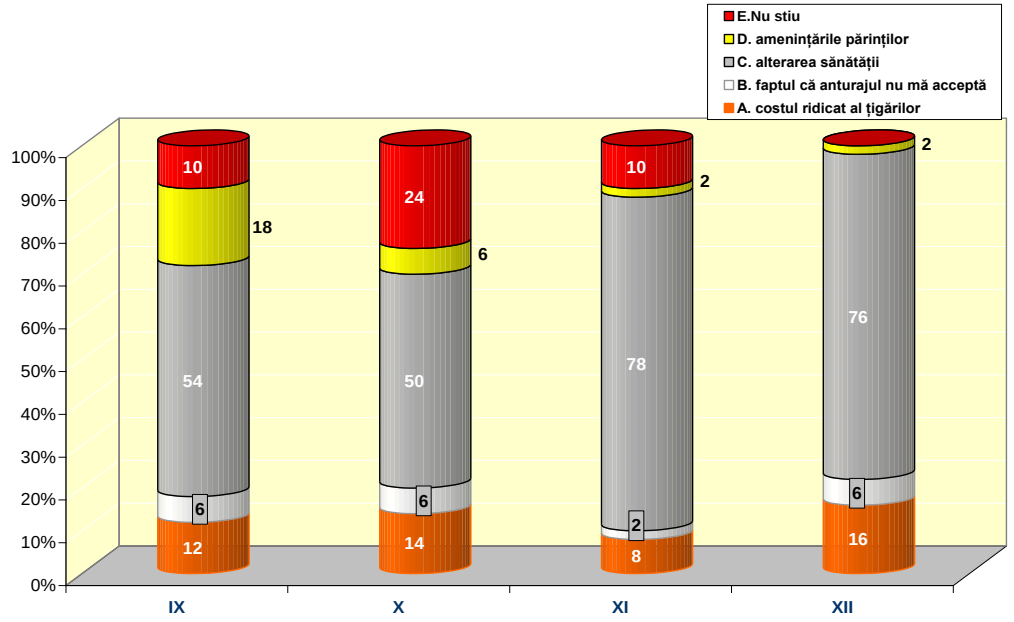

Fig. 1. Ce i-ar putea determina pe elevii de liceu să renunţe la fumat ?

Consumul de alcool

Dintre băuturile alcoolice preferate de către adolescenţi se remarcă berea. Astfel, 71,5% dintre toţi elevii incluşi în studiu au consumat maximum de două ori bere în ultima lună, fără să existe diferenţe între o clasă sau alta. Abuzul de bere este apanajul băieţilor, fără deosebiri legate de vârsta consumatorului.

O situaţie similară întâlnim şi în cazul vinului unde 72,5% dintre elevi au consumat de maximum două ori vin în ultima lună. Un aspect interesant este acela că toate fetele chestionate nu au consumat vin de mai mult de 5 ori în ultimele 30 de zile.

În ceea ce priveşte băuturile spirtoase se remarcă un aspect comun celor chestionaţi şi anume acela că 64,5% nu au consumat deloc astfel de băuturi în ultima lună şi doar 20% le-au consumat de 1-2 ori.

O consecinţă imediată dar şi cu posibile efecte pe termen mediu şi lung este starea de ebrietate. Cu cât aria de căutare este mai mare cu atât răspunsurile sunt mai puţin exacte, cele mai exacte (sincere) fiind răspunsurile elevilor din clasa a IX-a.

Un alt detaliu legat de consumul de băuturi alcoolice este „vârsta primului pahar". Analizând acest moment, dar în conexiune cu tăria alcoolică a băuturii (grade de alcool pur), putem remarca pentru bere tentaţia consumului precoce înainte de 11 ani la aproximativ un sfert dintre toţi cei chestionaţi.

Dacă la cei din clasele mediane nu sunt diferenţe între băieţi şi fete, la clasele extreme băieţii care au consumat bere înainte de 11 ani sunt de 2, respectiv de 12 ori, mai numeroşi comparativ cu fetele.

Consumul unui pahar de vin pentru prima dată mută accentul spre 14 ani la băieţi şi spre 15 ani la fete.

Băuturile spirtoase nu au fost (cel puţin declarativ) consumate de 48, 44, respectiv 30% dintre elevii cei mai mici şi doar de 14% dintre elevii mari ($\chi 2=13,37$ şi $p=0,0002$).

Băuturile alcoolice dulci nu au fost consumate de 40% dintre elevii cei mai mici şi doar de 14% dintre elevii mari ($\chi^2=8,48$ şi $p=0,003$).

Ultimul aspect analizat de chestionarul nostru privind consumul de alcool a fost „vârsta primei beţii". Proporţia celor care neagă că ar fi ajuns la starea de ebrietate scade, pe măsură ce cresc, de la 20% la 12% pentru băieţi şi de la 26% la 16% pentru fete. Evenimentul este plasat după vârsta de 15 ani pentru cei din clasa a IX-a şi peste 16 ani pentru ceilalţi. Jumătate dintre elevii din anul terminal recunosc că au ajuns în stare de ebrietate, băieţii fiind de 2,5 ori mai mulţi comparativ cu fetele.

Consumul de droguri

Ultima secţiune a chestionarului aplicat celor 200 de adolescenţi a fost dedicată consumului de droguri „ilicite" sau de substanţe psihotrope.

Consumul de droguri este cel mai redus la elevii de clasa a IX-a şi semnificativ mai bine reprezentat în clasele a X-a ($\chi^2=5,019$ şi $p=0,02$), a XI-a ($\chi^2=7,087$ şi $p=0,007$) şi a XII-a ($\chi^2=4,069$ şi $p=0,043$) (Fig. 2).

De asemenea, sunt diferenţe semnificative între proporţia băieţilor şi cea a fetelor care consumă droguri la clasa a X-a (de 3 ori mai mulţi) şi la clasa a XII-a (de 4,5 ori mai mulţi).

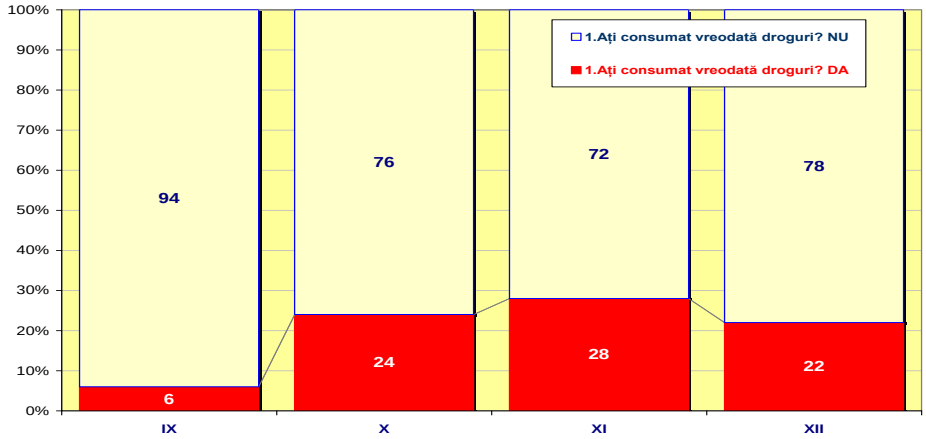

Fig. 2. Distribuţia elevilor din clasele de liceu în funcţie de consumul de droguri

Legat de tipul de drog care este preferat de fiecare clasă în parte, se observă că **marijuana** este drogul exclusiv pentru cei din clasa a IX-a care afirmă că sunt consumatori, dar deține supremația și la consumatorii din celelalte clase: 71,43% la clasa a X-a, 76,92% la clasa a XI-a și 58,34% la clasa a XII-a (Fig. 3).

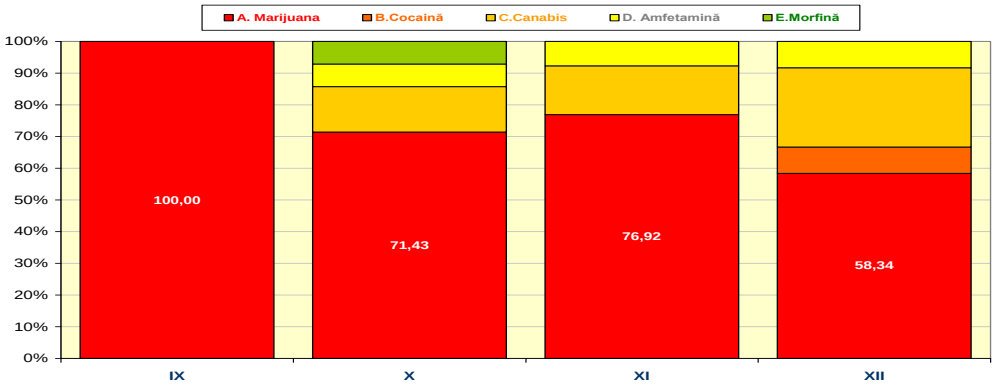

Fig. 3. Distribuția consumului diferitelor droguri în funcție de clasa în care se află elevii chestionați

Frecvența declarată a consumului indică pentru clasa a IX-a *doar o singură încercare* drept predominantă, pe când la clasele mai mari predomină *consumul ocazional*: 58,33% la clasa a X-a, 64,28% la clasa a XI-a și 81,81% la clasa a XII-a.

Motivația primului contact cu substanțele psihotrope și apoi repetarea experienței la cei chestionați constă în special în *generarea unei stări de bine* indiferent de vârstă: la 75% dintre elevii consumatori de clasa a IX-a; la 63,64% dintre elevii consumatori de clasa a X-a; la 92,30% dintre elevii consumatori de clasa a XI-a și la toți elevii consumatori de clasa a XII-a (Fig. 4).

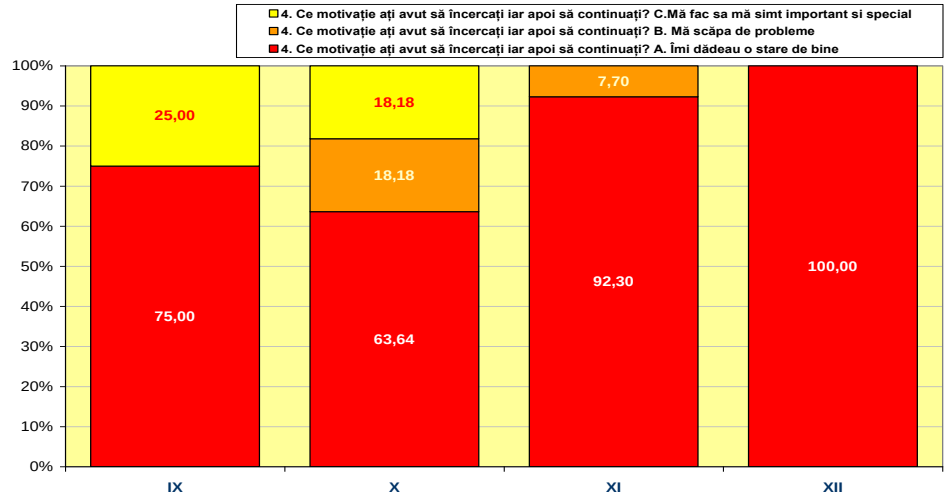

Fig. 4. Motivația consumului de substanțe psihotrope la elevii de liceu chestionați

Un alt detaliu extrem de interesant al investigaţiei noastre a fost reprezentat de nivelul de informare a elevilor din clasele de liceu despre posibilele efecte ale consumului de substanţe psihotrope asupra stării lor de sănătate:

- dependenţa fizică şi psihică este cunoscută de 58% dintre respondenţi, fără diferenţe semnificative între clase;
- tulburările psihice sunt cunoscute de 24,5% dintre respondenţi, fără diferenţe semnificative între clase;
- afectarea memoriei este cunoscută de 17,5% dintre respondenţi, fără diferenţe semnificative între clase;
- afectarea inimii este cunoscută de 13,5% dintre respondenţi, cu diferenţe semnificative între clasa a IX-a şi a X-a (χ^2=5,65 şi p=0,017)

Grila de tip asociativ, care a urmărit să scoată în evidenţă dacă elevii ştiu ce acţiune imediată au diferitele substanţe psihotrope, a fost completată cel mai corect de către elevii din:

- clasa a IX-a pentru amfetamine (70%) şi barbiturice (68%)
- clasa a X-a pentru barbiturice (62%) amfetamine şi marijuana (câte 56%)
- clasa a XI-a pentru barbiturice (64%) şi amfetamine (60%)
- clasa a XII-a pentru barbiturice (76%) şi cocaină (70%)

Acest fapt relevă existenţa unui nivel de informare şi/sau interes peste medie pentru barbiturice (indiferent de clasă), amfetamine (clasele a IX-a - a XI-a) şi cocaină doar la elevii din clasa a XII-a.

Elevii din clasele de liceu privesc diferit posibilitatea renunţării la consumul de droguri. Elevii de clasa a IX-a sunt semnificativ mai puţin convinşi că ar putea renunţa fără ajutor specializat comparativ cu elevii din clasa a X-a (χ^2=6,55 şi p=0,0104), a XI-a (χ^2=9,79 şi p=0,00175) şi a XII-a (χ^2=3,849 şi p=0,0484).

Asocierea riscurilor pe care le reprezintă consumul de alcool, tutun şi droguri este reprezentată diferit în acest grup de adolescenţi şi evoluează în diverse moduri, pe măsură ce elevii cresc şi se maturizează într-un sens pozitiv sau nu pentru existenţa lor viitoare.

Astfel, putem identifica 7 subgrupuri de elevi care consumă cu diferite frecvenţe:

- doar alcool (berea fiind elementul comun pentru toate clasele) (53%);
- alcool şi tutun (27,5%);
- alcool, tutun şi ocazional droguri (9,5%);
- alcool, tutun şi cel puţin o dată au încercat să consume şi droguri (3,5%);
- alcool şi ocazional droguri (3,0%);
- alcool, tutun şi zilnic droguri (2%);
- alcool şi cel puţin o dată au încercat să consume şi droguri (1,5%) (Fig. 5)

Prin aplicarea testului χ^2 am identificat următoarele aspecte:

- sunt semnificativ mai mulţi elevii care consumă doar alcool în clasa a IX-a comparativ cu clasa a XII-a (χ^2=20,19 şi p=0,0000007), tendinţa fiind de reducere a monoconsumului pe măsură ce adolescentul creşte.
- sunt semnificativ mai mulţi elevii care asociază alcoolul cu tutunul în clasa a XII-a comparativ cu clasa a IX-a (χ^2=7,821 şi p=0,0051).

La clasa a XII-a se observă o distribuţie extrem de „variată” comparativ cu clasele mai mici în ceea ce priveşte asocierile de droguri:

- 44% beau şi fumează;
- 36% doar beau;
- 12% beau, fumează şi ocazional se droghează;
- 4,0% beau, fumează şi au încercat cel puţin o dată drogurile;
- 4,0% beau şi ocazional se droghează.

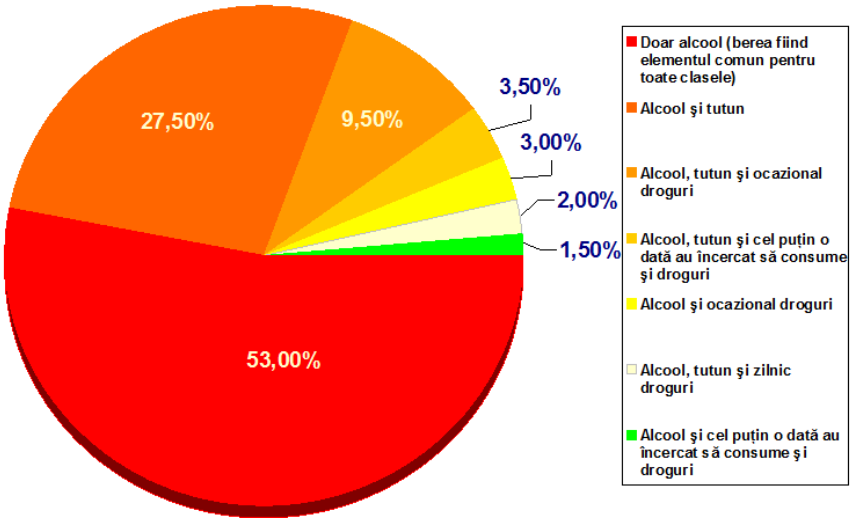

Fig. 5. Asocierea diferitelor tipuri de droguri la cei 200 de elevi chestionaţi

CONCLUZII

Consumul de tutun este cel mai redus la elevii de clasa a IX-a şi este semnificativ mai bine reprezentat la elevii din clasele mai mari.

Nu sunt diferenţe semnificative între proporţia băieţilor şi cea a fetelor care fumează, indiferent de clasă.

La peste o treime din toţi elevii, indiferent de clasă se remarcă pentru consumul de tutun o similitudine motivaţională, şi anume curiozitatea sau absenţa unei motivaţii.

Riscurile pe care le presupune fumatul (schimbarea aspectului/culorii dinţilor şi cel de apariţie a neoplaziei) sunt cunoscute de peste 80% dintre elevii chestionaţi.

Dintre motivele care ar putea determina renunţarea la fumat predomină la elevul de clasa a IX-a ameninţarea părinţilor (semnificativ mai puternică decât la cei de clasa a XI-a şi a XII-a), spre deosebire de elevii mai mari, care valorizează mai mult o posibilă alterare a propriei lor stări de sănătate.

Preţul de cost al ţigărilor nu este un motiv suficient de percutant pentru a determina adolescentul să renunţe la fumat la nici o vârstă, între 14 şi 19 ani.

Majoritatea elevilor chestionaţi asupra fumatului (91,5%) sunt de acord că fumatul nu aduce nici un beneficiu real consumatorului adolescent.

Alcoolul, considerat drogul social cel mai acceptat, este şi preferatul adolescenţilor mai ales dacă vorbim de bere (cea mai agreată) sau de vin şi mai puţin de băuturile spirtoase cu tărie alcoolică peste 40º.

În privinţa debutului consumului de alcool, corelat cu tăria alcoolică a băuturii (grade de alcool pur), putem remarca la aproximativ un sfert dintre toţi cei chestionaţi tentaţia consumului precoce (înainte de 11 ani) de bere.

La clasele extreme băieţii care au consumat bere înainte de 11 ani sunt de două, respectiv de 12 ori, mai numeroşi comparativ cu fetele. Referitor la acest aspect la elevii din clasele a X-a şi a XI-a, între băieţi şi fete nu sunt diferenţe semnificative statistic.

Consumul pentru prima dată al unui pahar de vin este identificat în jurul vârstei de 14 ani la băieţi şi spre 15 ani la fete.

Marijuana este drogul exclusiv folosit de către consumatorii de clasa a IX-a, dar care deţine supremaţia şi la celelalte clase.

Aproximativ 2/3 dintre elevii chestionaţi au informaţii corecte despre ce sunt barbituricele şi amfetaminele şi ce efecte imediate produc consumul lor.

Consecinţele pe termen mediu şi lung cunoscute de cei mai mulţi elevi (58%) sunt dependenţa fizică şi cea psihică.

Asocierea consumului de alcool, tutun şi droguri este un proces dinamic, tendinţa fiind de reducere a monoconsumului pe măsură ce elevii cresc şi se maturizează.

Deşi chestionarul a fost confidenţial se poate observa o nuanţă fină de neîncredere la subiecţii intervievaţi, când se abordează probleme cum ar fi starea de ebrietate şi, mai ales, consumul de droguri ilicite, tentaţia fiind de mistificare a realităţii.

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NOUTĂȚI

MANAGEMENTUL CONSERVATOR AL TRAUMATISMELOR CONTUZE HEPATICE: CARE ESTE DURATA OPTIMĂ A SPITALIZĂRII?

Atitudinea terapeutică curentă pentru traumatismele contuze hepatice la pacienţi stabili hemodinamic este non-chirurgicală. Cu toate acestea, durata optimă a spitalizării pentru aceşti pacienţi nu este încă stabilită. Într-un centru de traumatologie la care externarea pacienţilor cu traumatisme contuze hepatice a fost hotărâtă exclusiv pe baza criteriilor clinice au fost analizate retrospectiv registrele de trauma. Datele obţinute au inclus informaţii despre severitatea leziunilor, gradul leziunii hepatice, durata spitalizării, rata de succes a tratamentului conservator şi, acolo unde a fost necesar, motivul modificării atitudinii terapeutice de la conservatoare la chirurgicală. Între 2002 şi 2007, 591 de pacienţi au fost internaţi consecutiv unui traumatism contuz hepatic; în 35 de cazuri atitudinea a fost chirurgicală, în special consecutiv hemoragiei datorate leziunilor organelor vecine. Durata medie de spitalizare pentru restul cazurilor a fost de 2,2 zile. Nu s-au înregistrat consecinţe nefaste consecutiv externării pe baza criteriilor clinice. Concluziile studiului indică utilitatea examinării clinice în evaluarea duratei de spitalizare a traumatismelor contuze hepatice; autorii susţin că acestea ar trebui să fie criteriu unic în condiţiile unui examen abdominal normal la un pacient hemodinamic stabil, indiferent de gravitatea traumatismului. (Parks NA, Davis JW, Forman D, Lemaster D. Observation for nonoperative management of blunt liver injuries: how long is long enough? *J Trauma*. 2011 Mar; 70(3):626-9)

Teodora Alexa

INFLUENȚA OBICEIURILOR VICIOASE ASUPRA DEZVOLTĂRII APARATULUI DENTO-MAXILAR

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INFLUENCE OF VICIOUS ORAL HABITS IN THE DEVELOPMENT OF DENTO-MAXILLARY SISTEM (Abstract): Main features of the dentomaxillary sistem (breathing, chewing, swallowing) are provided by the rhythmic movements of the mandible, tongue, lips, mimic muscles, learned during ontogenesis and coordinated neuromusculary and largely automated. **Aim:** The aim of our study was to prove the role of oral habits in the development of dentomaxillary anomalies. We made experimental investigations - model studies , fotostatic test for face and side face , radiological examinations, clinical general and ENT exam for accurately assess of the factors which contribute to the emergence and development of dento – maxillary anomalies. **Material and methods:** The study group consisted of 175 children (94 girls and 81 boys) aged between 7 and 14 years. We made individual sheets for recording neuromuscular and oropharyngeal balance starting from a patient's overall clinical appearance. **Results:** The diagnosis of most teenagers was first Angle's class of malocclusion with tongue thrusting and mouth breathing. Oral habits disrupt the muscular balance and bone growth producing changes in the dental arch and occlusal characteristics. The high prevalence of dentomaxillary anomalies (52.57%) and obvious interrelationship with functional imbalances of the dentomaxillary sistem (62.82% of subjects examined) supports the implication of functional factors in those etiology .**Conclusions:** Suppression of vicious habits by methods of awareness, myogymnastic and functional reducing exercises are the first steps of early functional treatment for dentomaxillary anomalies **Key words**: TONGUE THRUSTING, MOUTH BREATHING, ANGLE MALOCCLUSION

Funcţiile principale ale aparatului dento–maxilar (respiraţia, masticaţia, deglutiţia) sunt asigurate prin mişcările ritmice ale mandibulei, limbii, buzelor, muşchilor mimicii, deprinse în cursul ontogenezei şi coordonate neuro – muscular şi în mare parte automatizate.

Aceste funcţii, la rândul lor, stimulează dezvoltarea normală a maxilarelor şi modelează forma lor în vederea adaptării la condiţiile de mediu. Mişcările rezultate din funcţionabilitatea aparatului dento – maxilar condiţionează în mod egal morfologia şi poziţia dinţilor (1)

Se consideră că direcţia de creştere a arcadelor şi apofizelor alveolare se face într-un culoar muscular în care forţele izvorâte din acţiunea celor două grupe musculare antagoniste (muşchii jugali şi orbiculari ai buzelor - extraoral şi de muşchii limbii situaţi intraoral) se echilibrează.

Modificarea ritmului, secvenţei şi intensităţii contracţiei unor fascicule musculare în timpul exercitării funcţiilor aparatului

dento – maxilar creează un dezechilibru funcţional cu afectarea dezvoltării arcadelor alveolo – dentare cu apariţia unor rezultante spre vestibular sau oral faţă de această linie a arcadelor.(2)

Respiraţia orală, tulburările masticatorii, deglutiţia atipică şi celelate tulburări funcţionale sunt considerate obiceiuri vicioase. În afara acestor obiceiuri vicioase provenite din desfaşurarea anormală a unor stereotipe funcţionale, există o grupă aparte de obiceiuri vicioase propriu-zise determinate de exercitarea unor atitudini posturale (dormitul cu capul în poziţii anormale sau compresiunea anormală pe maxilare în diferite faze de activitate) sau de exercitarea unor obiceiuri vicioase (de sugere, muşcare, roadere).

Obiceiurile vicioase rup echilibrul muscular şi tulbură creşterea oaselor, dând o nouă direcţie de creştere a acestora, în sensul tracţiunii şi presiunii, producând modificări de poziţie a dinţilor, arcadelor şi oaselor maxilare.(3)

MATERIAL ŞI METODĂ

Lotul de studiu a fost alcătuit din 175 copii (94 de fete şi 81 de băieţi) cu vârste cuprinse între 7 şi 14 ani.

Pentru evaluarea exactă a factorilor care concură la apariţia şi evoluţia anomaliilor dento – maxilare, după realizarea examenului clinic individual şi punerea unui diagnostic prezumtiv, s-a apelat la o serie de investigaţii experimentale: studii de model, examen fotostatic de faţă şi profil, examene radiologice, examen clinic general şi ORL.

S-au întocmit fişe individuale pentru consemnarea bilanţului neuro – muscular, oro – faringian pornind de la aspectul clinic general al pacientului, modul de desfpşurare a funcţiilor aparatului dento – maxilar, evidenţierea disfuncţiilor şi obiceiurilor vicioase, continuând cu examenul musculaturii jugale, buzelor şi limbii.

Distribuţia subiecţilor cu anomalii dento – maxilare şi obiceiuri vicioase, precum şi interpretarea şi raportarea datelor s-au facut pe clase de vârstă şi sex. Ţinânad cont de multitudinea de forme clinice de anomalii dento – maxilare şi de tipuri de clasificare, pentru studiul nostru am folosit clasificarea ocluzionistă Angle, clasificare curent utilizată de şcolile de ortodonţie în cercetare şi practică.

REZULTATE ŞI DISCUŢII

Din analiza statusului anomaliilor dento – maxilare rezultă că jumatate din populaţia examinată (52,57%) prezintă anomalii, din care; 41,14% malocluzii Clasa I Angle; la diferenţă de aproximativ 30 de procente; 5,14% subiecţi cu malocluzii Clasa II_1 şi 6,28% subiecţi cu malocluzii Clasa II_2.

Analiza pe clase de sex şi vârstă arată un procentaj mai mare de fete cu anomalii decât de baieţi la toate grupele de vârstă cu excepţia ultimei grupe (12 – 13 ani). Repartiţia subiecţilor pe clase de anomalii arată o situaţie inversă, cu valori mai mari la băieţi decât la fete în majoritatea grupelor de vârstă pentru anomaliile de Clasa I Angle şi primele două grupe de vârstă pentru Clasa II_2 Angle. Procentajul de subiecţi afectaţi de una sau mai multe obiceiuri vicioase se situează la valori medii foarte ridicate (62,85%) din care 47,42% sunt la originea malocluziilor Clasa I Angle si 15,42% a malocluziilor Clasa II_1 Angle (tab. I).

Analiza tipurilor de obiceiuri vicioase arată o pondere a subiecţilor cu deglutiţie atipică (45,14%) şi la fete (57,44% fete faţă de 30,86% la băieţi) urmată la diferentă de 30 de procente de subiecţi cu respiraţie orală (16,57%) la valori apropiate fete şi băieţi (fig. 1,2,3).

TABELUL I

Distribuția subiecților după tipurile de obiceiuri vicioase pe grupe de vârstă, sex şi clase de anomalii Angle

		Clasa Angle		Clasa I	Clasa II_1		$I + II_1$			
Grupa de vârstă	**Sex**	**Respirație orală**	**Deglutiție atipică**	**Mușcarea buzei**	**Respirație orală**	**Deglutiție atipică**	**R**	**D**	**M**	**T**
7-8 ani	F	11.11	33.33							
	B	12.5	12.5							
	T	11.76	23.52	0	0	0				
8-9 ani	F	53.84	100	15.38						
	B	62.5	75	0						
	T	58.62	86.20	6.89	0	0				
9-10 ani	F	8.69	30.43		28.08	69.56				
	B	9.09	36.36		0	0				
	T	8.88	33.33	0	13.33	35.55				
10-11 ani	F					33.33				
	B					0				
	T	0	0	0	0	18.51				
11-12 ani	F		0							
	B		33.33							
	T	0	20	0	0	0				
12-13 ani	F	11.53	38.46							
	B	0	0							
	T	8.10	27.02	0	0	0				
Total	F	10.63	35.10	2.12	6.38	22.34	17.02	57.44	2.12	76.59
	B	16.04	30.86	0	0	0	16.04	30.86	0	46.91
	T	13.14	33.14	1.14	3.42	12	16.57	45.14	1.4	62.85
		47.42%		**15.42**						

Fig. 1. Deglutiție atipică

Fig. 2. Deglutiție atipică

Fig. 3. Respirator oral

Fig. 4. Obicei vicios de muşcare a buzei inferioare

Cele mai mari valori se inregistrează la clasa de vârstă 8 – 9 ani când, alături de cele două disfuncţii enunţate se evidenţiază şi obiceiul vicios de muşcare a buzei inferioare. (fig. 4)

Distribuţia tipurilor de disfuncţii pe clasă de anomalie şi grupă de vârstă arată că doar doua grupe de vârstă (9 – 10 ani şi 10 – 11 ani) se înscriu în Clasa I şi II1 Angle, celelalte înscriindu-se în Clasa I Angle.

După cum afirmă Moss, mişcările rezultate din funcţionalitatea aparatului dento – maxilar condiţionează în mod egal morfologia maxilarelor şi poziţia dinţilor.Cum zona incisivă este cea mai vulnerabilă la acţiunea factorilor disfuncţionali, ea pune în evidenţă primele semne de anomalie în pierderea echilibrului muscular endobucal şi exobucal. Este influenţată direcţia de implantare a dinţilor şi de creştere a proceselor alveolare, ele fiind deviate sagital (grade diferite de inocluzie sau proalveolodenţie sau/şi vertical (supraocluzie sau inocluzie verticală)

Distribuţia subiecţilor afectaţi după modificarea zonei frontale (semne clinice) arată o pondere a modificărilor sagitale exprimate prin gradul de inocluzie: 43,42% subiecţi cu overjet redus (2 – 4 mm), 8% subiecţi cu overjet mediu (4 – 6 mm) şi 1,71 % subiecţi cu overjet mai mare de 6 mm. (tab. II)

TABELUL II

Distribuţia subiecţilor (%) după semne clinice şi tipuri de anomalii

Anomalii dento – maxilare	**Clasa I**			**Clasa II_1**			**Clasa II_2**		
	F	B	T	F	B	T	F	B	T
Overjet 2-4 mm	51.06	23.45	38.3	7.44	2.46	5.14	58.5	25.9	43.42
Overjet 4-6 mm	3.19	9.87	6.28	3.19	0	1.7	6.38	8.87	8
Overjet >6 mm	-	-	-	0	3.7	1.7	3.19	7.4	5.14
Bipro Alveolo denţie	0	3.7	1.7	0	7.4	3.42	3.19	7.4	5.14
Inocluzie verticală	7.44	11.11	9.14	6.38	2.46	4.57	13.8	13.6	13.7

În ordine cronologică inocluzia verticală (13,7%) urmează la diferenţă remarcabilă (de aproximativ 30 de procente) imediat după inocluzia sagitală uşoară.

Încadrarea semnelor clinice în clasa de anomalie Angle arată că 55,42% dintre subiecţi aparţin Clasei I Angle şi doar 12,57% Clasei II1 Angle unde se semnalează de altfel prin overjet-ul mai mare de 6 mm anomaliile scheletale.

CONCLUZII

Forme clinice variate de anomalii dento – maxilare caracteristice fiecărei etape de vârstă se datorează asocierii diferitelor obiceiuri vicioase şi intensităţii sau duratei acestor tulburări funcţionale.

Datele prelucrate pe parcursul studiului nostru confirmă datele din literatura, interrelaţia obiceiuri vicioase cu anomaliile dento – maxilare fiind susţinută de preva-

lenţa crescută a obiceiurilor vicioase şi a anomaliilor dento – maxilare de cauză disfuncţională.(4, 5)

Prevalenţa crescută a anomaliilor dento – maxilare (52,57%) precum şi interrelaţia evidentă cu dezechilibrele funcţionale ale aparatului dento – maxilar (62,82% din subiecţii examinaţi susţin implicaţia factorilor funcţionali în etiologia anomaliilor dento – maxilare.

Suprimarea obiceiurilor vicioase prin metode de conştientizare, miogimnastică şi reducere funcţională reprezintă aspecte de intervenţie ale tratamentului funcţional asupra formelor incipiente sau deja constituite de anomalii dento – maxilare.(6)

Dispensarizarea activă a colectivelor de copii preşcolari şi şcolari este forma ideală de combatere şi interceptare a disfuncţiilor şi obiceiurilor vicioase şi, prin urmare, verificarea reală în masă a conceptului de anomalie dento – maxilară.

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OBŢINEREA ACIDULUI SUCCINIC PRIN FERMENTAŢIE CU STRAT MOBIL DE *ACTINOBACILLUS SUCCININOGENES*

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SUCCINIC ACID PRODUCTION USING MOBILE BED OF IMMOBILIZED *ACTINOBACILLUS SUCCINOGENES* IN ALGINATE (Abstract) : This work investigates the production of succinic acid by immobilized *A. succinogenes* cells, using a bioreactor with stirred/mobile bed of biocatalysts. **Material and method** : The experiments have been carried out for different sizes of biocatalysts particle, under substrate and product inhibitory effects. **Results** : The results indicated that the inhibitory effects could be diminished by cells immobilization, the substrate consumption rate increasing with the increase of biocatalyst particle size. Moreover, the biocatalysts can be used for many fermentation cycles. **Key words :** *ACTINOBACILLUS SUCCINOGENES*, FERMENTATION, SUBSTRATE INHIBITION, SUCCINIC ACID

Acidul succinic, numit şi acid butandioic, este un acid dicarboxilic cu numeroase aplicaţii in industria chimică (reactivi, polimeri biodegradabili, solvenţi verzi, cerneluri), în agricultură (pesticide, factori de creştere, stimulatori), în industria farmaceutică şi alimentară (aminoacizi, antibiotice, surfactanţi, vitamine şi aditivi) (1,2,3,4).

In prezent, la nivel industrial, acidul succinic se obţine utilizând drept materie primă butanul din gazul petrolier lichefiat, prin intermediul anhidridei maleice. Costul obţinerii prin sinteză a acestui acid variază între 5,9 - 9 $/kg, în funcţie de puritate, aportul materiei prime la acesta fiind de 1,03 $/kg acid succinic. În afara dificultăţii etapelor de separare şi purificare a acestui acid, utilizarea tehnologiilor de sinteză chimică creează probleme serioase privind protecţia mediului ambiant (4). Din acest motiv, a crescut interesul pentru obţinerea acidului succinic pe cale fermentativă, cu costuri reduse, conform conceptului de „biotehnologie albă". Acesta a fost introdus cu ocazia Conferinţei Internaţionale "European Bioperspectives - En Route to a Knowledge-Based Bio-Economy" (Cologne, Germania, 31 mai – 1 iunie 2007), şi susţine prioritatea folosirii de materiale regenerabile pentru producţia de diverşi compuşi chimici prin procese biotehnologice ieftine ce protejează mediul înconjurător (5). Prin prisma acestor precepte, au fost studiate numeroase microorganisme cu potenţial producător de acid succinic raportate în literatură : bacterii (*Veillonella parvula, Selenomonas ruminatum*, *Succiniclasticus ruminis*, *Corynebacterium glutamicum, Enterococcus fecalis*, *Actinobacillus succinogenes*, *Actinobacillus succiniproducens*, *Mannheimia succiniproducens*, *Escherichia coli*), drojdii (*Saccharomyces cerevisiae*) şi fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Byssochlamys nivea*, *Lentinus degener*, *Paecilo-*

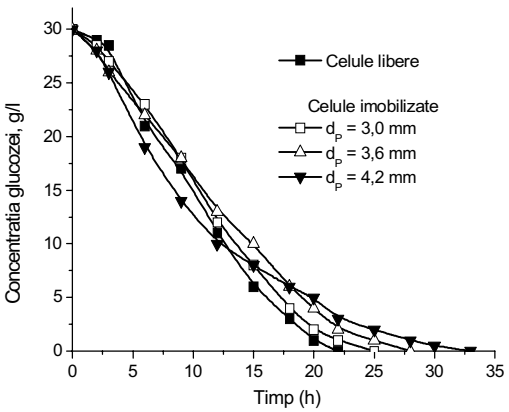

Fig. 1. Consumul glucozei pentru *A. succinogenes* liberă şi imobilizată, ciclul I de fermentaţie

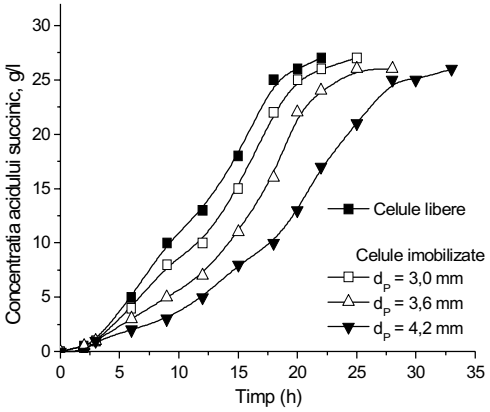

Fig. 2. Variaţia concentraţiei acidului succinic pentru *A. succinogenes* liberă şi imobilizată, ciclul I de fermentaţie

myces varioti, *Penicillium viniferum*) (6).

Majoritatea sistemelor de fermentaţie pentru obţinerea acidului succinic au utilizat celule libere de *Actinobacillus succinogenes*, procesul putând fi afectat de fenomene de inhibiţie de substrat şi produs (7,8).

Utilizarea microorganismelor sau a enzimelor imobilizate oferă o serie de avantaje, precum rezistenţă crescută la forţele de forfecare, rezistenţă chimică şi termică ridicată, diminuarea sau evitarea proceselor de inhibiţie, recuperarea uşoară a biocatalizatorilor din mediul de fermentaţie şi creşterea numărului de cicluri repetate de fermentaţie utilizând aceiaşi biocatalizatori (9).

În acest context, prin experimentele din această lucrare s-a realizat o analiză comparativă a obţinerii acidului succinic cu ajutorul celulelor libere şi celulelor imobilizate în alginat, în strat mobil. De asemenea, pentru sistemul cu celule bacteriene imobilizate şi în condiţii de inhibiţie de substrat şi de produs, au fost investigate transferul de masă al substratului în exteriorul şi în interiorul granulei de biocatalizator, evidenţiindu-se implicarea difuziei interne în desfăşurarea acestui proces.

MATERIAL ŞI METODĂ

Experimentele s-au realizat în bioreactoare anaerobe cu capacitatea de 100 mL (80 mL volum util) simultan, fiecare conţinând 54 mL mediu cu următoarea compoziţie (pe litru): glucoza 30 g; extract de drojdie 5 g; $NaH_2PO_4 \cdot H_2O$ 1,16 g; Na_2HPO_4 0,31 g; NaCl 1,0 g; $MgCl_2 \cdot 6H_2O$ 0,2 g; $CaCl_2 \cdot 2H_2O$ 0,2 g; vitamină B_{12} 1 μg; biotină 20 μg; acid folic 20 μg; tiamină 50 μg; riboflavină 50 μg; niacină 50 μg; pantotenat 50 μg; p-aminobenzoat 50 μg; acid lipoic 50 μg; vitamină B_6 100 μg; $MgCO_3$ 10 g; antispumant siliconic 1 ml. Bioreactoarele au fost plasate pe un agitator rotativ la 100 rpm şi incubate la 37°C pe întreaga durată a fermentaţiei. În timpul procesului de fermentaţie, în mediu a fost barbotat CO_2 cu un debit de 0,1 L/l mediu pe minut.

În aceste experimente a fost folosită bacteria *A. succinogenes* ATCC 55617 imobilizată în alginat. Microorganismul a fost furnizat de American Type Culture Collection şi păstrat în frigider la -70°C.

Imobilizarea s-a realizat prin incluziunea celulelor bacteriene într-o matrice de alginat. Prepararea biocatalizatorilor s-a realizat în condiţii aseptice, separat pentru fiecare bioreactor. În acest sens, s-au adăugat 6 ml de inocul peste 20 ml soluţie 5% alginat de sodiu. Granulele de biocatalizator au fost obţinute prin picurarea acestei suspensii sub presiune constantă, prin capilare, sub

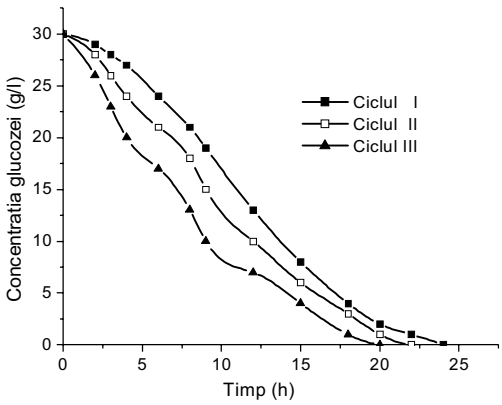

Fig. 3. Variaţia concentraţiei substratului în timpul fermentaţiei cu *A. succinogenes* celule imobilizate

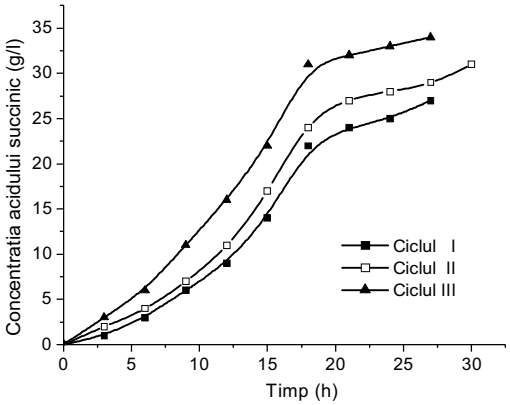

Fig. 4. Variaţia concentraţiei produsului în timpul fermentaţiei cu *A. succinogenes* celule imobilizate

agitare, într-o soluţie 0,2% clorură de calciu. Au fost utilizate 3 capilare de dimensiuni diferite, astfel încât au fost obţinute granule de *A. succinogenes* imobilizate cu 3 diametre: 3; 3,6 şi 4,2 mm. În toate cazurile fracţia volumică a celulelor în mediul de fermentaţie a fost de 0,28.

Fermentaţia s-a considerat încheiată atunci când substratul a fost consumat în întregime sau când concentraţia acestuia nu s-a modificat timp de 12 ore. Concentraţia substratului a fost măsurată la fiecare 2 sau 3 ore pe parcursul fermentaţiei cu ajutorul analizorului de glucoză de tip Analox GL 6.

Conţinutul de acid succinic din lichidul de fermentaţie a fost dozat folosindu-se tehnica HPLC (echipament Star Varian Chromatography Workstation), utilizând ca fază mobilă o soluţie de acid trifluoracetic 0,1%. Coloana de tip Shimadzu CTO-6A a funcţionat la temperatura de 60°C.

REZULTATE ŞI DISCUŢII

Datorită difuziei interne a substratului in interiorul particulelor de biocatalizator, concentraţia substratului este diminuată în apropierea centrului activ, ceea ce duce la reducerea vitezei proceselor biochimice (creşterea biomasei şi formarea produsului) comparativ cu sistemele ce folosesc bacterii libere. Apariţia difuziei interne, în cazul imobilizării celulelor, ar putea atenua efectele inhibitorii.

Astfel, din figura 1 se poate observa că durata fermentaţiei succinice pentru bacteria *A. succinogenes* liberă este de aproximativ 22 de ore, perioada lag fiind de 3 ore. Durata procesului de fermentaţie a crescut prin utilizarea celulelor imobilizate devenind de 25 de ore pentru granulele cu diametrul de 3 mm, 28 de ore pentru cele de 3,6 mm şi 33 de ore pentru granulele cu diametrul de 4,2 mm. Această variaţie este consecinţa difuziei interne a glucozei în granula de biocatalizator, proces care reduce concentraţia substratului în interiorul particulelor şi, implicit, viteza de consum a glucozei.

Aparent, perioada lag nu este atât de evidentă în cazul bacteriei *A. succinogenes* imobilizate, posibil datorită difuziei glucozei dinspre faza lichidă spre biocatalizatori. Însă, pentru primele 12 ore, cea mai ridicată viteză de utilizare a substratului este atins pentru granulele de biocatalizator cu diametrul de 4,6 mm. Acest rezultat evidenţiază efectul pozitiv al difuziei interne în fermentaţia succinică în condiţiile inhibiţiei de substrat. După 12-15 ore, atunci când concentraţia glucozei este mai mică decât cea care ar induce efectul de inhibiţie, difuzia internă devine un factor limitativ pentru procesul de fermentaţie, iar viteza de consum a

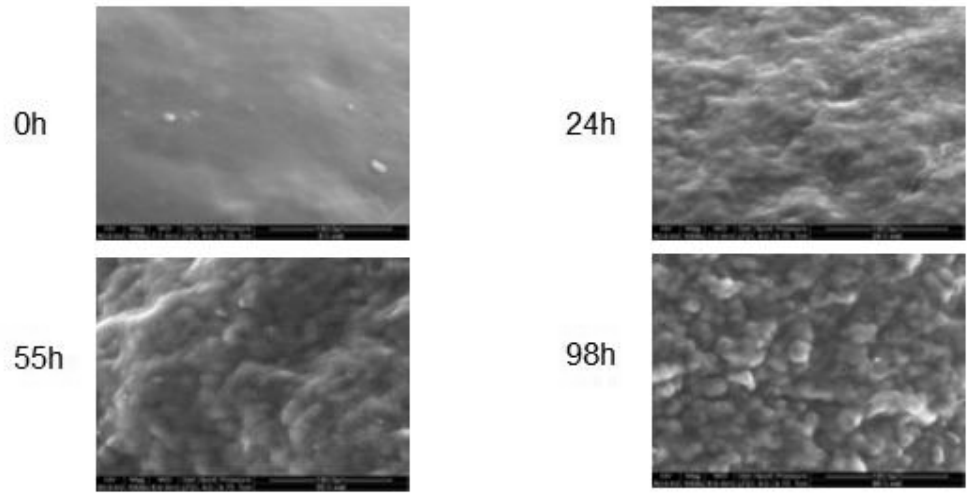

Fig. 5. Imagini SEM ale granulei de biocatalizator

substratului se reduce, fenomen mai evident la dimensiuni mai ridicate ale granulelor.

În acelaşi timp, s-a constatat că prin utilizarea bacteriilor imobilizate şi prin creşterea diametrului granulelor de biocatalizator se reduce viteza de producere a acidului succinic, datorită acumulării sale în interiorul granulelor şi apariţiei fenomenului de inhibiţie de produs (fig. 2).

Aceiaşi biocatalizatori au fost folosiţi pentru 3 cicluri succesive de fermentaţie succinică şi s-a constat că, pentru aceleaşi granule, viteza de consum a glucozei şi viteza producerii acidului succinic cresc de la un ciclu la altul, fenomen ce se datorează dezvoltării bacteriilor în interiorul granulelor (fig. 3, 4).

Dezvoltarea bacteriilor în interiorul biocatalizatorilor a cauzat distrugerea granulelor la finalul celui de-al treilea ciclu, fapt evidenţiat prin măsurarea turbidităţii mediului. De asemenea, dezvoltarea biomasei în interiorul granulelor de biocatalizatori este dovedită şi de imaginile SEM obţinute în diferite momente pe parcursul celor trei cicluri de fermentaţie succinică (fig. 5).

CONCLUZII

Experimentele prezentate au abordat fermentaţia succinică într-un bioreactor cu strat mobil de *A. succinogenes* imobilizate în alginat, în condiţiile inhibiţiei de substrat şi produs. Studiile s-au concentrat pe efectul imobilizării celulelor bacteriene asupra vitezelor de consum a glucozei şi producere a acidului succinic, precum şi pe posibilitatea realizării mai multor cicluri de fermentaţie succesive.

Rezultatele obţinute au indicat faptul că efectul inhibitoriu al substratului poate fi diminuat prin imobilizarea bacteriilor, gradul de consum al glucozei crescând odată cu creşterea dimensiunii particulelor de biocatalizator.

Studiile viitoare vor urmări transpunerea proceselor realizate din sistemul cu strat mobil de biocatalizator într-un sistem ce foloseşte stratul fix tip „basket”.

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FLAVONOIDELE – COMPONENTE BIOACTIVE DIN SUCURI DE FRUCTE

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FLAVONOIDS - BIOACTIVE COMPOUNDS IN FRUITS JUICE (Abstract): Since 1989, grapefruit juice is reported to modify, with clinical implications, the pharmacokinetic of a series of drugs such as calcium channel blockers that are dihydropyridine derivates and/or some HMGCoA reductase inhibitors. All these drugs are metabolized to a large extent by the most abundant human isoform of cytocrome P450, CYP3A4. Grapefruit inhibits the cytocrome P450 3A4 enzyme system responsible for the oxidative metabolism of many orally administered drugs. The most important compounds of GFJ considered to be involved in the pharmacokinetic interaction are flavonoids (naringin, naringenin, narirutin, quercetin, kaempferol, hesperidin, neohesperidin, didymin, and poncirin), furanocoumarins (6',7'-dihydroxy-bergamottin, bergamottin, bergamottin-6',7'-epoxide, bergapten, epoxy-bergamottin) and sesquiterpens (nootkatone). **Material and method**: The experimental researches had as object the comparative study of total content of flavonoidic compounds, like naringin in different fruit juices: citric fruit (white, pink and red grapefruit), apples, pears - industrial or laboratory prepared juices (by squeezing). They praised a different content in flavonoidic compounds for citric and for the rest of analyzed fruits. **Results**: For grapefruit juices the concentrations in flavonoidic compounds are higher in total fruit juice: 143.86 mg/100 mL in red, 131.47 mg/100 mL in pink and 84.21 mg/100 mL in white grapefruit juice. In juice prepared from fruit pulp, the concentrations were 81.92 mg/100 mL, 108.23 mg/100 mL and 65.76 mg/100 mL, respectively. The content in naringin, the most important flavanone in citrus fruit, varied between 1.98 and 51.2 mg/100 mL juice. **Key words**: GRAPEFRUIT, FLAVONOID, NARINGIN, PHARMACOKINETIC INTERACTION

Produsele alimentare de origine vegetală conţin, pe lângă componentele cu rol biologic cunoscut, indispensabile în alimentaţia omului (macro- şi micronutrienţi), şi alte substanţe (pigmenţi naturali, acizi organici, enzime, uleiuri volatile) în concentraţii foarte mici care pot îndeplini, în aliment sau în organism, roluri specifice. Aceste substanţe sunt denumite cu un termen generic "substanţe bioactive"; trebuie precizat că o parte dintre acestea prezintă interes nutriţional şi se numesc "fiziologic active", iar altă parte prezintă, mai ales interes terapeutic (in domeniul fitoterapiei) - substanţe "farmacologic active"(1).

Sucul de grapefruit (SGF) este consumat frecvent pentru gustul său plăcut, pentru valoarea nutritivă, dar şi pentru aportul de substanţe bioactive (flavone, furanocumarine, vitamine). Tradiţional, sucul de grapefruit este consumat pentru acţiuni multiple: antioxidant, antinitrozaminic, antiseptic, cardiotonic, detoxifiant, hipocolesterolemiant, sedativ, stomahic (2,3). Studii experimentale

(4,5) au sugerat că sucul de grapefruit reduce formarea plăcilor aterosclerotice şi inhibă proliferarea unor celule canceroase (cancer mamar, pulmonar, de colon). Recent au fost publicate numeroase date care confirmă acţiunea hipocolesterolemiantă a componentelor bioactive din sucul de grapefruit (6,7).

Existenţa interacţiunii între sucul de grapefruit şi unele medicamente blocante ale canalelor de calciu (felodipina şi nifedipina) a fost evidenţiată din întâmplare în anul 1991, în urma unui studiu al interacţiunii etanol-medicament în care sucul de grapefruit a fost utilizat ca vehicul pentru alcoolul folosit în studiu (8). De atunci au fost publicate rezultatele a numeroase studii privind interacţiunea sucului de grapefruit cu diferite medicamente şi importanţa clinică a acestora, mecanismul interacţiunii, componentele bioactive din produsul alimentar responsabile de producerea interacţiunii (2).

Medicamentele care interacţionează cu sucul de grapefruit sunt supuse în organism unui metabolism oxidativ, sub acţiunea citocromului P450, în peretele intestinal sau în ficat. Sucul de grapefruit conţine flavonoide care afectează sistemul citocrom P450 (CYP), în special izoenzimele CYP1A2 şi CYP3A4, prin ataşare la izoenzimă, ca substrat, şi astfel este afectată prima etapă a metabolismului prin inactivare directă sau prin inhibarea enzimei. Efectul major asupra enzimelor CYP constă într-o inhibare selectivă a CYP3A4 din intestinul subţire (9). Intensitatea interacţiunii dintre sucul de grapefruit şi medicamente din clase terapeutice diferite, cu structuri chimice variate, este influenţată de modul de preparare al sucului (pulpa, pulpă şi albena, fruct integral) şi de varietatea de fruct (3,10,11) utilizată (grapefruit alb, roz sau roşu).

În prezent este confirmat faptul că şi alte componente bioactive din produse de origine vegetală (legume, fructe, plante medicinale), pot interacţiona cu unele medicamente. Astfel, Banfield et al. (12) a evidenţiat modificarea marcantă a parametrilor farmacocinetici (aria de sub curbă, concentraţia maximă, timp de înjumătăţire) la administrarea simultană la subiecţi umani de fexofenadină şi suc de mere. Concluzia care de desprinde din aceste rezultate este aceea că se impune o nouă abordare privind interacţiunile dintre medicamente şi sucurile din produse vegetale.

Cercetările experimentale prezente au ca obiectiv studiul comparativ al conţinutului total în flavonoide, respectiv în naringină pentru diferite sucuri de fructe : citrice (grapefruit alb, roz şi roşu, portocale, mandarine), mere, pere - preparate industrial sau în laborator.

MATERIAL ŞI METODĂ

Probe de analizat

Probele supuse analizei (tab. I) au fost sucuri de grapefruit (alb, roz şi roşu), de portocale, de mandarine, de pere şi de mere - preparate industrial (din comerţ) sau în laborator, utilizând storcătorul de fructe. Sucurile de citrice preparate în momentul utilizării (în laborator) s-au realizat în trei variante : suc din pulpa fructului (I), din pulpa fructului şi albena (II) şi din fructul integral (III). Sucurile preparate industrial au fost achiziţionate din reţeaua comercială a oraşului Iaşi.

Studiul cromatografic

Identificarea flavonoidelor din sucurile de fructe s-a realizat prin cromatografie pe strat subţire pe suport de silicagel, în sistem ascendent (metoda standardului extern). Ca substanţe de referinţă s-au utilizat rutozida şi naringina.

Determinarea cantitativă a flavonoidelor

Determinarea conţinutul total în flavonoide exprimat în naringină s-a realizat prin tehnica prezentată în Farmacopeea Română, ediţia a X-a, monografia *Cynarae folium* (13). Calculul conţinutului în flavonoide al

TABELUL I
Probele de sucuri de fructe analizate

Nr. probei	Denumirea probei	Nr. probei	Denumirea probei
1	Suc grapefruit roşu I*	14	Suc portocale I*
2	Suc grapefruit roşu II*	15	Suc portocale II*
3	Suc grapefruit roşu III*	16	Suc portocale III*
4	Suc grapefruit roz I*	17	Nectar portocale cu pulpă (Santal)**
5	Suc grapefruit roz II*	18	Suc mandarine I*
6	Suc grapefruit roz III*	19	Suc mandarine II*
7	Suc grapefruit alb I*	20	Suc mandarine III*
8	Suc grapefruit alb II*	21	Suc mandarine 100%**
9	Suc grapefruit alb III*	22	Suc mere*
10	Suc grapefruit alb (Fortuna)**	23	Suc de mere 100% (Cappy)**
11	Suc grapefruit roşu (Costa)**	24	Suc pere *
12	Suc grapefruit alb (Hortex)**	25	Suc pere 100% (Santal)**
13	Nectar grapefruit roşu cu pulpă (Tymbark)**		

* preparat în laborator ** procurat din comerţ

probelor de suc, exprimat în mg naringină/ 100 mL s-a realizat cu ajutorul curbei de calibrare trasate în prealabil, pentru domeniul de concentraţii 0-50 μg. Separarea flavonoidelor din probele de sucuri de fructe în vederea determinării spectrofotometrice s-a realizat prin tratare cu metanol anhidru (1 : 1, v/v) şi centrifugare timp de 15 minute la 3000 RTM. Cotă parte din lichidul supernatant a fost prelucrat în modul indicat la trasarea curbei de etalonare.

Determinarea conţinutului în naringină

Pentru probele de suc de fructe luate în studiu s-a determinat conţinutul în naringină prin metoda aplicată la determinarea flavonoidelor totale după separarea acesteia prin cromatografie preparativă pe strat subţire (standard extern), pe suport de poliamidă, cu dublă developare în sistemul de solvenţi metanol – nitrometan (2 : 5). Revelarea cromatoplăcilor s-a realizat cu soluţie de triclorură de aluminiu 1% în alcool etilic şi expunere la lumină Wood. Banda corespunzătoare naringinei a fost raclată de pe placa cromatografică, adusă într-o eprubetă uscată şi extrasă cu 1,5 – 2 ml amestec metanol - etilenglicol - apă - hidroxid de sodiu 4N (25 : 22,4 : 2,6 : 1). Calculul conţinutului în naringină al probelor de sucuri

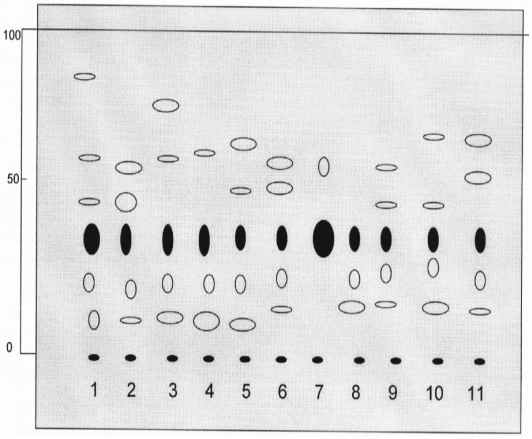

Fig. 1. Cromatograma pe strat subţire pentru flavonoide în sucuri de citrice. Suport : plăci cromatografice Polyamide P-6, 20/20cm ; Sistem de solvenţi : metanol – nitrometan (2 : 5) ; Revelare : triclorură de aluminiu, soluţie 1% în alcool etilic şi expunere la lumină Wood. Legenda : 1. Proba 3 ; 1. Proba 6 ; 3. Proba 9 ; 4. Proba 10 ; 5. Proba 11 ; 6. Proba 13 ; 7. Naringina etalon ; 8. Proba 16 ; 9. Proba 17 ; 10. Proba 20 ; 11. Proba 21

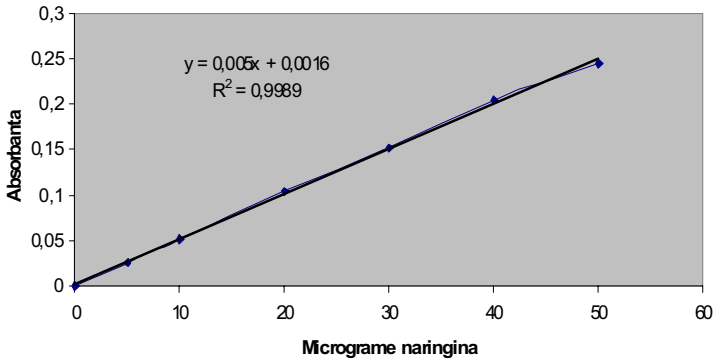

Fig. 2. Curba de calibrare pentru determinarea cantitativă a naringinei

analizate s-a realizat cu ajutorul curbei de calibrare trasate în prealabil, pentru intervalul de concentraţii 0 – 50 μg.

REZULTATE ŞI DISCUŢII

Tabloul cromatografic pentru identificarea prezenţei flavonoidelor în probele de sucuri de fructe este prezentat în fig. 1.

Spoturile corespunzătoare naringinei sunt suficient de delimitate şi au permis o bună separare a benzilor corespunzătoare acestei componente.

Curba de calibrare pentru determinarea flavonoidelor totale exprimate în naringină este prezentată în fig. 2.

Coeficientul de corelaţie R^2 obţinut are o valoare de 0,99899 care permite calculul conţinutului în naringină, respectiv în flavonoide totale al probelor analizate.

Conţinutul în flavonoide şi în naringină pentru probele de suc de grapefruit preparate în laborator este prezentat în fig. 3.

Se constată valori mai crescute ale flavonoidelor totale pentru sucul de grapefruit roşu preparat din fructul integral – 143,86 mg/100 mL; în sucurile preparate din pulpa fructului, concentraţiile variază de la 65,76 mg/100 ml pentru SGF alb la 108,23 mg/100 mL pentru SGF roz. Aceste valori sunt în concordanţă cu cele raportate de alţi autori (3,14,15) care au determinat în 9 sorturi diferite de suc concentraţii de flavonoide cuprinse între 19,44 şi 84,28 mg/100 mL.

Pentru sucurile de grapefruit preparate industrial (fig. 4) concentraţiile în flavonoide totale sunt cuprinse între 96,21 şi 102,54 mg/100 mL. Aceste valori sunt apropiate de cele determinate de alţi autori în sucuri preparate din fructul integral (14). Nectarul de grapefruit preparat industrial (proba 13) conţine 83,12 mg flavonoide la 100 mL produs.

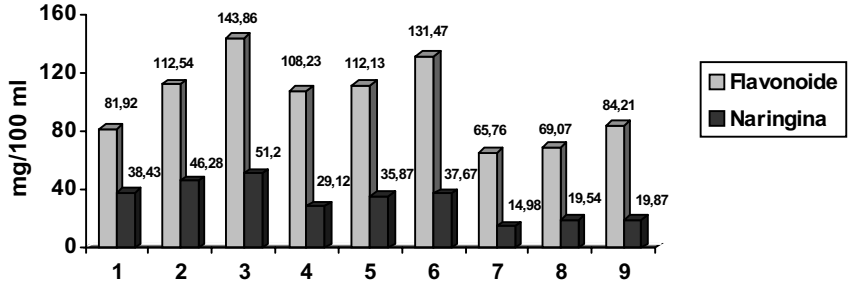

Fig. 3. Concentraţiile flavonoidelor totale şi ale naringinei în probele de suc de grapefruit preparate în laborator (probele 1-9)

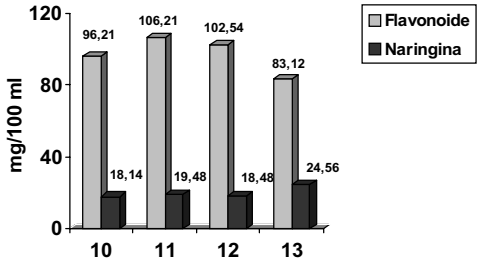

Fig. 4. Concentraţiile flavonoidelor totale şi ale naringinei în probele de suc de grapefruit preparate industrial (probele 10-13)

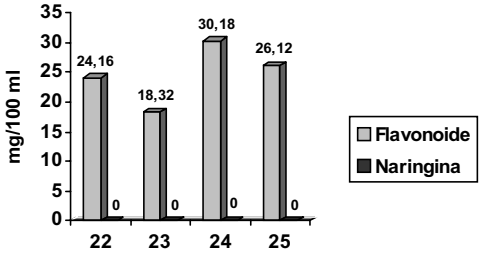

Fig. 6. Concentraţiile flavonoidelor totale în probele de suc de mere şi de pere (probele 22-25)

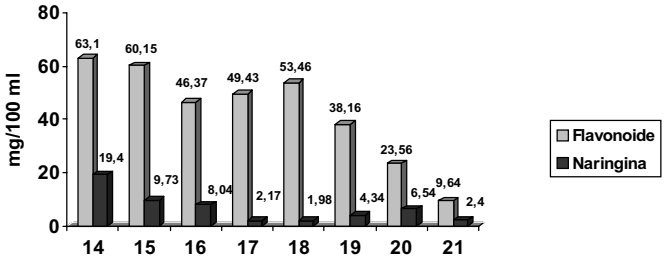

Fig. 5. Concentraţiile flavonoidelor totale şi ale naringinei în probele de suc de portocale şi de mandarine (probele 14-21)

În fig. 5 sunt prezentate concentraţiile flavonoidelor în sucurile de portocale şi de mandarine preparate în laborator sau procurate din comerţ. Pentru sucurile de portocale (probele 14-17) valorile concentraţiilor flavonoidelor variază între 46,37 mg/100 mL (suc de portocale preparat din pulpa fructului) şi 63,18 mg/100 mL (suc preparat din fructul integral). Pentru sucurile de mandarine (probele 18-22) valorile sunt ceva mai mici şi variază de la 9,64 mg/100 mL la sucul preparat industrial la 53,46 mg/100 mL la sucul preparat în laborator din fructul integral.

Pentru sucurile de pere şi de mere (fig. 6) s-au determinat concentraţii ale flavonoidelor totale cuprinse între 18,32 mg/100 mL şi 30,18 mg/100 mL produs.

Concentraţiile în naringină determinate în paralel cu conţinutul total în flavonoide (fig. 3-6) variază între 14,98 mg/100 mL pentru SGF alb preparat din pulpa fructului şi 51,20 mg/100 mL pentru SGF preparat din grapefruitul roşu, fructul integral. Sucurile de grapefruit preparate industrial (din fruct integral) conţin între 24,56 mg şi 29,48 mg naringină pentru 100 mL produs. Valorile conţinutului în naringină pentru probele de suc obţinute din portocale şi mandarine variază de la 1,98 mg/100 mL pentru sucul de mandarine preparat numai din pulpa fructului la 21,17 mg/100 mL pentru nectarul de portocale cu pulpă. În probele de sucuri de mere şi de pere nu a fost determinată naringina. Cromatografia pe strat subţire indică absenţa acestui compus din compoziţia sucurilor menţionate, rezultat în concordanţă cu datele din literatura de specialitate (16).

CONCLUZII

Determinarea conţinutului în flavonoide totale în sucuri de fructe (citrice, mere, pere) preparate industrial sau în laborator a evidenţiat valori diferite atât în funcţie de natura fructului, cât şi în funcţie de modul

de preparare al sucului. Sucul preparat din fructul integral este mai bogat în flavonoide, respectiv în naringină, comparativ cu sucul preparat din pulpa fructului.

Sucurile preparate industrial, din fruct integral, prezintă valori ale conţinutului în flavonoide apropiate de cele preparate în laborator. Diferenţele constatate între concentraţiile în componentele bioactive ale sucurilor de fructe explică intensitatea diferită a interacţiunilor farmacocinetice care se manifestă la administrarea simultană cu medicamente din clase terapeutice diferite.

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ASPECTE DE SĂNĂTATE OCUPAŢIONALĂ ÎN ÎNTREPRINDERILE MICI ŞI MIJLOCII DIN BACĂU

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SOME ASPECTS REGARDING OCCUPATIONAL HEALTH IN SMALL AND MIDDLE ENTERPRISES (Abstract): The objective of the study was to assess occupational health services in small and medium enterprises (SME's) in Bacău, between 2007 and 2008, in order to increase the quality of services' management. **Material and method**: Five hundred seventy nine SME's with 21,815 employees have been studied, with a number of employees varying from ten to 250 per enterprise. About 4478 employees, out of which 587 with different occupational exposures, have no occupational health services provided on a contract basis. **Results**: The high number of occupational diseases and work related accidents in SME's show a low level of concern for occupational health and safety matters, compared to big enterprises. The employees' opinions showed that the role of occupational health is not known, there is no concern for the medical adaptation of new employees or old employees rehired after a long break, the employees do not consult occupational health services on their own accord. The occupational services do not fully satisfy the need. The study offers useful data to physicians and SME's management in order to improve the occupational health management. **Key words**: OCCUPATIONAL HEALTH SERVICES, SMALL AND MIDDLE ENTERPRISES, INTERVENTION RESEARCH

Din 1989 toate statele Uniunii Europene au avut ca obligaţie, pe măsura aderării, transpunerea în legislaţia naţională a Directivei Consiliului 89/391/CEE din 12 iunie 1989 privind punerea în aplicare de măsuri pentru promovarea îmbunătăţirii securităţii şi sănătăţii lucrătorilor la locul de muncă (1,2). Angajatorul are doar obligaţia de a asigura protecţia stării de sănătate a angajaţilor în condiţiile existente la locul de muncă.

Activităţile de medicina muncii sunt organizate în raport de consideraţiile economice şi pot fi practicate de asistenţi medicali specializaţi, medici de medicină generală sau specialişti de medicina muncii (3,4,5).

MATERIAL ŞI METODE

În studiu s-au inclus informaţiile privind numărul întreprinderilor şi al angajaţilor acestora, tipurile şi nivelurile riscurilor pentru sănătate, situaţia bolilor profesionale şi accidentelor de muncă, şi opiniile angajaţilor privind riscurile şi natura îngrijirilor pentru sănătate. De asemenea, Direcţia Generală a Finanţelor Publice, Bacău a furnizat datele privind asigurarea cu servicii de medicina muncii pe bază de contract.

Evidenţele Direcţiei de Sănătate Publică

Bacău şi ale Compartimentului Accidente de Muncă şi Boli Profesionale din cadrul Inspectoratului Teritorial de Muncă Bacău, au permis realizarea unor aprecieri privind situaţia existentă în IMM-uri, în raport de particularităţile grupurilor de angajaţi (6).

REZULTATE ŞI DISCUŢII

Particularităţi ale structurilor incluse în studiu. În anii 2007 şi 2008, au fost în activitate 579 IMM cu 21815 angajaţi, dintre care 451 întreprinderi mici cu 9079 angajaţi, şi 128 întreprinderi mijlocii cu 12736 angajaţi, cu variate profiluri de activitate şi de asistenţă pentru sănătate (tab. I).

Serviciile de medicina muncii. Din total angajaţi în IMM-uri, 20,5% nu beneficiază de asistenţa medicului de medicina muncii,

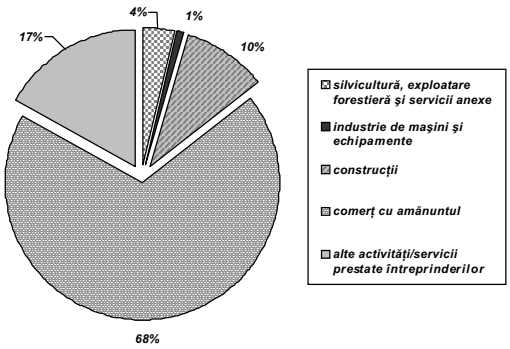

Fig. 1. Distribuţia angajaţilor pe profiluri de activitate neasiguraţi cu servicii de medicina muncii, prin contract

TABELUL I

Numărul angajaţilor pe profiluri de activitate şi gradul de acoperire cu medici de medicina muncii, prin contract

Nr. crt.	Profil de activitate	Total salariaţi	Număr de salariaţi cu medic	Număr de salariaţi fără medic
1.	Comerţ	6441	3371	3070
2.	Construcţii	3949	3497	452
3.	Fabricare produse textile si îmbrăcăminte	2696	2696	0
4.	Alte servicii/ activităţi prestate	1286	531	755
5.	Transporturi terestre	1091	1091	0
6.	Fabricare articole marochinărie	967	967	0
7.	Industria alimentara şi a băuturilor	811	811	0
8.	Fabricare construcţii metalice	678	678	0
9.	Fabricare produse din hârtie/ tipografie	499	499	0
10.	Recuperare deşeuri	392	392	0
11.	Industria metalurgica	343	343	0
12.	Industria de maşini si aparate electrice	336	336	0
13.	Servicii personale	327	327	0
14.	**Silvicultura, exploatare forestieră, servicii anexe**	278	121	157
15.	Producţia mobilier	253	253	0
16	Industria altor mijloace de transport	238	238	0
17.	Hotel/ restaurante	233	233	0
18.	Industria de maşini si echipamente	199	155	44
19.	Sănătate si asistenta sociala	191	191	0
20.	Creştere animale	186	186	0
21.	Prelucrare mase plastice si sticla	182	182	0
22.	Captare/ distribuţie apa	99	99	0
23.	Fabricare produse din lemn	98	98	0
24.	Fabricare produse din ţiţei	26	26	0
25.	Producţia de caroserii	16	16	0
TOTAL		**21815**	**17337**	**4478**

TABELUL II

Numărul angajaţilor expuşi la noxe şi situaţia asigurării cu servicii de medicina muncii, prin contract

Nr. crt.	Tip noxe	Număr angajaţi expuşi la noxe	Neasiguraţi prin contract	Asiguraţi prin contract
1.	Suprasolicitare vizuală	2558	38	2520
2.	Suprasolicitare locomotorie	2020	72	1948
3.	Pulberi textile	1700	0	1700
4.	Zgomot	820	54	766
5.	Muncă la înălţime	538	22	516
6.	Iritanţi/ alergizanţi ai pielii	518	40	478
7.	Gaze si vapori iritanţi	406	43	363
8.	Alţi solvenţi organici	390	22	368
9.	Agenţi biologici	292	30	262
10.	Ciment	227	0	227
11.	Vibraţii	221	26	195
12.	Microclimat cald	219	0	219
13.	Microclimat rece	159	0	159
14.	Pulberi silicogene	130	130	0
15.	Alte pulberi	117	61	56
16.	Pulberi organice	116	0	116
17.	Suprasolicitare laringe	91	0	91
18.	Oxid de carbon	36	6	30
19.	Alţi compuşi organici	32	31	1
20.	Crom şi compuşi	11	9	2

situaţie întâlnită îndeosebi în sectoarele de comerţ, construcţii şi servicii (fig. 1).

Asigurarea cu servicii de medicina muncii rămâne, în continuare, pentru angajatorul român, doar o obligaţie legală (Legea nr. 319 din 14 iulie 2006, a securităţii şi sănătăţii în muncă) (7).

Dacă toţi cei 12 medici care îşi desfăşurau activitatea în teritoriul la care ne referim, ar fi încheiat contracte cu IMM-urile cu peste 10 angajaţi, fiecăruia i-ar fi revenit 1855 de angajaţi.

Riscurile ocupaţionale pentru sănătate au făcut obiectul unui alt studiu, din care rezultă că prezenţa factorilor de risc a fost constatată la 22% din angajaţii întreprinderilor mici şi la 31% din angajaţii celor mijlocii. Pe primele 3 locuri s-au situat suprasolicitarea vizuală, musculo-osteo-articulară şi expunerea la pulberi textile.

Măsurătorile de noxe au arătat că LMA sunt depăşite în proporţie de 35%, îndeosebi pentru pulberile cu SiO_2 şi de lemn, precum şi pentru zgomot şi solvenţii organici. Deşi s-a constatat că 10,1% din angajaţii expuşi la noxe nu sunt supravegheaţi medical, în relaţie cu riscurile ocupaţionale, s-a evidenţiat implicarea angajatorilor în supravegherea lucrătorilor expuşi la noxe (tab. II).

Percepţia negativă asupra condiţiilor de muncă a fost exprimată în medie de 14,35% dintre angajaţi, cu proporţii diferite, în funcţie de profilul muncii (1,75-85%).

Evaluări pentru perioada 2005-2007. În cei 3 ani luaţi în studiu, au fost declarate 23 cazuri de boli profesionale, reprezentând 79,31% din totalul de 29 declarate în toate întreprinderile din municipiu. În proporţie de 90% acestea provin din industria metalurgică, secţia de turnătorie (silicoză, hipoacuzie / surditate şi iritaţii ale căilor aeriene superioare). Au fost înregistrate şi cazuri de astm bronşic (confecţii, panifi-

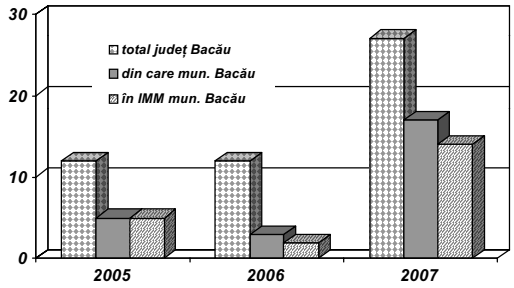

Fig. 2. Repartiţia numărului de cazuri de boli profesionale declarate în perioada 2005-2007

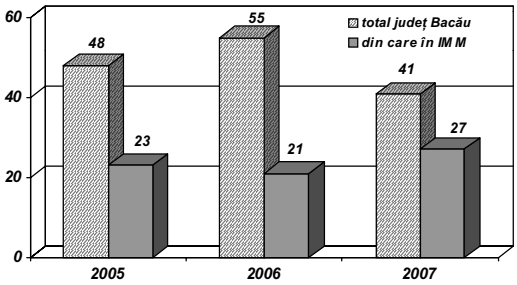

Fig. 3. Numărul accidentelor de muncă declarate în perioada 2005-2007

caţie), boli musculo-osteo-articulare (silvicultură, industria chimică), şi iritaţii ale căilor aeriene superioare (construcţii, prelucrare petrol) (fig. 2).

În aceeaşi perioadă (2005-2007), în IMM au fost înregistrate 71 accidente de muncă, reprezentând 49,3% din totalul de 144 pentru toate întreprinderile din municipiu (fig. 3).

S-a remarcat faptul că, în întreprinderile mijlocii, numărul accidentelor este de 3 ori mai mare decât în cele mici, ele reprezentând 2/3 din totalul accidentelor din municipiu. Situaţia se explică prin numărul mare de întreprinderi de construcţii, în care riscul de accidentare este crescut.

Numărul mai mare al cazurilor de boli profesionale şi accidente de muncă în IMM faţă de cel din întreprinderile mari reflectă nivelul scăzut al organizării şi preocupărilor de promovare a sănătăţii şi securităţii în muncă.

Oferta de servicii de medicina muncii. În municipiul Bacău, în anii 2007 şi 2008, asistenţa de medicina muncii era asigurată de trei tipuri de unităţi: 4 cabinete medicale individuale, 4 clinici particulare, şi cabinetul de medicina muncii din ambulatoriul spitalului judeţean. În aceste unităţi, erau încadraţi 12 medici de medicina muncii care asigurau servicii pentru salariaţii întregului judeţ. La aceştia se adăugau 20 medici de alte specialităţi care şi-au câştigat dreptul de a practica medicina de întreprindere prin cursuri speciale. Se apreciază că oferta de servicii de medicina muncii pentru angajaţii din IMM, cât şi pentru celelalte întreprinderi, nu corespunde cerinţelor. Legislaţia din România nu prevede un număr maxim de salariaţi, care poate fi asistat de medicul de medicina muncii. Studiul nostru oferă medicilor, angajatorilor şi legiuitorilor, o bază de date în scopul creşterii, în viitor, a volumului şi calităţii managementului sănătăţii ocupaţionale în IMM.

CONCLUZII

Studiile privind nevoile şi oferta de servicii de medicina muncii pentru angajaţii din IMM sunt încă în număr redus. Datele pe această temă din ţările Uniunii Europene pot fi utile, impunându-se şi studiul particularităţilor din România. Oferta şi calitatea serviciilor de medicina muncii se cere a fi reconsiderată şi adaptată nevoilor care apar, în concordanţă cu transformările socio-economice ale IMM, care prezintă mare mobilitate şi instabilitate. Prin destrămarea marilor întreprinderi şi absorbţia forţei de muncă de IMM-uri, municipiul Bacău poate fi luat ca exemplu din acest punct de vedere. În aceste condiţii, o cerinţă de prim ordin pentru Direcţia de Sănătate Publică este de a asigura oferta de servicii de medicina muncii tuturor angajaţilor din IMM, pe bază de contract. Tabloul polimorf al activităţilor din IMM, al riscurilor pentru sănătate şi producerea unor boli, impune organizarea de structuri diversificate de medicina mun-

cii, care să satisfacă nevoile de sănătate ocupaţională ale angajaţilor.

Cazurile de boli ocupaţionale şi accidentele de muncă, semnificativ mai numeroase în IMM decât în întreprinderile mari, ca şi existenţa unor riscuri reale pentru sănătate, sunt consecinţa minimalizării preocupării angajatorilor pentru sănătatea şi securitatea în muncă. În acelaşi timp, angajaţii nu sunt educaţi pentru a înţelege importanţa măsurilor prevenţionale şi de asistenţă prin medicina muncii, la care apelează doar în caz de îmbolnăvire, iar cei cu boli cronice solicită rar ajutorul medicului de medicina muncii, putând evidenţia relaţia dintre unele aspecte ale muncii şi anumite stări patologice.

În prezent, se impun măsuri care să amelioreze interrelaţiile medicilor de medicina muncii şi de alte specialităţi, în rezolvarea problemelor complexe pe care le ridică promovarea sănătăţii ocupaţionale.

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NOUTĂŢI

REZULTATELE STUDIULUI DE MONITORIZARE A REZISTENŢEI LA ANTIBIOTICE (SMART) ÎN EUROPA

În perioada 2002-2008 s-a înregistrat o creştere semnificativă (de la 4,3% în 2002 la 11,8% în 2008, $p < 0,001$) a tulpinilor de *Escherichia coli* producătoare de beta-lactamaze cu spectru extins (ESBL), izolate din infecţii intr-abdominale în Europa ; aceasta creştere nu a fost înregistrată în cazul tulpinilor de *Klebsiella pneumoniae* : 16,4% faţă de 17,9%, $p > 0,05$. Tulpinile izolate din spital au fost mult mai frecvente decât cele din comunitate : 14% *versus* 6,5% pentru *Escherichia coli,* respectiv 20,9% *versus* 5,3%, în cazul tulpinilor de *Klebsiella pneumoniae* (Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Cantón R, Baquero F. Incidence and antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* with extended-spectrum â-lactamases in community- and hospital-associated intra-abdominal infections in Europe : results of the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother* 2010 ; 54 (7) : 3043-3046).

Cristina Tuchiluş

MANAGEMENT ŞI PREVENŢIE PRIVIND MANIFESTĂRILE ORALE ÎN DISCRAZIILE SANGUINE ŞI PATOLOGIA CU ETIOLOGIE VIRALĂ

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MANAGEMENT AND PREVENTION OF ORAL MANIFESTATIONS FOR BLOOD DYSCRASIA AND VIRAL PATHOLOGY (Abstract): The study outlines the dentist's attitude in dealing with two of the many serious diseases throughout the body, HIV infection and acute leukemia. Many systemic diseases have oral manifestations which may appear at the beginning of any developed pathology, thus early detection can occasionally occur at the dental clinic. Gingival lesions are the most common oral phenomenon known for leukemia and HIV infection. In this case an early detection of pathology at the dental clinic was based on an uncommon and single presenting sign of massive spontaneous gingival bleeding. The objectives are demonstrate this unusual sign of blood dyscrasia and HIV infection in oral cavity and establishing management and prevention of oral manifestations for blood dyscrasia and pathology with viral etiology. **Material and method**: We investigated 225 cases had pathology with viral etiology. The patients entered the study HIV / AIDS in Botoşani, Suceava, Neamţ during 2004-2008. All patients had a detailed medical history and examination as well as a thorough oral examination, which were all done without prior knowledge of the patient's HIV serostatus. **Results**: Oral disease is frequently associated with HIV. While nearly all oral disorders associated with HIV infection also occur in other conditions characterized by immunosuppression, no other condition is associated with as wide and significant a spectrum of oral disease as is HIV infection. Many HIV associated oral disorders occur early in HIV infection, not infrequently as the presenting sign or symptom. Thus, early detection of associated oral disease should, in many cases, result in earlier diagnosis of HIV infection. Likewise, awareness of the variety of oral disorders which can develop throughout the course of HIV infection, and coordination of health care services between physician and dentist, should improve overall health and comfort of the patient. This paper reviews the clinical, diagnostic and therapeutic aspects of HIV associated oral disorders. **Conclusions**: Diseases with a particular viral etiology infectiousness have a special resurgence. For the dentist, their recognition has a particularly importance for the quick and accurate determination of the diagnose and for the establishing, together with the infectious disease physician, of the appropriate treatment. Working with the department of epidemiology is useful to define the preventive measures among the community. **Key words**: ORAL MANIFESTATION, HIV INFECTION, BEHAVIORAL STRATEGIES, PREVENTION, ACUTE LEUKEMIA

POLYMERIC MATERIALS AS NANOFIBERS USED IN THE TREATMENT OF BURNS

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POLYMERIC MATERIALS AS NANOFIBERS USED IN THE TREATMENT OF BURNS (Abstract): This paper deals with a short review on the state-of-the-art of the use of nanofibers as wound dressing materials. Nanofibers have potential for use in a wide range of applications due to their biodegradability, low cost and high efficiency. Given the complexity of burn therapy, there is an increasing need for investigating the therapeutic options of wound healing process. The use of nanofibrous materials for burn care is preferable due to their structural similarity whit that of native extracellular matrix. The preparation methods of nanofibers have been extensively studied and some techniques have been reported to produce materials for different applications. Special attention was focused on the preparation of micro- and nanofibrous materials with antibacterial and antifungal activity. **Keywords:** BURN THERAPY, WOUND HEALING, POLYMERS, NANOFIBERS, ELECTRO-SPINNING.

Wound healing is one of the most complex biological events, as a result of the interaction of different tissue structures with a large number of infiltrating cell types as leukocyte subsets (neutrophils, macrophages). The normal healing response begins when the tissue is injured and continues with tissue repair process which involves re-epithelialization, formation of granulation tissue and contraction of underlying wound connective tissues (1). During the healing process, collagen production is essential for epithelial migration and proliferation in the first days after injury (2). Given the complexity of burn therapy and the high incidence of burn injuries and burn-related deaths (approximately 322.000/year) (3), there is an important need for experimental studies on the therapeutic options of wound healing and tissue regeneration.

In the field of wound care, wound dressings have brought the attention of researchers, so far. The treatment of burns requires a material that will restore the epidermal function and will promote the

healing process (2). The ideal wound dressing must have properties that allow the absorption of exudates, inhibition of the growth of microorganisms (4) and also must have high porosity for respiration. Traditional dressings such as natural or synthetic bandages have been extensively used, but they have limited swelling capacity and moisture vapor permeability. In this context, the use of nanofibrous materials is preferable due to their structural similarity to that of native extracellular matrix (5).

On the other hand, nowadays polymeric materials play an important role in many applications, starting from daily needs to biomedical applications.

TECHNIQUES USED TO OBTAIN NANOFIBRES

Nanofibers have been extensively studied and some techniques for producing this type of materials for biomedical applications have been reported. Of these, electrospinning is the most studied technique as it seems to generate the most promising results for biomedical applications (fig. 1).

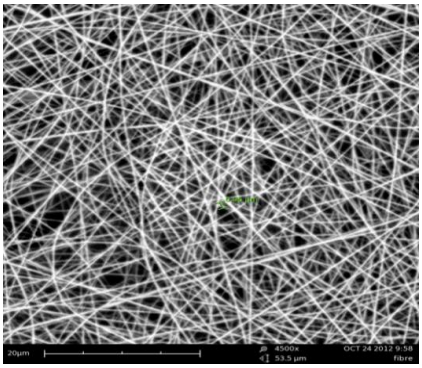

Fig. 1. SEM images of Chitosan nanofibers

The Force-spinning method has been successful used to obtain nanofibers of poly-ethylene oxide, poly-lactic acid, polypropylene, acrylonitrile-butadiene-styrene, polyvinyl pyrrolidone and polystyrene (6). This method uses centrifugal force and parameters such as rotational speed of spinneret, collection system and temperature to control the morphology of the obtained nanofibers.

Template synthesis uses a nanoporous membrane as a template to make nanofibers. The disadvantage of this method is that the process takes a relatively long time to transfer the solid polymer into the nanoporous foam (7).

Mechanical treatments such as cryocrushing (8), grinding (9) and high-pressure homogenizing (10) could be also applied for getting nanofibers.

Chemical treatments such as *acid hydrolysis* provide preparation methods of stable nanofibers. Acid hydrolysis of the biomass involves the use of mineral acids like sulphuric acid, hydrochloric acid and phosphoric acid and produces micro- and nanofibers with a high degree of crystallinity by removing the amorphous regions of the material (11).

Biological treatments, such as enzyme assisted hydrolysis, may decrease the processing cost by lowering the number of passes through the homogenizer and has main advantages from environmental point of view as compared with chemical methods (12).

Electrospinning method. Starting with 1930, electrospinning has been used as a particularly important technique applied to generate fibrous materials used in a variety of applications, such as: filters, skin masks, semipermeable membrane, clothing and medical materials such as dressings, vascular grafts, controlled drug delivery systems, absorbable sutures (13).

In the electrospinning process, fibers can be produced by applying an electric

potential which provides a charge to the polymeric solution. The charged jet forms nanofibers that can be collected on a stationary or rotating metallic collector (13). (fig. 2).

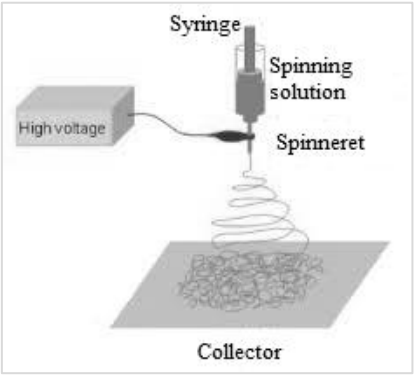

Fig. 2. Electrospinning system

However, this technique attained a considerable recognition since the 90's, when the term electrospinning was invented (14). Polymer solution properties like viscosity, surface tension or conductivity, determine the nanofiber diameter and decrease the risk of bead formation. The nanofibers obtained using this method have gained a particular attention due to their specific properties such as high surface area resulting from the nano- to micro-size, high porosity and good mechanical strength (15). More than this, nanofibers have a high capacity for loading drugs or active materials such as gold (16) or silver nanoparticles (17).

Since bacterial contamination of wounds is a serious problem and a great concern, the selection of a proper antimicrobial agent is essential to inhibit a wide range of pathogens (18). Due to the local interruption of blood flow in burns, systemic infection prevention has low effect, so the use of topical treatment is very important. In the 17th century, silver nitrate was the first silver salt used in the treatment of wounds and ulcers (19). In the prevention process of wound contamination, the use of antimicrobial dressings can help to promote normal wound healing. Lately, a special attention has been given to the preparation of micro- and nanofibrous materials with antibacterial and antifungal activity, as it is the case of antimicrobial nanofibers containing metronidazole (20) or electrospun polycaprolactone containing ampicillin (21).

NATURAL POLYMERS FOR WOUND DRESSINGS

Natural polymers such as collagen, chitosan, elastin, fibrinogen are biocompatible substrates similar to macromolecules recognized by the human body and are involved in the repair of damaged tissues and skin regeneration (22). Also, polymers such as pullulan, dextran, starch, cellulose are used for obtaining electrospun materials with application in the tissue engineering (23).

Pullulan is a linear α-glucane, constituted from three glucose units (fig. 3), being an extracellular microbial polysaccharide produced by the fungus-like yeast *Aureobasidium pullulans*. Several types of nanofibers containing pullulan were prepared using electrospinning technique (24).

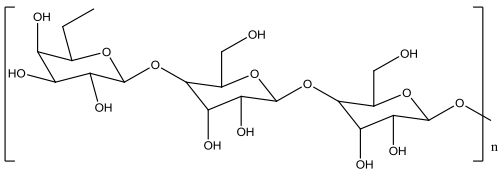

Fig. 3. Structure of *Pullulan*

Dextran is a polysaccharide (fig. 4) with high biocompatibility and biodegradability. It is a proper polymer for development of antimicrobial nanofibers, by up-

loading antimicrobial agents such as moxifloxacin (25).

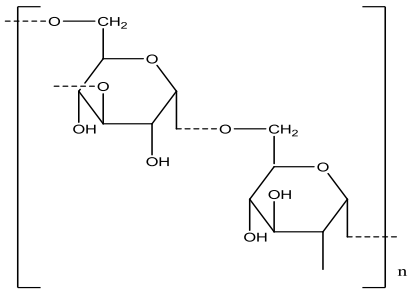

Fig. 4. Structure of *Dextran*

Cellulose (fig. 5) is widely used because of its availability, biocompatibility, degradability and sustainability. It has a large number of applications, from cigarette filters to fibers or films for biomedical fields. It is used especially as healing scaffold for wound dressing (26).

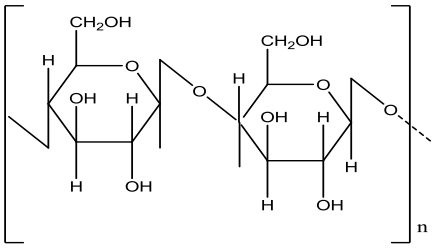

Fig. 5. Structure of *Cellulose*

Chitosan is a naturally derived cationic polysaccharide comprising of N-acetyl glucosamine and D-glucosamine units (fig. 6). Its low toxicity with versatile biological activities such as antimicrobial, low immunogenicity and wound-healing have provided ample opportunities for further development of biomaterials for wound dressing applications (27).

Due to its polycationic nature, chitosan inhibits the growth of a wide variety of bacteria and fungi, showing broad spectra of antibacterial activity, high killing rate and low toxicity (28).

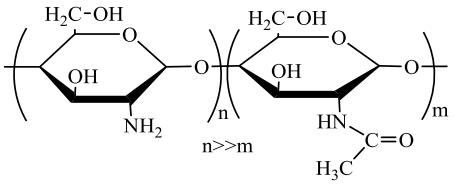

Fig. 6. Structure of *Chitosan*

Carrageenan (fig. 7) is a generic name for a family of polysaccharides, obtained by extraction from some species of red seaweeds (*Rhodophyta*). Carrageenans are widely utilized due to their excellent physical properties such as thickening, gelling and stabilizing abilities (29). Over time, carrageenan was formulated in the form of wound dressings for successful wound healing (30).

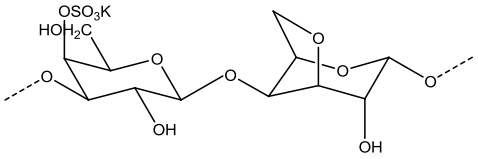

Fig. 7. Structure of *Carrageenan*

SYNTHETIC POLYMERS FOR WOUND DRESSINGS

Nanofibers based on synthetic polymers are prepared by several techniques, especially by electrospinning. Synthetic electrospun scaffolds containing materials such as *poly-caprolactone* (PCL) were found to have good mechanical properties and good biodegradability (31). In addition, other polymers such as *lauric acid*, *polyethyleneglycol*, *polyvinylidene fluoride* have been obtained *via* electrospinning process and the resulting fibers were found to have good mechanical strength and high thermal stability (32).

Poly-(lactic-co-glycolic acid) (PLGA) (fig. 8) is an excellent material for tissue engineering scaffolds because the high surface area allows a high degree of cellular attachment, as well as multiple focal adhesion points on nano-sized fibers (33).

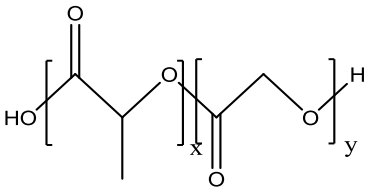

Fig. 8. Structure of *Poly-(lactic-co-glycolic acid)*

Poly-(ethylene-oxid) (PEO) (fig. 9) is a synthetic polymer easy to spin, and has been used for different applications such as drug delivery. Over time, various studies on electrospun PEO/water solutions were aimed at determining the effects of electrospinning process (34).

Fig. 9. Structure of *Poly-(ethylene-oxid)*

Poly-(vinyl alcohol) (PVA) (fig. 10) is a water soluble polymer, nontoxic, biocompatible, biodegradable which has been widely used in biomedical, plastics and textile fields. In the medical field, PVA nanofibrous matrix has been also used for biomimetic wound dressings (35).

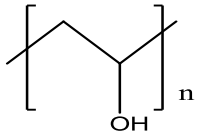

Fig. 10. Structure of *Poly-(vinyl alcohol)*

INFLUENCE OF SOLVENTS ON THE FORMATION OF NANOFIBERS

Solvents used in making polymer solutions are selected on the basis of polymer solubility. Even in these conditions, the solvent with the highest solubility is not always preferable for the electrospinning process. Fluid parameters, like solution surface tension, viscosity, density, conductivity, dielectric constant, are affecting the size and morphology of the electrospun fibers (36).

Some experiments for testing the effect of solvents on the morphology of electrospun polymers have been made. Thus, solvents like acetone, chloroform, dichloromethane, tetrahydrofuran, toluene, hexafluoro-2-propanol, trifluoroethanol have been used to obtain clear polymer solutions to be then processed in nanofibers form (37). The low boiling points of solvents such as of dichloromethane (40^0C) or chloroform (62^0C) determine fast solvent evaporation which leads to porous appearance (fig. 11)

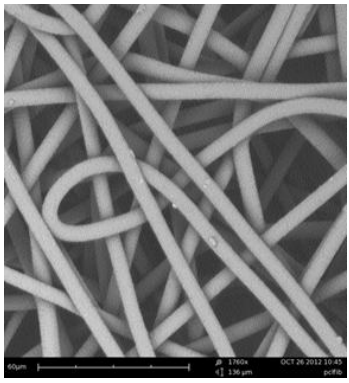

Fig. 11. PCL nanofibers from chloroform

CONCLUSIONS

Based on the complexity of wound healing process it is essential to have an ideal membrane which can combine all the above

mentioned features into one single design, to address the different aspects of a dynamic wound dressing biomaterial. In this context, the use of three-dimensional polymeric scaffolds plays an important role in the treatment of wounds. The attention of researchers, focused on the study of biocompatible and biodegradable natural or synthetic polymers has produced new types of dressings to be used in regenerative medicine. However, for the treatment of burns the most favorable materials are based on natural polymers such as chitosan, dextran or cellulose.

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ANTIOXIDANT AND PHYTOBIOLOGICAL STUDIES ON TWO *ALLIUM CEPA* L. EXTRACTS

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ANTIOXIDANT AND PHYTOBIOLOGICAL STUDIES ON TWO *ALLIUM CEPA* L. EXTRACTS (Abstract). **Aim:** To investigate the antioxidant potential of two *Allium cepa* L. extracts. **Material and methods:** The antioxidant activity of the two extracts (encoded EC1 and EC2) was assessed using two methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity and determination of their reducing power; phytobiological studies were performed using grains of *Triticum aestivum* L., *Falmura* variety**. Results and discussions:** EC1 and EC2 extracts had a particular affinity for binding DPPH radicals. At equivalent concentrations, the reducing power of EC1extract was about 2 times higher compared to EC2, increasing with the concentration of the analyzed sample. The phytobiological study demonstrated that the investigated extracts had no cytotoxic and genotoxic potential. **Keywords:** ANTIOXIDANT ACTIVITY, *ALLIUM CEPA*, PHYTOBIOLOGICAL

Onion (*Allium cepa* L.) is widely used around the world as a food product, but also for its medicinal applications. Onion contains many active sulfur compounds, mainly cysteine derivatives, as S-alkyl cysteine sulfoxides, which decompose into a variety of thiosulfinates and polysulfides under the action of the enzyme alliinase (1). They possess antidiabetic, antibiotic, hypocholesterolemic, fibrinolytic and other various biological actions. Approximately 90% of the soluble organic bound sulfur is present as γ-glutamylcysteine peptides, which are not substrates for alliinase (2, 3).

Flavonols are present in onion tissues in the form of free quercetin and kaempferol and as the glycosides of those compounds. The main sapogenins from onion are: sitosterol, oleanolic acid, diosgenin, β-chlorogenin and cepagenin (4).

Over time, onion has received a special attention from researchers, who observed its traditional therapeutic uses. Numerous studies have revealed a multitude of biological actions: antibiotic activity (antibacterial and antifungal), action on the cardiovascular system (antiplatelet, lipid lowering), on the respiratory system, antidiabetic, anticancer, antiinflammatory activities (5, 6).

MATERIAL AND METHODS

Extract preparation: Onion extracts

were prepared using fresh bulbs of *Allium cepa* L. (*Wolska* variety) purchased from a local market. The onion bulbs were peeled, cut into small pieces and allowed to stand for 18 h. Then, the product was soaked in absolute ethanol for 10 days at room temperature, with intermittent shaking. The extractive solution was concentrated using a rotary evaporator (under reduced pressure) at 40 °C. The extract encoded EC1 was obtained with a DER (drug extract ratio) of 5:2.

In order to obtain the extract encoded EC2, the onion bulbs were peeled and homogenized in cold water in a blender. The homogenized mixture was filtered through cheesecloth, the filtrate was centrifuged and the clear supernatant was diluted to 100 ml with cold distilled water. The EC2 extract had a DER of 1:2. The aqueous extract was stored at -20°C until use.

DPPH scavenging activity: The scavenging activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. When a solution of DPPH is mixed with an extract that can donate a hydrogen atom, the reduced form of the DPPH radical is generated, leading to the reduction of the violet color (7). Briefly, 50 µL of sample (EC1 and EC2) was mixed with 2.95 mL DPPH solution (4 mg %). After 5 minutes, the absorbance was recorded at 517 nm using a V-550 Jasco spectrophotometer. Quercetine was used as positive control. The radical scavenging activity was expressed as percentage of inhibition and was calculated as follows:

Scavenging effect (%) = (Absorbance of control-Absorbance of test)/Absorbance of control) X100

Reducing power: The reduction potential of EC1 and EC2 extracts was determined taking into account the fact that substances which act like reducing agents can react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex, that has a maximum of absorption at 700 nm (8). Ascorbic acid was used as positive control.

Phytobiological studies: During our research on EC1 and EC2 extracts, phytobiological studies using grains of *Triticum aestivum* L., *Falmura* variety were performed (9). Three experimental samples (encoded V1, V2 and V3) were used for each set of tests, these representing different concentrations of extract (tab. I).

TABLE I
Experimental samples for phytobiological studies

Treatment	EC1 (mL%)	EC2 (mL%)
Control sample (M)	-	-
V1	1	1
V2	2.5	2.5
V3	5	5

The studied aspects were the influence of EC1 and EC2 extracts on plantlet germination, elongation and biomass accumulation and the impact of the extracts on mitosis in root tissues.

Groups of 200 wheat grains were used for both the control and each dilution; these were soaked for 24 hours in 50 mL of each experimental sample and in the same volume of distilled water for the control. 150 wheat seeds were washed and placed uniformly upon a system made of sieves, with plastic vessels filled with water placed below. The cultivation was performed in an air-conditioned chamber at an average air temperature of 22±2ºC and constant light of 2.500 lux. After 7 days, the germination was stopped, the seed germination rate was

calculated and the shoot and root length was measured. Seedlings having less than 50 mm in length (both root and stem) were excluded from the experiment. Dry weight of roots and shoots for each treatment version was determined, after oven drying at 40 ºC for 24 h.

On day 10 the roots and shoots of the wheat seedlings were detached and weighed. After the determination of their fresh weight (FW) the seedlings were maintained in an oven at 105 ^{0}C for 24 h and weighed again in order to obtain the dry weight (DW). Their average fresh and dry mass was calculated for each experimental sample and also for the control. But, in order to objectively assess to what extent the biostimulatory or bioinhibitory effect was real, the ratio DW/FW x 100 was calculated.

The remaining 50 pre-imbibed grains were placed in 10 cm diameter Petri dishes, on filter paper wet with distilled water. After 72 h, the primary roots were cut off and a squash preparation stained with Schiff reagent was made. Both normal ana- and telophases and those with different types of aberrations (bridges, fragments, fragments and bridges, tri- and tetrapolar anaphases, chromosomes in ring) were counted.

RESULTS AND DISCUSSION

Considering the mechanisms that express the antioxidant activity, the ability to give up a hydrogen atom (or an electron) of the investigated EC1 and EC2 extracts was lower compared to that of 5 mM quercetine, used as control.

Qualitative and quantitative differences concerning the active ingredients with antioxidant potential (flavones, polyphenolic acids, sulfur compounds) present in EC1/EC2 extracts (10) explained why the DPPH scavenger activity was higher for EC1 extract compared to EC2 (fig. 1).

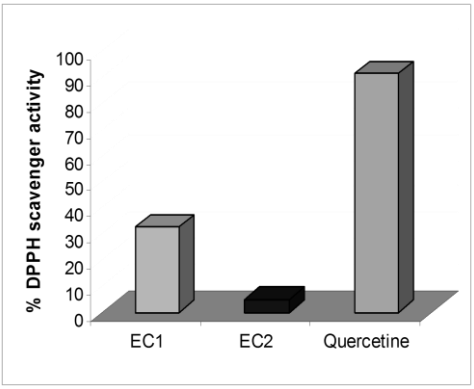


Fig. 1. DPPH scavenging activity of EC1 and EC2 extracts

The increase in absorbance was directly correlated with the reducing power, parameter that can serve as a significant indicator of the antioxidant potential. Data analysis suggested that at the same concentration the investigated extracts had different reducing abilities: at the concentration of 500 mg/mL EC1 extract had a reducing potential equal to that of 6.7 mmol/L ascorbic acid, while the EC2 reducing potential corresponded to that of 3.76 mmol/L ascorbic acid (fig.2).

The experimental data showed a low percentage of inhibition of *Triticum* seed germination for the investigated extracts. The influence of the treatment with onion extracts on the growth of *Triticum aestivum* plantlets was studied by length measurement of roots and shoots (tab. II).

As to the increase in root length, this parameter was generally positively influenced, except for sample V1 for EC1 and EC2 extracts.

The influence of EC1 and EC2 extracts on biomass accumulation is shown in table III.

In the case of EC1 extract, sample V2 caused a slight increase in root biomass accumulation, in both fresh and dried

plants. Fresh and dry shoot biomass accumulation was similar but lower than in the control (fig. 3, 4).

According to the results presented in figs. 3 and 4, it can be stated that the treatment of the caryopses with EC1 and EC2 extracts did not significantly influence the biomass accumulation in the wheat plantlets.

The cytogenetic analysis showed that the frequency of chromosomal aberrations did not exceed 4% for all treatment samples, this value being considered normal for chromosomal aberrations during *in vivo* phytotoxicity testing (fig. 5).

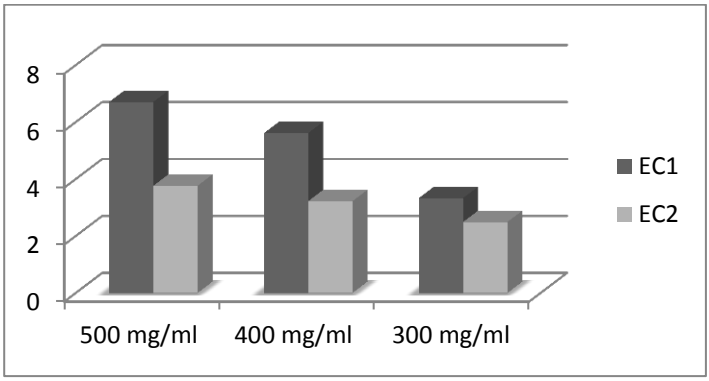


Fig. 2. Reducing power of the investigated extracts

TABLE II

Effect of EC1 and EC2 extracts on germination rate and root and shoot length

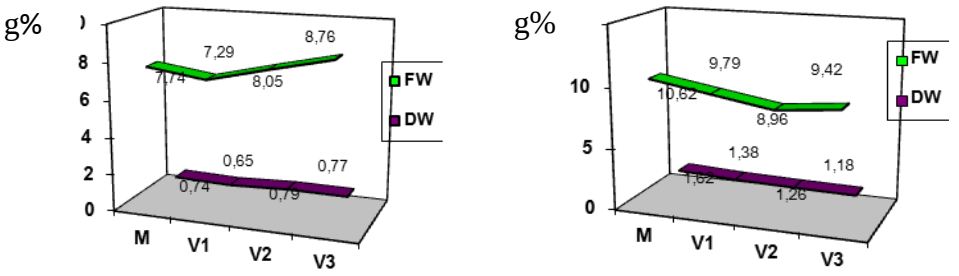
Treatment	Sample of treatment	Germination rate %	Root length (mm) ± SD	Shoot length (mm) ± SD
Water	M	93	119.97 ±12.40	149.34 ±12.17
EC1	V1	92	108.55±11.13	144.28 ±14.44
	V2	91	134.07±13.66	146.93±15.28
	V3	85	139.8 ±17.44	148.8 ±15.08
EC2	V1	87	114.76±13.19	146.5 ±14.06
	V2	90	138.8 ±15.19	143.80±16.86
	V3	68	128.2 ±13.82	144.36±12.35

TABLE III

Influence of EC1 and EC2 extracts on biomass accumulation

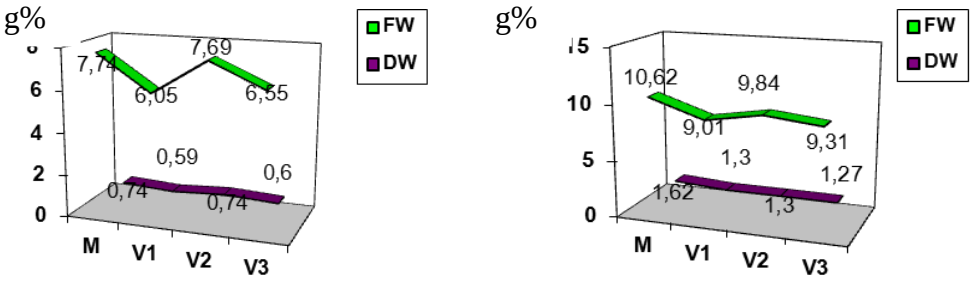
Treatment	Sample of treatment	Root		Shoot	
		FW (g)	DW (g)	FW (g)	DW (g)
water	M	7.74	0.74	10.62	1.62
EC1	V1	7.29	0.65	9.79	1.38
	V2	8.05	0.79	8.96	1.26
	V3	8.76	0.77	9.42	1.18
EC2	V1	6.05	0.59	9.01	1.30
	V2	7.69	0.74	9.84	1.30
	V3	6.55	0.60	9.31	1.27

FW=fresh weight; DW=dried weight



a b

Fig. 3. Fresh and dry biomass accumulation in *Triticum* roots (a) and shoots (b) under EC1 treatment



a b

Fig. 4. Fresh and dry biomass accumulation in *Triticum* roots (a) and shoots (b) under EC2 treatment

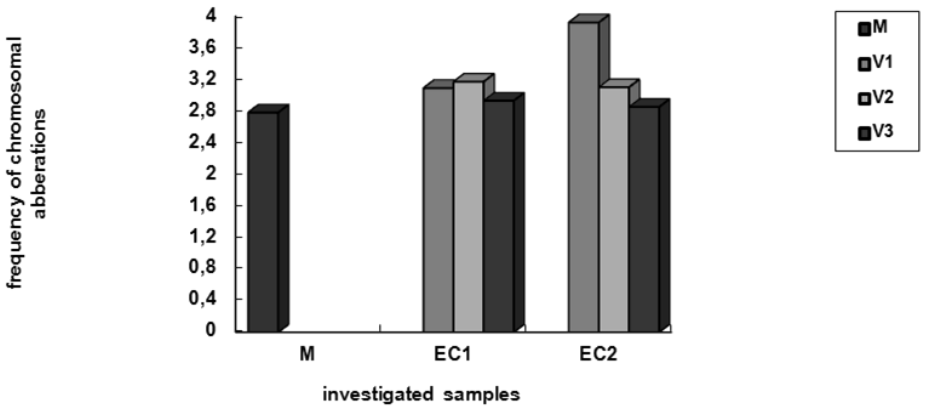


Fig. 5. Frequency of chromosomal aberrations in *Triticum aestivum* root meristem induced by treatment with EC1 and EC2 extracts

CONCLUSIONS

The results revealed that EC1 and EC2 extracts possess an interesting antioxidant activity.

According to the phytobiological studies, the biostimulatory or bioinhibitory

effects of the investigated extracts were not significant compared with the blank.

The results indicated that the extracts were well tolerated by plant cells, revealing the lack of cytotoxicity and mutagenicity.

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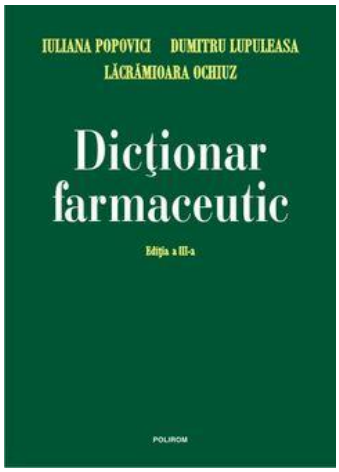
BOOK REVIEW



PHARMACEUTICAL DICTIONARY, Third Edition, 2014

Authors: Iuliana POPOVICI, Dumitru LUPULEASA, Lacramioara OCHIUZ

Preface: Prof. Dr. Medic Valeriu RUSU, member of the Romanian Academy of Medical Science

Publisher: Polirom, Iaşi, BIOS Collection

Description:

The Pharmaceutical Dictionary is a complex work that contains a multi-, inter-, and cross-disciplinary approach that has the purpose of forming and improving the scientific vocabulary of the new generation of pharmacists according to the latest standards of the field. The dictionary contains the latest and most up to date pharmaceutical and medical terminology. Its target audience consists of primarily the students of the Pharmacy and Medicine faculties, but also of pharmacists, physicians, MA graduates, resident physicians, PhD students, scientists working in the cosmetic and pharmaceutical industries, academic personals, researchers on drug development and other professionals working or studying in fields akin to those of medicine and pharmacy.

The third edition of the Pharmaceutical Dictionary has been updated thoroughly and contains eight sections: Introduction; The Evolution of Medical and Pharmaceutical Terminology; Abbreviations; Synonyms and Symbols; Etymologic Guide; Index Terms; Index of Patronyms; Annexes and Bibliography.

The *Dictionary* in Numbers and Figures:

- authors: 3;
- devised with the combined contributions of 45 experts;
- six Universities of Medicine and Pharmacy are represented (Iaşi, Bucureşti, Târgu–Mureş, Timişoara, Arad and Chişinău – Republic of Moldova), as well as pharmaceutic companies;
- it contains:
  - over 18,000 terms;
  - short biographies of over 390 important people in this field;
  - 134 annexes;
  - 126 bibliographic sources;
  - 943 pages.

Contents:

The Pharmaceutical Dictionary contains essential terminology belonging to the fields of analytic chemistry, anatomy, anorganic chemistry, bio-chemistry, bio-pharmacy, bio-informatics, biology, clinical pharmacy, cosmetology, dermopharmacy, environmental and food hygienics, ergonomy, genetics, homeopathy, immunopharmacology, immunology, informatics, inframicrobiology, law,

mathematics, mathematical analysis, medicine, medicine analysis, microbiology, organic chemistry, pathology, parasitology, pharmaceutic bio-technology, pharmaceutical chemistry, pharmaceutical deontology, pharmaceutical marketing and management, pharmaceutical practice, pharmaceutical propaedeutics, pharmaceutical technology, pharmacodynamics, pharmacogenomics, pharmacognosis, pharmacokinetics, pharmacology, physiology, physiopathology, psychology, physics, social pharmacy and patient communication, spectroscopy, statistics, dental medicine, therapy, toxicology, virusology and so on.

Relevant new additions:

- The presentation of the evolution of medical and pharmaceutical terminology;
- The introduction of an etymological guide that explains the origin and composition of each term;
- An explanation of the abbreviations, synonyms and symbols that are frequently encountered in foreign scientific texts;
- The dictionary contains pharmaceutical and medical terms as well as a number of modern words and concepts that are frequently used in other languages; these terms are translated into Romanian and presented in alphabetical order, including their etymology, synonyms, abbreviations and definitions;
- An alphabetical list of patronyms – the most important and influent people in the history of medical and pharmaceutical sciences since antiquity to this day;
- An impressive number of annexes, grouped into eight sections, in tables containing exact or suggestive data, towards ensuring clear and accurate explanations for any pharmacist;
- The most recent and relevant bibliography and sources.

As noted by Prof. Dr. Valeriu Rusu in the preface of the *Pharmaceutical Dictionary*, the current context of international cooperation, built on the constant change of information and the continuous advancement of knowledge, urges any professional who strongly desires a career in the vast field of medicine to always be kept updated with the most recent developments in his or her field of practice. This makes the *Dictionary* a most important asset in the formation of any such professional.

PROTECTIVE EFFECT OF GRAPE SEED EXTRACT IN EXPERIMENTAL MENOPAUSAL SYNDROME

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PROTECTIVE EFFECT OF GRAPE SEED EXTRACT IN EXPERIMENTAL MENOPAUSAL SYNDROME (Abstract) Grape seed extract (GSE) is a natural product obtained from *Vitis vinifera* seeds; it has been used as adjuvant therapy in wide range of pathological conditions. **Aim:** In the present study, we investigated the ability of GSE to improve the mental status on ovariectomized rats. **Material and methods**: GSE (10 mg/kg/day, 60 days long) was administrated orally on ovariectomized adult female rats; then the animals were investigated in open field. Additionally, were measured the hematological parameters, the plasma biochemistry, and the oxidative stress markers. **Results and discussion**: Treatment of GSE improved the loco motor activity in the open field, the number of crossings were almost twice in GSE treated rats (22.25±8.75) compared to placebo group (13.00±2.62) ($p<0.05$). Lipid peroxides were reduced up to 51.57% ($p<0.05$), while hematology and plasma biochemistry remained almost unchanged. **Conclusion:** GSE improve the mental status and reduced the level of oxidative stress in rats subject to experimental ovariectomy. **Keywords:** POLYPHENOLS, OXIDATIVE STRESS, OVARIECTOMY, OPEN FIELD

Menopause is the cessation of spontaneous menstrual cycling; the average age of menopause in women is approximately 50 years, resulting a post reproductive period for one third of their lives. A number of other physiological systems are also affected by the sudden withdrawal of hormonal support associated with menopause, including bone density, cardiovascular health, cognition and possibly some cancers. Finding an appropriate model is a debated subject, but the use of rodent models is manifold, because they have a relatively low life span, ovarian function is well characterized and homogenous laboratory strains allowed controlled experiments (1). This experimental model is widely used to prove the efficiency of various plant compounds against menopause associated pathology; polyphenols from green tea proved to have a protective effect against bone loss in on ovariectomized female rats in non dose dependent manner, the same osteprotective effect was provided by phytoestrogens like coumestrol and zearalanol (2); on the other hand, soy isoflavone prevent oxidative changes in hepatocytes (3) and flavones from *Radix puerariae* reduce the hepatic

accumulation of triglycerides (4).

Grape seed extract (GSE) is a natural product obtained from *Vitis vinifera* seeds; and they are a byproduct of wine. Nowadays, the interest for GSE is fully justified, they contain polyphenols, mainly flavonoids, proanthocyanidins, galic acid, cathechins etc. The most important constituents are the polyphenoles, which act not only as effective free radical scavenges, but they modulate de activity of antioxidant enzymes, block hormone receptors, and reduced the activity of various mutagens. Experimental and clinical studies show that polyphenols protect the blood vessel, prevent the platelet aggregation, and lower the LDL-cholesterol.

MATERIAL AND METHODS

Nutritive supplement. Grape seed extract (GSE) was a commercial capsulated product 100 mg / capsule, a generous gift of TC Alsifcom Ltd. The hydro soluble premix (extracted with hydro alcoholic solutions), was manufactured by Eurochem Feinchemie GmbH (batch number 4001103K001). The analysis bulletin was performed by high performance liquid chromatography (HPLC); its composition was as follow catechins 2-3%, epicatechins 2-3 %, proanthocyanidin oligomers (bi- and trimetric) 6-9%, proanthocyanidin (polymers) 89 %, total of proanthocyanidin oligomers were over 92%. The product was within acceptable range of microbiological contamination and residues. The animals in study received 10 mg/kg/day, dissolved in water, immediately before administration, for all experiment long.

Animals. Experiments and animals welfare were according to the *Guide for the Care and Use of Laboratory Animals* (Department of Health Education, and Welfare, National Institute of Health, 1996), and followed the guidelines of European Communities Council Directive (86/609/1986) and Ordinance No. 37 of the Romanian Government from 2nd February 2002. The animal tests and experiments were allowed by the Bioethical Board of the Faculty of Veterinary Medicine Cluj-Napoca. The animals were caged in polycarbonate cages, at controlled temperature of 21-22ºC, humidity (40-60%) and 12/12h light/dark cycle. Standard lab chow, soy free (to avoid any source of estrogens), provided by National Institute for Research and Development Cantacuzino Bucharest, and water were freely available.

Experimental protocol. Experiment was carried out on 24 albino Wistar female rats, 232.75±8.52 g body weight, divided into tree equal groups, of 8 animals each. Groups I was the sham operated group, it represents the control. Groups II and III were subject to ovariectomy, and, additionally, 5 days after surgery, group 3 received GSE therapies orally, by using a lubricated catheter, daily, for 60 days long, until the end of the study. The other two groups received isotonic saline solution as placebo. In the end, the animals were subject to open field test; blood samples were drawn under deep narcosis and, than, euthanasia was made. Immediate latter, the animals were subject to gross examination and uterus weight was determined.

Surgical procedure. The surgery was performed under isoflurane narcosis, supplemented by neuroleptanalgesia by xylazine 0.2% (Narcoxyl) 0.2 ml/kg and ketamin 10% 0.3 ml/kg injected intramuscularly. A longitudinal incision was made along vetromedian area, between umbilical region and pubis; the ovaries were exteriorized, and excised. Abdominal muscles and skin were sutured with Dexon and polyacrilate respectively. The sham operated

animals were subject to laparotomy, but whiteout removal of the ovaries. The subjects were allowed 5 days to recover from surgery before starting the experiments.

Open Field Test. Open field test was run within in a box measuring 60x40x50 cm, whose floor was divided by lines into 12 equal squares. Animals were placed facing the rear left corner of the arena and observed for 3 min. Number of squares crossed with the four paws from one square to another was indicative of motor activity. Measuring score included defecation, urination and ambulations.

Laboratory analysis. Blood hematology was investigated immediately, using EDTA anticoagulated blood, with Abacus Junior Vet, automatic analyzer Diatron Messtechnik, Budapest, Hungary. For biochemical analysis, the blood drawn in clot activator vacutainers, was centrifuged at 2000 rpm for 10 min to separate the serum, immediately after separation serum samples were frozen at – 20 ºC. Blood chemistry (cholesterol, triglyceride, alkaline phosphatase etc.) was measured using screen point semiautomatic analyzer, STAT – FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd. Ramsey Minnesota, 55303 USA, by using special determination kits.

Lipid peroxides were determined, by measuring the production of tiobarbituric reactive substances according to the method of Burge and Aust, protein carbonyl were evaluated by using guanidine chloride. Ravin method (parafenildiamin dichloride) was the choice for measuring the plasma ceruloplasmin, while Hatano (diphenyl picrichydroxil) method was preferred for measuring hydrogen donor ability.

Statistics. All data are reported as the mean ± SEM. The Gaussian distribution was checked by Shapiro-Wilk normality test. One-way analysis of variance ANOVA, followed by post hoc Dunnett's range test procedure was done Statistical significance was at $p<0.05$ (95% confidence interval). Statistical values were obtained using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego California USA.

RESULTS AND DISCUSSION

The mean body mass increased significantly in both ovariectomized groups (II and III) compared to group I ($p<0.05$), body weight gain was associated with high amount of abdominal fat. Increasing in body weight and abdominal fat accumulation is a widely accepted side effect of ovariectomy as a consequence of sudden drop in estrogen level (4). Expectedly, the uteri of rats from groups II and III revealed severe atrophy, highly significant as compared to group I ($p<0.001$). GSE therapy has no influence the body weight or the degree of uterus involution, therefore GSE does not seem to exert any estrogenic effect on the genital organs or other body areas (tab. I).

TABLE I

Variation of body weight and uterus weight

	b.w. initial (g)	*b.w. final (g)*	*dif b.w..*	*Uterus*	*% of b.w..*
Control	215.20±18.09	225.00±28.78	09.80 ±27.10	0.700±0.200	0.310±0.080
Ovariectomy	248.75±05.30	286.50±17.67	37.75*±12.37	0.195±0.007**	0.068±0.006*
Ovariectomy +GSE	177.33+11.45	222.83+13.24	45.50+21.54	0.180+0.070	0.081+0.033

Result expressed as mean ± SD, b.w. = body .weight. GSE = Grape seed extract

* and ** = statistically significant as compared to Control group at $p<0.05$ and $p<0.001$

Oxidative stress markers were increased following ovariectomy, but significant results we found only for malonildialdehyde ($p<0.05$). The therapy was effective, the values in group III were significant lower than in group II ($p<0.05$), and they were similar to group I rats. In menopausal women, is already proved a clear relationship between the plasma levels of malonildialdehyde and the logarithm of the plasma estradiol concentrations, which was best demonstrated with a quadratic regression (5), estrogens having a well known antioxidant protective effect. The antioxidant protective effect of GSE is not surprising; it was already proved on various experimental models (tab. II).

TABLE II

Variation of oxidative stress markers

	Malonildialdehyde (nmol/ml)	Protein carbonyl (nmol/ml)	Hydrogen donors ability (%)
Control	5.8±1.74	1.54±0.76	31.06±6.95
Ovariectomy	11.13±2.95*	1.20±0.35	34.66±10.13
Ovariectomy +GSE	5.74±1.29†	0.80±0.15	28.24±8.66

Result expressed as mean ± SD, GSE = grape seed extract

* = statistically significant as compared to Control group at $p<0.05$

† = statistically significant as compared to Ovariectomy group at $p<0.05$

Plasma biochemistry showed increase serum alkaline phosphatase (AP) activity in both groups II and III (184.7±73.5 u/l, and 164.7±53.3 u/l, respectively) as compared to I (137.8±37.2 u/l,) ($p<0.05$). The relation between the loss of bone density and elevated activity for AP is widely accepted; so, antiosteoporotic therapy also down regulates the AP activity (2). Similar values of PA between groups II and III suggest that GSE failed to prevent the bone destruction. Other blood enzymes (GGT, ASAT and ALAT) reveal no difference among groups in study, so were the other biochemical compounds (triglycerides, cholesterol, urea, creatinine, glucose, total bilirubin, total protein, and calcium). Hematology was also, within normal limits for all groups. In female Guinea pigs, subject of ovariectomy and a high cholesterol diet, the GSE supplementation reduced the plasma triglycerides. Even that cholesterol levels remained high, the VLDL cholesterol was diminished, and, by down regulation of cholesterol acyle transferase, GSE prevents the cholesterol accumulation into the aortic walls (6). Furthermore, GSE proanthocyanidins prevent the posprandial peroxidation of lipids, and peroxidation of LDL (7). Clinical studies also confirm the protective effect of GSE against menopausal associated dyslipidemia; it reduced the plasma triglycerides and LDL, by diminishing the systemic inflammatory status and oxidative stress (8). In our findings, the ovariectomy was not associated with any obvious impairment of excretory, liver or metabolic function, probably because the animals received a balanced diet, whiteout atherogenic compounds, which unrevealed a possible protective effect of GSE on lipid metabolism. Our findings correlated to literature data, suggest once again that, in addition to hormonal imbalance, dyslipidemia associated with menopausal syndrome have an important alimentary etiology.

TABLE III
Results of the *Open field* test

	N. of rearing	No.of defecations	No.of crossings
Control	7.75±2.92	3.13±1.13	27.00±7.45
Ovariectomy	2.87±1.55††	0.75±0.89††	13.00±2.62††
Ovariectomy +GSE	5.00±4.30	6.25±2.65** †	22.25±8.75*

Result expressed as mean ± SD, GSE = grape seed extract
* and ** = statistically significant as compared to *Ovariectomy* group at $p<0.05$ and $p<0.01$
† and †† = statistically significant as compared to *Control* group at $p<0.05$ and $p<0.01$

Ovariectomy was responsible for reducing the motility, reflected in number of rears, in squares crossings and also the emotive expressed in defecations ($p<0.01$). It not a widely accepted opinion that ovariectomy is able to induce the decreasing of motility, Monteiro, *et al*. (9) assumed that ovariectomy did not affect the number of crossings, but other studies, relate the response to open field test to number hormonal status (10). However, the GSE proved a significant ameliorative effect, the number of defecations and crossings were improved in a significant manner ($p<0.05$), and distinctly significant manner ($p<0.01$) respectively.

Even various plant compounds have antidepressant properties; relatively few studies on GSE were done. Therefore, the mechanism remain obscure, but is rational to suppose that this improvement in mental status is subsidiary to general tonic effect provided by the plant compounds, because clinical evaluation revealed a much better condition in treated animals as compared to other groups, including sham operated animals.

In post menopausal women, the impairment of cognitive functions is not only induced by the estrogenic withdrawal, but also with degenerative phenomena inherent to aging process, many related to increased oxidative damage. Diminished efficiency of brain antioxidant systems in elderly rats leads to an intense formation of lipids peroxides (11, 12), carbonyl proteins, reduction of total donor hydrogen ability (11), functional impairment of cholinergic system (12), and consequently, loss of memory (11). GSE prevents, to some extent, these side effects of aging, not as a simple antioxidant only, but as an inductor of the antioxidant enzymes CAT and SOD (12). The inductor mechanism of GSE was further proved on gene expression of antioxidant enzymes *in vitro*, on HepG2 hepatocarcinoma cell line (13).

CONCLUSIONS

On rat ovariectomy model, GSE prevented the plasma lipid peroxidation and improved the cognitive functions, but it did not affect the level of uterine involution and the body weight gain. These findings recommend it as possible alternative remedy against menopausal associated pathology.

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ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF EXTRACTS FROM *GLINUS OPPOSITIFOLIUS* AERIAL PARTS

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ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF EXTRACTS FROM *GLINUS OPPOSITIFOLIUS* AERIAL PARTS (Abstract): In Malian traditional medicine, aerial parts of *Glinus oppositifolius* are used in the treatment of many disorders, particularly inflammations and joint pains. **Aim**: The present study was initiated to investigate the antioxidant potential of *Glinus oppositifolius*. **Material and methods**: Aqueous and ethanol extracts were prepared from the dried aerial parts. Both extracts were examined for the phenolic content and antioxidant activity. Total phenolic content was quantified by Folin-Ciocalteu method. Antioxidant activity was studied *in vitro* by evaluating the ability to scavenge different free radicals (ABTS, superoxide and hydroxyl radicals, nitric oxide) and to chelate ferrous ions. **Results**: Aqueous extract was found to contain a higher level of total phenols than ethanol extract (1.27±0.04 *vs.* 0.94±0.05 g GAE/100 g extract). Both extracts lacked the ability to scavenge superoxide anion radical but scavenged ABTS and hydroxyl radicals, nitric oxide and chelated ferrous ions in a concentration-dependent manner. According to the EC_{50} values, aqueous extract showed stronger antioxidant effects than ethanol extract (174.32±1.49 *vs.* 276.76±1.52 µg/mL in ABTS radical cation scavenging assay, 119.91±1.70 *vs.* 240.57±0.97 µg/mL in nitric oxide scavenging assay, 1.99±0.01 *vs.* 6.54±0.08 mg/mL in ferrous ion chelating assay). In hydroxyl radical scavenging assay, aqueous extract had an EC_{50} value of 0.86±0.00 mg/mL while the EC_{50} value of ethanol extract was higher than the highest concentration that was tested (3.75 mg/mL). **Conclusions**: Our results clearly indicate the antioxidant potential of *Glinus oppositifolius* aerial parts and suggest that the anti-inflammatory activity might be partly related to the antioxidant potential. **Keywords**: *Glinus oppositifolius*, INFLAMMATION, OXIDATIVE STRESS, FREE RADICAL SCAVENGING ACTIVITY, FERROUS ION CHELATING ACTIVITY

Glinus oppositifolius (L.) Aug. DC. (*Aizoaceae*) is native to tropical and subtropical regions of the world, its leaves and aerial parts being used either as food or medicine in different ailments. In Malian traditional medicine the plant has been used in the treatment of joint pains, inflammations, fever, skin disorders, wounds, urinary

infections, abdominal pains, diarrhoea, intestinal parasitosis, malaria. In an attempt to valorize Malian traditional medicines, researchers from School of Pharmacy, University of Oslo and Department of Traditional Medicine, Bamako, Mali carried out chemical and biological studies on the aerial parts of the species. Triterpenoid saponins and pectic polysaccharides have been isolated and characterized. Pectic polysaccharides showed immuno-modulating activity by fixing the complement system components and increasing the chemotaxis of macrophages, NK cells and T cells (1-3).

Glinus oppositifolius is one of the herbs Malian traditional healers use in the treatment of inflammations and joint pains. In addition to an enhanced immune response, inflammation is characterized by an increased oxidative stress (4, 5). The aim of the present study was to evaluate a possible antioxidant activity involved in the anti-inflammatory potential of *Glinus oppositifolius*. In this respect, the antioxidant activity of aqueous and ethanol extracts was studied by evaluating their ability to scavenge free radicals and to chelate ferrous ions. The total phenolic content was also determined.

MATERIAL AND METHODS

Chemicals.

Tris(hydroxymethyl)aminomethane (Tris), ferrous chloride, sodium nitroprusside were purchased from Merck (Darmstadt, Germany). 2, 2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulphonic acid monosodium salt (ferrozine), glutathione, pyrogallol, Folin-Ciocalteu's phenol reagent were supplied by Fluka (Steinheim, Germany). Iron (II) sulfate heptahydrate, hydrogen peroxide, L-ascorbic acid, N-(1-naphtyl) ethylenediamine dihydrochloride, sodium salicylate, sulfanilamide, were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Gallic acid monohydrate and potassium persulphate were purchased from Riedel-de Haën (Seelze, Germany).

Plant material. Aerial parts of *Glinus oppositifolius* (L.) Aug. DC. were supplied by the Department of Traditional Medicine, Bamako, Mali where a voucher specimen is deposited.

Extraction. Dried and powdered aerial parts (100 g) were refluxed with 2 × 2 L ultrapure water at 50°C, each time for 4 h or extracted by stirring with 2 × 2 L ethanol at room temperature, each time for 72 h. The combined aqueous and ethanol extracts were concentrated under reduced pressure at 50°C (Büchi R-210 rotary evaporator, Büchi V-850 vacuum controller, Büchi V-700 vacuum pump, Büchi B-490 heating bath) and then freeze-dried (Unicryo TFD 5505 freeze-dryer).

Total phenolic content. Total phenolic content was estimated by Folin-Ciocalteu method (6). The results were expressed as g gallic acid equivalents (GAE)/100 g extract.

ABTS radical cation scavenging assay. ABTS assay was performed according to the method developed by Re *et al*. (7). Glutathione was the positive control.

Superoxide anion radical scavenging assay. The assay was performed as described by Wang and Luo (8). Glutathione was used as positive control.

Hydroxyl radical scavenging assay. The assay was done according to the method described by Jeong *et al.* (9). L-Ascorbic

acid was used as positive control.

Nitric oxide scavenging assay. The assay was performed as reported by Tsai *et al.* with minor changes (10). L-Ascorbic acid was the positive control.

Ferrous ion chelating ability assay. The assay was done according to Dinis *et al.* (11). EDTA was used as positive control.

Statistical analysis. All experiments were performed in triplicate. The results were expressed as means±standard deviations. The EC_{50} values were calculated by linear interpolation between values above and below 50% activity.

RESULTS

Extraction. After removing the solvents, 40 g aqueous extract (GA) and 23.7 g ethanol extract (GE) were obtained.

Total phenolic content. The total phenolic contents of GA and GE were found to be 1.27±0.04 and 0.94±0.05 g GAE/100 g, respectively.

ABTS radical cation scavenging assay. Both extracts scavenged ABTS radical cation in a concentration dependent-manner. At 100 µg/mL, GA and GE showed scavenging effects of 35.42±0.47 and 27.37±0.66%, respectively. At 400 µg/mL, the scavenging activity of GA and GE increased significantly to 73.75±0.15 and 65.12±1.01%, respectively. Within the same concentration range (100-400 µg/mL), glutathione completely scavenged the radical (99.47±0.29-99.68±0.02%) (fig. 1). According to the EC_{50} values, the abilities to scavenge ABTS radical cation varied as follows: glutathione > GA > GE (tab. I).

Superoxide anion radical scavenging assay. Within this assay, GA and GE showed no scavenging effects on superoxide anion radical.

Hydroxyl radical scavenging assay. GA showed a higher hydroxyl radical scavenging activity than GE. At the highest concentration tested (3.75 mg/mL), GA scavenged the radical in a percentage of 91.69±0.09% while GE showed only 31.33±0.74% scavenging activity. GA was less active than the positive control, L-ascorbic acid; at concentrations of 0.47-3.75 mg/mL, the latter exhibited 100% scavenging activity (fig. 2). With regard to the EC_{50} values, the hydroxyl radical scavenging activity decreased as follows: L-ascorbic acid > GA > GE (tab. I).

Nitric oxide scavenging assay. Both extracts showed dose-dependent nitric oxide scavenging effects. The scavenging activity of GA increased significantly from 42.10±0.57% at 83.33 µg/mL to 83.47±0.17% at 1333.36 µg/mL. At the same concentrations, GE exhibited scavenging effects of 22.37±0.70 and 86.59±0.46%, respectively. The effects of L-ascorbic acid were superior to those of GA and GE at all concentrations tested (fig. 3). According to the EC_{50} values, the nitric oxide scavenging effects decreased in the following order: L-ascorbic acid > GA > GE (tab. I).

Ferrous ion chelating ability assay. Ferrous ion chelating abilities of both extracts increased with concentration. At all concentrations tested, the chelating ability of GA was greater than that of GE but lower than that of EDTA. At the highest concentration tested (6.62 mg/mL), the chelating effects of GA and GE were 76.53±0.48 and 50.40±0.75%, respectively; at the same concentration, EDTA completely chelated the ferrous ions (fig. 4). The ferrous ion chelating ability decreased as follows: EDTA > GA > GE (tab. I).

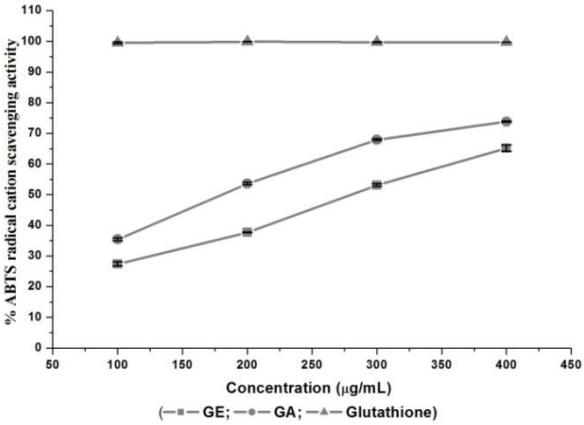

Fig. 1. ABTS radical cation scavenging activity

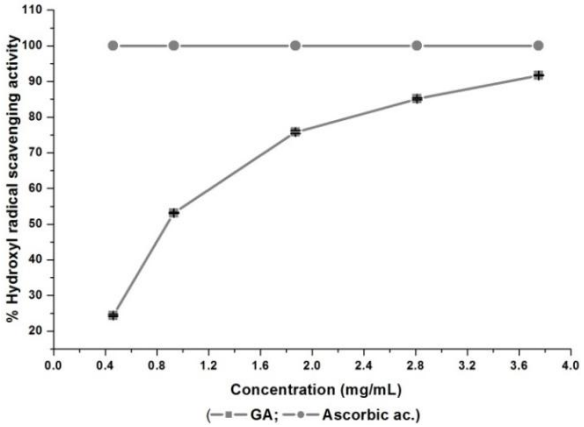

Fig. 2. Hydroxyl radical scavenging activity

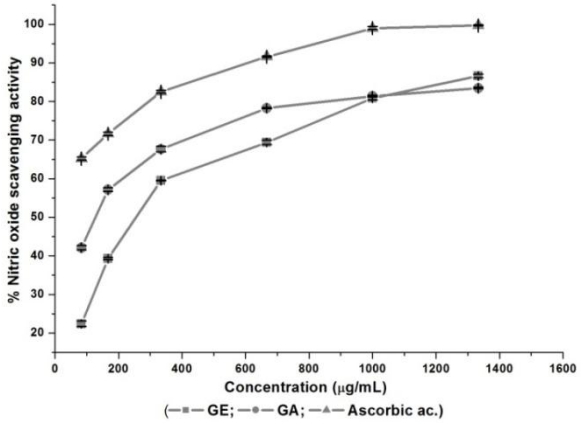

Fig. 3. Nitric oxide scavenging activity

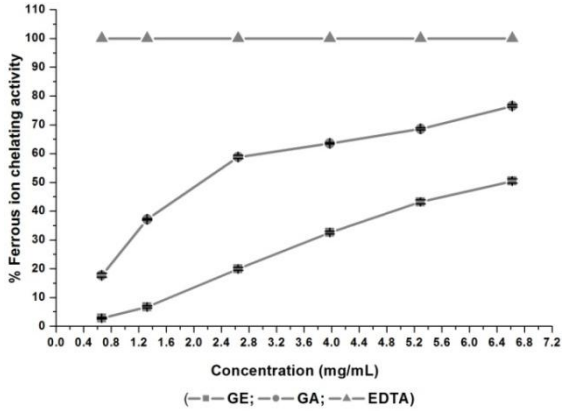

Fig. 4. Ferrous ion chelating activity

TABLE I

Antioxidant activities of *Glinus oppositifolius* (L.) Aug. DC. aerial part extracts

Extract/ Positive control	EC_{50}			
	ABTS radical cation scavenging activity	Hydroxyl radical scavenging activity	Nitric oxide scavenging activity	Ferrous ion chelating activity
GA	174.32 ± 1.49*	0.86 ± 0.00**	119.91 ± 1.70*	1.99 ± 0.01**
GE	276.76 ± 1.52*	> 3.75**	240.57 ± 0.97*	6.54 ± 0.08**
Glutathione	3.35 ± 0.01*	> 3.75**	-	-
L-Ascorbic acid	-	0.12 ± 0.00**	45.56 ± 0.45*	-
EDTA	-	-	-	6.3 ± 0.0*

GA – aqueous extract; GE – ethanol extract

* results expressed in μg/mL; ** results expressed in mg/mL

DISCUSSION

Numerous studies have reported the involvement of reactive oxygen species in inflammation. After infiltration to the inflammatory site, polymorphonuclear leukocytes and macrophages release high levels of superoxide anion radical and hydrogen peroxide (respiratory burst). Superoxide anion radical is able to release iron from ferritin as ferrous ions. Ferrous ions promote the generation of hydroxyl radical *via* Fenton reaction. Hydroxyl radical is the most aggressive reactive oxygen species being responsible for protein, lipid and DNA damage (4, 5, 12). In inflammation, inducible nitric oxide synthase (iNOS) generates high levels of nitric oxide. Nitric oxide enhances the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), but also of collagenases thus promoting the progression of inflammation (12). Nitric oxide in excess generates enhanced levels of reactive nitrogen species which target cellular macromolecules causing additional tissue damage (10).

This study indicated that *Glinus oppositifolius* extracts have antioxidant activity. GA and GE scavenged free radicals such as hydroxyl radical and nitric oxide and chelated ferrous ions. GA was found to be more active than GE. The stronger antioxidant activity of GA cannot be justified solely by its higher phenolic content; other

phytochemicals may also contribute to the antioxidant potential of GA. GA scavenged hydroxyl radicals and chelated ferrous ions with EC_{50} values of 0.86±0.00 and 1.99±0.01 mg/mL, respectively; in the same assays, GE showed weaker effects (EC_{50} > 3.75 mg/mL, EC_{50} = 6.54±0.08 mg/mL). In nitric oxide scavenging assay, GA (EC_{50} = 119.91±1.70 µg/mL) was only 2.6 times less active than L-ascorbic acid (EC_{50} = 45.46±0.45 µg/mL). As L-ascorbic acid is a strong nitric oxide scavenger (13), it is obvious that GA exhibited a very good scavenging activity. The high scavenging activity against nitric oxide, the ability to eliminate hydroxyl radicals and chelate ferrous ions make GA a good candidate for *in vivo* studies. Neither GA nor GE scavenged superoxide anion radicals. The lack of superoxide scavenging effects is surprising given that both extracts scavenged other free radicals. It is possible that certain phytochemicals in extracts act as prooxidants under the conditions of this assay thus masking the superoxide scavenging activity. It is worthy to note that there have already been reported prooxidant effects for certain flavonoids, alpha-tocopherol, coenzyme Q10, clove essential oil and butylated hydroxytoluene when tested in this assay (14, 15).

CONCLUSIONS

It can be concluded that the anti-inflammatory potential of *Glinus oppositifolius* is partly related to its antioxidant properties.

Further studies are needed to evaluate the *in vivo* significance of the antioxidant activity and its role in the regression of inflammation.

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NEWS

CEREBROPROTECTIVE EFFECTS OF IBUPROFEN ON DIABETIC ENCEPHALOPATHY IN RATS

A group of researchers from Xuzhou Medical College and Nanjing General Hospital of Nanjing Military Command, Jiangsu, China investigated the effects of ibuprofen on diabetic encephalopathy and the potential mechanisms. Diabetes was induced through a single intraperitoneal injection of streptozotocin (60 mg/kg). The diabetic rats were randomly divided into two groups: the control and the treated groups. The treatment was initiated 7 days after induction of diabetes and consisted in oral administration of ibuprofen (40 mg/kg) for 8 weeks. Cognitive performances were evaluated using Morris water maze. The temporal cortex and hippocampus were obtained to evaluate the levels of advanced glycation endproducts (AGEs) and their receptor (RAGE), the activity, protein expression, and mRNA levels of β-amyloid precursor protein cleaving enzyme 1 (BACE1), the protein and mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ), and the protein expression of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS). Blood was obtained for the evaluation of interleukin 1β level. The research revealed that chronic ibuprofen treatment reduced the mean escape latency of diabetic animals and significantly prevented the decline in learning and memory ability of diabetic rats. Ibuprofen treatment recovered the enzymatic activity of BACE1 to the control level in the temporal cortex, while markedly reduced enzymatic activity in the hippocampus of diabetic rats. Moreover, ibuprofen treatment reduced the activity, protein, and mRNA levels of AGE level, protein expression of RAGE, COX-2, and iNOS in the brain, and interleukin 1β level in serum, while increasing the protein and mRNA expression of PPARγ in the brain of diabetic rats. The present study demonstrated that ibuprofen ameliorated diabetic encephalopathy caused by type 1-diabetes, as evidenced by the improvement of cognitive dysfunction and the loss of neurons in the hippocampus. Furthermore, the amelioration of diabetic encephalopathy by ibuprofen was achieved by the down-regulation of BACE1, the suppression of the AGE/RAGE axis and inflammation in the brains of diabetic rats. (Liu Y-W, Zhu X, Zhang L, Lua Q, Zhang F, Guoa H, Yin X-X, Cerebroprotective effects of ibuprofen on diabetic encephalopathy in rats. *Pharmacol Biochem Behav* 2014; 117: 128-136)

Ioana Vasincu

FUNCTIONAL ANATOMY OF THE TEMPORO-MANDIBULAR JOINT (II)

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FUNCTIONAL ANATOMY OF THE TEMPORO-MANDIBULAR JOINT (II) (Abstract): Jaw movement is analyzed as an action between two rigid components jointed together in a particular way, the movable mandible against the stabilized cranium. Opening and closing movements are symmetrical; that is, both sides of the cranio-mandibular articulation are making the same movements. Protrusive and retrusive movements may also be symmetrical. The mandibular muscles determine all the complicated postures and movements of the jaw. Their behavior can be greatly clarified by restating certain fundamentals crucial to purposive muscular activity. The joint derives its arterial supply from the superficial temporal artery and the maxillary artery. Branches of the auriculo-temporal and masseteric nerves and post-ganglionic sympathetic nerves supply the tissues associated with the capsular ligament and the looser posterior bilaminar extension of the disc. **Keywords**: TEMPORO-MANDIBULAR JOINT, JAW MUSCLES, POSTURES, MOVEMENTS, MANDIBLE.

Physicists point out that the most general type of motion a body can undergo is a combination of translation and rotation (1). Translation is defined as a motion in which all points of a rigid body move in the same direction with the same velocity, at any given instant, relative to the same point outside the body. Rotation is a motion in which some points of a rigid body move in one direction while other points move in the opposite direction, at any given instant.

Now it can be readily seen that a partially lowered mandible can translate, that is, more forward or backward over the articular eminence with all points of the jaw moving in the same direction at the same rate, at any given instant. Thus in simple lowering and rising of the mandible, the definitive statement must be that the condyle rotates relative to the disc while the disc is rotating relative to the articular eminence with concurrent translation of the mandible relative to the temporal articular faces. It is simply a problem of relative motion, and mandibular movements are completely consistent with the general observations in the physics of motion (2).

MOVEMENTS

Opening and closing movements are symmetrical; that is, both sides of the cranio-mandibular articulation are making the same movements. Protrusive and retrusive movements may also be symmetrical. In this case disc and condyles of both sides slide downward and forward and backward

and upward, respectively, with condyles and discs always, as in all joints of the body, in firm contact with their opposing articular surfaces. Movements may also be executed asymmetrically, which then produces a lateral movement, a swinging of the jaw to one side. In this case one disc, accompanied by its condyle, slides downward, forward, and medially on to the preglenoid and medial glenoid planes.

The disc and condyle on the side to which the jaw is moving (left condyle for left lateral movement) do not travel far from their starting positions on the posterior slope of the eminence. Here the condyle orbits very slightly downward then outward in a small arc around a vertical axis somewhere behind the condyle. This imparts a transverse component to the excursion and thus produces a direct lateral shift of the entire mandible to the chewing side. This has been called the Bennett movement, or Bennett shift, and although it may be slight (probably never more than 1.5 mm) it has been considered significant in certain cases of restorative and prosthetic dentistry (2).

The lower joints of both sides work together as a common hinge joint. The two discs are the sockets, or „hooks", of the hinges, and each condyle forms the bar in the eye of the hinge on which the jaws swings open and closed. Because of the notable obliquity of the condyles to the frontal plane - the condylar axes converge posteriorly - the hinge movement of the mandible must be accompanied by adjusting movements between condyles and discs. The hinge movement occurs around a horizontal axe that runs approximately through the centers of the two condyles. Thus, during opening, the lateral pole of each condyle, situated in front of the hinge axis, must move slightly downward and backward. Concomitantly, the medial poles, situated behind the hinge axis, must move slightly upward and forward.

This information is highly significant clinically in radiographic interpretation. Ignorance of these anatomic facts has resulted in the belief that condyle and eminence does lose contact (separate) during normal jaw movements, since a dense lateral pole is seen radiographically to move away from the eminence. But, as pointed out previously, areas of articular contact are constantly shifting during movement in all joints of the body; thus contact is shifting toward the medial pole in simple opening. This prevents concentration of pressure at any point for any length of time (3). The broad areas of the close-packed position are adapted to withstand the peak of functional pressure.

Under normal conditions, sliding in the upper joints and rotating in the lower joints are probably always combined, but the magnitude of one or the other at a given time varies considerably. Both mandibular joints, always working together as a functional unit, can be classified as a hinge joint with a sliding socket (4). This definition clarifies the proper role or functional significance of the articular discs; they comprise the movable sockets of the hinge joint between the condyles and discs. The combination of two joints on the each side of the jaw gives a certain freedom of movement to the mandible in all planes of the space. But the fact that the articulating components always work in close contact obviously imposes some limitation of movement because of the two-sided articulation. Furthermore, the magnitude of these movements in not great, since they are effective limited by all the various fibers of the temporo-mandibular ligaments of each

side in the opened positions, to which is added the contact of the tooth plates in terminal closing positions.

RANGE OF MOVEMENTS

Beginning with the condyles retruded to the extreme hinge position, the mandible can perform a theoretically pure hinge rotation about a fixed axis passing through the approximate centers of both condyles. In opening, the interdental point swings down in an even arc of about 20 to 25 mm in length. Further opening requires a downward and forward excursion of condyles and discs along the articular eminences, with continued rotation of the condyles within the lower joint compartments. This activity within the joint abruptly changes the course of the incisal point. It then proceeds in an arc of greater curvature directed more anteriorly, in which the lower termination marks the limit of greatest jaw opening.

From this point closing proceeds directly upward and forward with the mandible strained forward in maximal protrusion during its entire path of movement. The interdental point now describes an arc of the least curvature in the sequence. Its upper termination is brought about by contact of the posterior teeth, since the lower anteriors reach forward beyond the upper anteriors with the jaw in its most protruted position. In making this movement, the condyles rotate backward in the lower compartments, and both condyles and discs move slightly posteriorly. From this point the circuit of extreme movement is completed by retrusion to the original point of departure at the most retruded hinge position of the jaw. The path is necessarily uneven because of the shifting contacts of the teeth. Proceeding form the most protruded contact position, where the lower incisors are anterior to the uppers, the path of the incisal point dips to the edge-to-edge contact of the incisors, rises to the position of complete occlusal interdigitation, then dips again as cusps are cleared to reach back to the terminal hinge position.

Movements in the transverse, or horizontal, plane can be similarly traced. Again beginning with the mandible in its most retruded (terminal hinge) position, the jaw is rotated to one side as far it will go with teeth just touching. In doing so it turns around a vertical axis somewhere behind the condyle on the side of the movement. If this is the left condyle it moves little, orbiting laterally in a small arc with a short radius of curvature. The condyle of the controlateral side, however, orbits in a long flat arc, since its radius of curvature is much longer. Thus the right condyle slides downward, forward, and inward to the anterior limit of the articular plane. This movement of the jaw carries the incisal point laterally and forward in a mild curve to the right. From this position the jaw is swung toward the midline in full protrusion. Thus the right condyle now slides downward, forward and inward to the interior limit of its articular plane. This movement carries the incisal point forward and medially in a mild curve to the same point of greatest protrusion visualized in the sagittal view (5).

The reverse action is now carried out by moving the jaw to the extreme of the left lateral excursion, the left lateral condyle thus moves up and back toward the most retruded position. Finally, the right condyle follows till mandible is returned to the original point of departure in the most retruded hinge position. The outline traced by the incisal point in this total excursion is

diamond-shaped, and it defines the marginal movements of the jaw in the horizontal (occlusal) plane. When these lateral movements are made with the jaw at increasing levels of opening, the diamonds become smaller and smaller until they disappear at the point of maximal opening identical with that visualized in sagittal view.

MUSCLES AND THE MOVEMENTS IN THE TEMPORO-MANDIBULAR JOINT

The mandibular muscles determine all the complicated postures and movements of the jaw. Their behavior can be greatly clarified by restating certain fundamentals crucial to purposive muscular activity. In the first place, there are three distinct roles that a muscle can play when activated: it can contract isotonically and shorten to act as a mover of a part; it can contract (tense) isotonically yet lengthen to act as a balancer for a moving part; and it can contract isometrically, hence neither shorten not lengthen, to act as a stabilizer of a movable part. In the second place, surprisingly large groups of distant muscles must act in the seemingly simplest functional movements.

When a jaw muscle contracts in the absence of a restraint, the mandible is pulled in the direction of the shortening muscle. The muscles have therefore been classified as protruders (lateral and medial pterygoids); retractors (posterior fibers of temporalis assisted by digastric and geniohyoid); elevators (anterior and middle fibres of temporalis, superficial and deep fibers of masseter and medial pterygoid); depressors (lateral pterygoids aided by digatric, geniohyoid and mylohyoid); lateral movers (medial and lateral pterygoids of each side).

All these muscles are active in all mandibular movements - some as movers, some as balancers, and some as stabilizers - shifting their role in accord with the progress of the movement. In close coordination with the above actions, muscles of the neck steady the cranium and hyoid bone to establish stable bases from which opening and closing muscles can pull (6). Since precise movements of the tongue are an integral part of masticatory activity, the hyoid bone is seen to move in rhythm with the jaw in chewing. It shifts continuously with firm stability to differing positions of mechanical advantage both for jaw opening and bolus manipulation.

Movements are described beginning from the centric occlusal posture with all teeth in even contact. In this position the elevators of the jaw are under active contraction, whereas in the rest position these muscles are safe to be in tonic contraction. There are two major movement sequences of the mandible in which some teeth come into contact: a cutting movement, such as that used by incisors and canines to bite off a piece of food, and a grinding movement, such as that used by molars and premolars to comminute a piece of food.

Both masticatory movement complexes - cutting and grinding - can be analyzed in three phases. The first, the opening or preparatory, phase is a free movement of the jaw. The second phase begins with the beginning closing action and ends at the approach of contact. The third phase is a truly articulatory movement. It occurs with the contact of teeth. The first phase requires little force. Considerable force may be applied during the second and third phases as these phases fuse to produce the masticatory stroke. Furthermore, in actual chewing true contact of teeth probably occurs rarely before many masticatory strokes have been made, depending on the

resistance of the food.

VASCULAR SUPPLY

The joint derives its arterial supply from the superficial temporal artery laterally and the maxillary artery medially. Veins drain the anterior aspect of the joint and associated tissues into the plexus surrounding lateral pterygoid, and posteriorly they drain into the vascular region that separates the two laminae of the bilaminar region of the disc (1).

In sagittal sections, the disc appears to possess a thin intermediate zone and thickened anterior and posterior bands, and its upper surface appears concavo-convex where it fits against the convex articular eminence and the concavity of the articular fossa. Posteriorly the disc is attached to a region of loose vascular and nervous tissue, which splits in two laminae, the bilaminar region. The upper lamina, composed of fibroelastic tissue, is attached to the squamo-tympanic fissure, and the lower lamina, composed of fibrous non-elastic tissue, is attached to the back of the condyle. The bilaminar region contains a venous plexus (1, 6, 7). Lymphatic drain deeply to the upper cervical lymph nodes is surrounding the internal jugular vein.

INNERVATIONS

The articular tissues and the dense part of the articular disc have no nerve supply. Branches of the auriculo-temporal and masseteric nerves and postganglionic sympathetic nerves supply the tissues associated with the capsular ligament and the looser posterior bilaminar extension of the disc. The temporo-mandibular joint capsule, lateral ligament and retroarticular tissue contain mechanoreceptors and nociceptors.

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SPECTROPHOTOMETRIC DETERMINATION OF ENALAPRIL THROUGH TERNARY COMPLEX FORMATION

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SPECTROPHOTOMETRIC DETERMINATION OF ENALAPRIL THROUGH TERNARY COMPLEX FORMATION **(Abstract):** Enalapril is an angiotensin-converting enzyme inhibitor widely used in the treatment of hypertension and heart failure. **Aim**: To design and validate a simple and sensitive spectrophotometric method. This method is based on a ternary complex formation between enalapril, copper (II), and bromothymol blue, in a slightly alkaline environment, complex that is extractable in chloroform, with an absorption maximum at 426 nm. **Materials and methods**: To a volume of 1 milliliter of standard enalapril drug solution in concentration range 200 ÷ 500 μg/ml, two milliliters of Cu (II) chloride solution 0.2 % followed by 2 ml bromothymol blue solution 0.01 % and 0.5 ml of pH 8 buffer solution (Britton-Robinson) were added. The complex was two times extracted with 2 × 1.5 ml chloroform. Ten minutes after extraction, absorbance was measured at 426 nm against blank. **Results**: Our spectrophotometric method was validated by determining linearity, limit of detection and quantification, system and method precision, and method accuracy. The method showed a good linearity in the range 200 ÷ 500 μg/ml (correlation coefficient r = 0.9993). Limit of detection (LD) was 9.907 μg/ml and limit of quantification (LQ) 33.024 μg/ml. Precision showed an RSD = 1.83%, and accuracy with mean recovery is 102.6 % in the range 98.8÷ 103.9. **Conclusions**: The ternary complex formed under the above-mentioned condition and measured spectrophotometrically can be regarded as an ion-association complex between the metal-drug cation and bromothymol blue anion. The experimental results demonstrated a good sensitivity. **Key words**: ENALAPRIL MALEATE, SPECTROPHOTOMETRIC METHOD, VALIDATION

Angiotensin converting enzyme inhibitors constitute one of the major classes of compounds used in the treatment of essential and renovascular hypertension and congestive heart failure. Enalapril is an ethyl ester of a long-acting ACE inhibitor, enalaprilat. It is basically a pro-drug, which after oral administration undergoes hydrolysis to yield enalaprilat and is used as its maleate salt in therapeutic indications. The oral absorption of enalapril is superior to that of enalaprilat. The official drug enalapril maleate is (1-[N-[(S)-1-carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1'-ethyl ester, maleate (1:1)) (1, 2).

Many analytical methods have been reported in the literature for the analysis of enalapril maleate. The techniques include

spectrophotometry (2), spectrofluorimetry (3), ^{1}H nuclear magnetic resonance (NMR) spectroscopy (4). A number of high performance liquid chromatography (HPLC) (5, 6, 7) and gas chromatography (GC) methods (8) have been reported for the determination of enalapril.

This article presents a method developed and fully validated for the determination of enalapril.

MATERIAL AND METHODS

Instrumentation

A UV-VIS Hewlett-Packard 8453 spectrophotometer with quartz cells (l=1 cm) was used. A vibration shaker IKA-Werke type VX2 and ultrasonic bath were also used.

Materials and reagents

- Stock solution of enalapril maleate 1 mg/ml: 0.1 g enalapril (99.9% Zhejiang Huahai Pharmaceutical co., LTD, China) was dissolved in 1 ml methanol by ultrasonic bath. After dissolution, it was brought quantitatively to 100 ml with distilled water. Standard solution in concentration range 200-500 μg/ml was prepared from stock solution.
- Bromothymol blue (BTB) (Merck KGaA) 0.01% (w/v) aqueous solution.
- Copper (II) chloride ($CuCl_2.2H_2O$, Sigma Aldrich) 0.2% (w/v) aqueous solution.
- Buffer solution pH 8 (Britton Robinson). Equal volumes of acetic acid 0.04 M, phosphoric acid 0.04 M, and boric acid 0.04 M were mixed. To 100 ml of this mixture 60 ml of sodium hydroxide 0.2 M was added.

General procedure

To 1 ml of the standard drug solution in concentration range 200-500 μg/ml enalapril were added 2 ml of Cu (II) chloride solution 0.2 % followed by 2 ml of BTB solution 0.01 % and 0.5 ml buffer solution pH 8. The complex was two times extracted with 2 × 1.5 ml chloroform. The solution was shaken for 2 min each time. Absorbance of combined chloroform solutions was measured 10 minutes after extraction. Absorbance of the resulting solution was determined at 426 nm against blank prepared in the same manner.

RESULTS AND DISCUSSION

Ternary complexes of general formula ($L_NM_XS_Y$) have been widely used in spectrophotometric analysis. The particularity of the ternary complexes in this study is that their main ligand L is a drug (enalapril maleate), the second ligand S is bromothymol blue, and M is copper (II) (fig. 1).

The experimental conditions were established by varying and observing the different parameters that affect the absorbance of the stained product. The optimum experimental conditions were found to be: 2 ml Cu (II) chloride 0.2%, BTB solution 0.01 %, and 0.5 ml pH 8 buffer solution (Britton-Robinson). Two extraction of 2-min duration were found to be necessary for the quantitative determination of the complex. The best solvent for extraction was chloroform. At a concentration of less than 250μg/ml, the complex is stable 10 to 15 minutes after extraction. At concentration greater than 400μg/ml, the complex is stable for 45 minutes.

A series of absorption spectra have been recorded for each component and their mixtures under the above mentioned experimental conditions. The spectra revealed that chloroform solution of bromothymol blue absorbs at λ_{max}=267 nm, while neither copper (II) chloride nor the drug have maximum absorbance in the UV-VIS region. Enalapril-bromothymol blue and copper (II) chloride - bromothymol blue mixtures have the same

maximum absorption as bromothymol blue at λ_{max}=267 nm, while enalapril-copper (II) chloride mixture do not have maximum absorption in UV-VIS region.

The formed complex had maximum absorption at 426 nm (fig. 2).

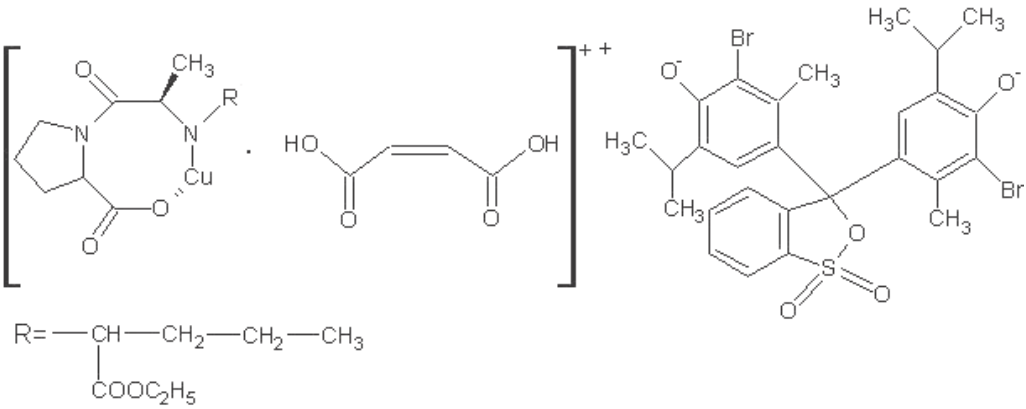

Fig. 1. Enalapril-Cu-bromothymol blue complex

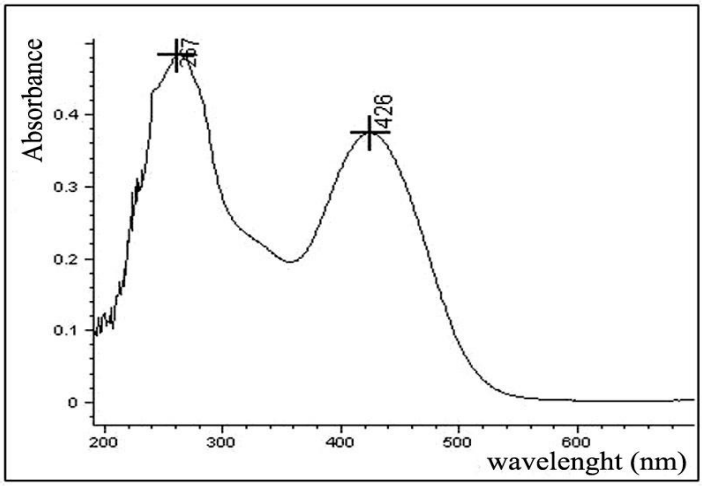

Fig. 2. Absorption spectrum of the ternary complex enalapril-Cu(II)-BTB

Calibration curves and validation

In order to validate the UV-VIS spectrophotometric method for enalapril assay, the following parameters have been studied: detection wavelength, linearity, detection and quantification limit, system and method precision, and the accuracy of the method (9).

For the stained compound, maximum absorption was at 426 nm.

A calibration curve for the formed complex was obtained by the standard procedure. A good linear relationship within the range 200-500 μg/ml was observed. For each concentration three determinations were made, and the mean absorbance was calculated and calibration curve drawn (fig. 3).

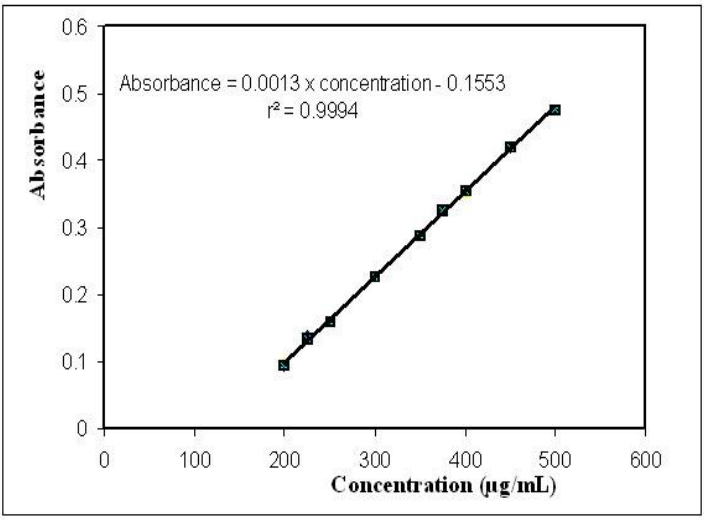

Fig. 3. Calibration curve

The analytical data, regression equation (Y = aX – b; a = slope and b = intercept), correlation coefficient (r), and regression coefficient (r^2) are summarized in tab. I.

To determine precision, system and method precision studies were performed.

In order to evaluate the precision of the system, ten determinations of the same sample (300 µg/ml) were done under the same experimental conditions, and then the relative standard deviation was calculated - RSD = 1.45, showing that the system is precise.

TABLE I

Linearity of the method and statistical data

Concentration (µg/ml)	Absorbance				Statistical data
	I	II	III	Average	
200	0.0902	0.0953	0.1008	0.0954	r = 0.9993 r^2 = 0.9994 SE = 0.0035 Intercept = - 0.1553 Slope = 0.0013 LQ = 33.024 µg/ml LD = 9.907 µg/ml
225	0.1394	0.1342	0.1319	0.1351	
250	0.1589	0.1596	0.1598	0.1594	
300	0.2297	0.2232	0.2295	0.2274	
350	0.2897	0.2895	0.2867	0.2886	
375	0.3245	0.3256	0.3244	0.3248	
400	0.3578	0.3542	0.3540	0.3553	
450	0.4187	0.4195	0.4202	0.4195	
500	0.4735	0.4785	0.4765	0.4762	

We have evaluated the precision of the method for a concentration range of ± 25% compared to the target value (300µg/ml). In order to validate the method (repeatability assessment) we analyzed two sets of samples, each on different day, with at least 9 determinations covering the specific concentration area (table II).

TABLE II

Precision of the method – first set and second set

Theoretical concentration (µg/ml)	Precision of the method First set			Precision of the method Second set		
	Absorbance (426 nm)	Calculated concentration (µg/ml)	Recovery (%)	Absorbance (426 nm)	Calculated concentration (µg/mL)	Recovery (%)
225	0.1377	225.4	100.2	0.1355	223.7	99.4
	0.1387	226.2	100.5	0.1398	227.0	100.9
	0.1329	221.7	98.5	0.1421	228.8	101.7
300	0.2457	308.5	102.8	0.2456	308.4	102.8
	0.2499	311.7	103.9	0.2502	311.9	104.0
	0.2468	309.3	103.1	0.2516	313.0	104.3
375	0.3459	385.5	102.8	0.3488	387.7	103.4
	0.3489	387.8	103.4	0.3512	389.6	103.9
	0.3497	388.4	103.6	0.3484	387.4	103.3
Statistical data		Mean	102.6		Mean	102.8
		SD	1.87		SD	1.64
		RSD	1.83		RSD	1.76

For the first set of determinations, we obtained RSD = 1.83, for the second set RSD = 1.76, values below 2%, but close to the precision of the system (RSD = 1.45). All these values confirm that the proposed method is precise.

In order to determine accuracy, we used the addition method at concentrations of: 225, 300 and 375µg/ml. Using the regression equation and the corresponding absorbance values, the concentration in µg/ml for each sample was calculated (table III).

TABLE III

The accuracy of the method

Theoretical concentration (µg/ml)	Absorbance (426 nm)	Calculated concentration (µg/ml)	Recovery (%)	Statistical data
225	0.1475	232.9	103.5	Mean = 103.2 Min = 101.4 Max = 104.9
	0.1514	235.9	104.9	
	0.1452	231.1	102.7	
300	0.2432	306.5	102.2	
	0.2402	304.2	101.4	
	0.2532	314.2	104.7	
375	0.3405	381.3	101.7	
	0.3545	392.1	104.6	
	0.3547	392.3	104.6	

CONCLUSIONS

The ternary complex formed under the above mentioned conditions and measured spectrophotometrically can be regarded as an ion-association complex between the metal, drug cation and bromothymol-blue anion.

The proposed method is simple, fast and, highly sensitive); the detection limit was 9.907 µg/ml, and quantification limit 33.024 µg/ml.

The analysis method was validated at 426 nm optimum detection wavelength. We also studied the linearity of the method in the range of 200-500 µg/ml enalapril/ sample; the correlation coefficient was r^2 = 0.9994, precision of the system (RSD = 1.45), precision of the method (RSD = 1.83), and accuracy (mean recovery = 103.2 %).

The proposed method is simple, accurate, highly sensitive, with good precision and accuracy and is inexpensive.

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PINI CEMBRAE CONI: STUDII HISTO-ANATOMICE ŞI CHIMICE, EVALUAREA ACȚIUNII ANTIOXIDANTE

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PINI CEMBRAE CONI: HISTO-ANATOMICAL AND CHEMICAL STUDIES, ASSESSMENT OF ANTIOXIDANT ACTIVITY (Abstract): This study aimed to identify some histo-anatomical features, to evaluate the polyphenolic content and antioxidant activity of cembran pine female cones. **Material and methods**: The cross sections, obtained by usual techniques, were examined using a Novex microscope with a Canon A95 digital camera. For chemical and antioxidant assays dried cones were extracted with 80% aqueous methanol. The total phenolics, flavonoids and proanthocyanidins in cone extract were quantified by spectrophotometric methods. The antioxidant activity was investigated using different *in vitro* assays: ABTS radical cation scavenging assay, reducing power assay and ferrous ion chelating ability assay. **Results**: The histo-anatomical study of the seed-bearing scale revealed many secretory channels. The cone extract had low contents of total phenolics (48.03 ± 0.10 mg/g), flavonoids (8.11 ± 0.27 mg/g) and proanthocyanidins (1.63 ± 0.03 mg/g). The extract scavenged ABTS radical cation and reduced ferric ions to ferrous ions with EC_{50} values of 64.6 ± 0.8 and 118.60 ± 1.89 μg/ml, respectively being less active than the positive control, quercetin (EC_{50}=1.16 ± 0.05 and 2.58 ± 0.00 μg/ml, respectively). In ferrous ion chelating ability assay the extract showed a good activity (EC_{50}=1.2 ± 0.0 mg/ml) while quercetin was inactive. **Conclusions**: The ability of cone extract to chelate ferrous ions encourages further studies for identification of bioactive constituents and evaluation of their protective effects against ferrous ion-induced oxidative stress. **Key words**: *PINUS CEMBRA* L., POLYPHENOLS, ABTS, REDUCING POWER, FERROUS IONS CHELATION

Conurile de pin au fost considerate mult timp reziduuri forestiere şi, din acest motiv, compoziția chimică şi posibilele lor efecte biologice au fost puțin studiate.

În medicina tradițională japoneză, extractul apos din conurile speciei *Pinus parviflora* Sieb. et Zucc. era utilizat în tratamentul cancerului gastric (1).

Micales *et al.* au studiat compoziția chimică a conurilor de *Pinus contorta* Dougl., *Pinus jeffreyi* Grev. & Balf., *Pinus lambertiana* Dougl. şi *Pinus sylvestris* L.. În extractele eterice, au identificat, prin gaz-cromatografie, 16 acizi rezinici dintre care acizii dehidroabietic, isopimaric, abietic şi pimaric au predominat cantitativ.

Extractele au fost active faţă de unii fungi fitopatogeni (*Ceratocystis coerulescens*, *Trametes versicolor*) (1).

În conurile unor specii de *Pinus* au fost identificate antocianozide (3-glucozidele cianidolului, delfinidolului, petunidolului şi peonidolului) (2).

Studiul chimic al conurilor provenite de la specii de *Pinus* răspândite în Turcia (*Pinus halepensis* Mill., *Pinus pinea* L., *Pinus sylvestris* L., *Pinus nigra* L., *Pinus brutia* Ten.) a evidenţiat un conţinut polifenolic redus (0,06-1,70 mg/g) în extractele acetonice. În toate extractele a fost identificată catehina în concentraţii variabile de la 0,03 mg/g (în extractul din conuri de *Pinus sylvestris* L.) la 1,26 mg/g (în extractul din conuri de *Pinus halepensis* Mill.). Alţi compuşi identificaţi au fost: vanilina, acidul 3,4-dihidroxibenzoic, galocatehina, taxifolina şi izolaricirezinolul (3).

Până în prezent nu au fost efectuate studii privind histo-anatomia, compoziţia chimică şi posibilele efecte biologice ale conurilor femele de zâmbru (*Pinus cembra* L., *Pinaceae*). Obiectivele principale ale prezentului studiu au fost: stabilirea unor caractere histo-anatomice, determinarea conţinutului în polifenoli şi evaluarea potenţialului antioxidant al unui extract din conuri femele de zâmbru.

MATERIAL ŞI METODE

Materialul vegetal luat în studiu a fost constituit din conuri femele de zâmbru recoltate de la un exemplar matur în Parcul Naţional Călimani (aproximativ 1650 m altitudine) în luna iunie 2011. Materialul vegetal destinat studiilor histo-anatomice a fost fixat şi conservat în alcool etilic 70%. Pentru studiile chimice şi de evaluare a acţiunii antioxidante, conurile au fost uscate la temperatura camerei, ferite de lumină şi umiditate.

Secţiunile, realizate cu microtomul manual conform metodologiei uzuale (4), au fost colorate cu verde de iod şi roşu de ruteniu şi examinate la un microscop optic Novex prevăzut cu cameră foto digitală Canon A95.

20 g material vegetal, uscat şi pulverizat, au fost extrase cu 200 ml metanol 80% (v/v) sub agitare continuă, la temperatura camerei, timp de o oră. Extracţia a fost repetată de două ori; extractele au fost filtrate, reunite, concentrate sub presiune redusă la 40°C şi liofilizate obţinându-se 2,01 g extract (EC) (randament 10,05%).

Studiul chimic a presupus determinarea cantitativă a polifenolilor totali, flavonoidelor şi proantocianidolilor din extractul obţinut. *Conţinutul în polifenoli totali* a fost determinat prin metoda Folin-Ciocâlteu (5); rezultatul a fost exprimat în echivalenţi acid galic (mg acid galic/g extract). Pentru *determinarea cantitativă a flavonoidelor* s-a folosit o metodă spectrofotometrică bazată pe proprietatea acestora de a forma complecşi cu Al^{3+} (6); rezultatul a fost exprimat în echivalenţi (+)-catehină (mg (+)-catehină/g extract). *Conţinutul în proantocianidoli* a fost determinat prin metoda descrisă de Porter *et al.* (7); rezultatul a fost exprimat în echivalenţi cianidol (mg cianidol/g extract).

Acţiunea antioxidantă a extractului EC a fost evaluată prin determinarea capacităţii de scavenger faţă de radicalul cation ABTS, a capacităţii reducătoare şi a capacităţii de chelatare a ionilor feroşi. Ca martor pozitiv s-a utilizat cvercetolul dihidrat. CE_{50} (concentraţia căreia îi corespunde o activitate de 50%) a fost calculată prin interpolarea liniară a valorilor activităţilor imediat superioară şi imediat inferioară activităţii de 50%. În testul de evaluare a capacităţii reducătoare CE_{50} a reprezentat concentraţia corespunzătoare unei absor-

bante de 0,5.

Pentru determinarea *capacităţii de scavenger faţă de radicalul cation ABTS* (sarea de diamoniu a acidului 2,2'-azino-bis-3-etilbenzotiazolin-6-sulfonic) a fost utilizată metoda descrisă de Re *et al.* (8, 9). Activitatea de scavenger a fost exprimată procentual cu ajutorul formulei: % activitate de scavenger = 100 x ($A_{control}$ – $A_{probă}$)/ $A_{control}$, unde $A_{control}$ reprezintă absorbanţa controlului şi $A_{probă}$ reprezintă absorbanţa extractului/martorului pozitiv. Activitatea antioxidantă a fost exprimată şi prin valoarea TEAC (Trolox Equivalent Antioxidant Activity, activitate antioxidantă exprimată în echivalenţi Trolox) conform metodologiei descrise de Re *et al.* (8).

Capacitatea de a reduce ionii ferici la ioni feroşi a fost determinată prin metoda bazată pe formarea ferocianurii ferice (albastru de Prusia) (10).

Capacitatea de chelatare a ionilor feroşi (%), determinată prin metoda descrisă de Dinis *et al.*, a fost calculată folosind formula: 100 x ($A_{control}$ - $A_{probă}$)/$A_{control}$, unde $A_{control}$ reprezintă absorbanţa controlului şi $A_{probă}$ = absorbanţa extractului/martorului pozitiv (11).

Toate determinările chimice şi de evaluare a acţiunii antioxidante au fost efectuate în triplicat; rezultatele sunt prezentate ca media ± deviaţia standard.

REZULTATE ŞI DISCUŢII

Studiul histo-anatomic. Secţiunea transversală prin solzul carpelar are un contur aproximativ semieliptic. Ţesutul protector este reprezentat de o epidermă ale cărei celule de mărimi diferite sunt izodiametrice şi cu pereţii puternic îngroşaţi; peretele extern este acoperit de o cuticulă subţire, uşor ondulată. Din loc în loc, pe faţa externă a solzului carpelar se observă peri tectori. În poziţie hipodermică se află o zonă de celule parenchimatice foarte mici, de grosimi diferite. Sub aceasta se diferenţiază un parenchim format din două categorii de celule: unele mai mari, alungite perpendicular faţă de epidermă, cu peretele îngroşat şi lignificat şi altele mult mai mici, fără meaturi între ele, în partea centrală a solzului carpelar (fig. 1). În parenchimul fundamental se observă mai multe canale secretoare de diametre diferite; canalul colector este înconjurat de un strat de celule epiteliale mici (fig. 2). Ţesutul conducător nu formează fascicule libero-lemnoase tipice, colaterale, ci grupe de celule xilemice şi floemice cu orientare diferită, uneori cu floemul faţă în faţă.

Fig. 1. Secţiune transversală prin solzul carpelar imagine de ansamblu

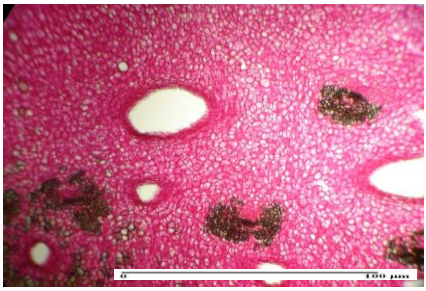

Fig. 2. Solzul carpelar – detaliu canale secretoare–

În secţiunea transversală de la nivelul ovulului tânăr se observă tegumentul care înconjoară nucela formată din celule poli-

gonale cu pereții foarte subțiri, fără meaturi între ele (fig. 3). Celulele sunt foarte tinere, cu caracter meristematic şi au nucleii mari. Pe seama unora dintre aceste celule, prin diviziuni succesive se va forma ulterior endospermul primar în care se vor edifica arhegoanele (fig. 4). Fiecare dintre acestea are partea bazală înconjurată de celule foarte mici; în arhegoane se vor forma gameții femeli.

Fig. 3. Secțiune transversală prin ovul – imagine de ansamblu

Fig. 4. Secțiune transversală prin ovul-începutul formării arhegoanelor

Studiul chimic. Studiul chimic cantitativ a evidențiat, pentru extractul EC, un conținut de 48,03 ± 0,10 mg polifenoli totali, 8,11 ± 0,27 mg flavonoide şi 1,63 ± 0,03 mg proantocianidoli/g extract. Rezultatele obținute sunt în concordanță cu date din literatura de specialitate conform cărora, în conurile speciilor de conifere, conținutul polifenolic este mai redus comparativ cu alte părți de plantă (scoarță, frunze) (3). Proantocianidolii, spre exemplu, se găsesc în cantități mari (până la 50%) în scoarțele de conifere (12).

Evaluarea acțiunii antioxidante. *Determinarea capacității de scavenger față de radicalul cation ABTS*. Rezultatele obținute sunt reprezentate grafic în figura 5. Extractul EC este mai puțin activ decât martorul pozitiv, cvercetolul. Astfel, extractul EC are o activitate de scavenger de 1,29 ± 0,09% la concentrația de 0,19 µg/ml; cvercetolul prezintă, la aceeaşi concentrație, o activitate de 9,64 ± 0,23%. La concentrația de 12,5 µg/ml cvercetolul inactivează complet radicalul în timp ce extractul EC are o activitate de numai 10,88 ± 0,39%. Această diferență de activitate este confirmată şi de valorile CE_{50} şi TEAC ale extractului EC şi cvercetolului (tab. I).

Determinarea capacității reducătoare. Valorile absorbanțelor obținute pentru extractul EC şi cvercetol în domeniul de concentrații 0,24 - 4 µg/ml sunt reprezentate grafic în fig. 6. Extractul EC prezintă o capacitate reducătoare mai mică decât martorul pozitiv, cvercetolul. Astfel, la o concentrație de 0,24 µg/ml, valoarea absorbanței amestecului de reacție, în cazul cvercetolului, este de 0,06 ± 0,00; la concentrația de 4 µg/ml valoarea absorbanței creşte la 0,71 ± 0,01. În acelaşi domeniu de concentrații, extractul EC este mai puțin activ. Astfel, la concentrația de 4 µg/ml, absorbanța amestecului de reacție este de numai 0,06 ± 0,00. În vederea calculării CE_{50} a extractului EC, au fost realizate diluții suplimentare (8,25 – 41,25 µg/ml). Capacitatea reducătoare scăzută a extractului EC este dovedită şi de valoarea CE_{50} a acestuia, mult mai mare decât cea a cvercetolului (118,60 ± 1,89 şi respectiv, 2,58 ± 0,00 µg/ml) (tab. I).

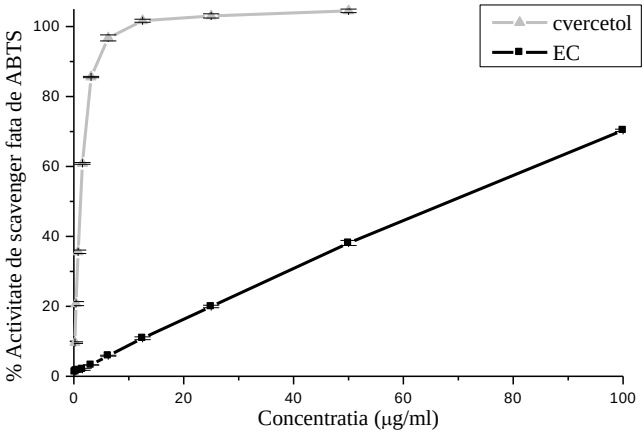

Fig. 5. Activitatea de scavenger faţă de radicalul cation ABTS (EC = extract din conuri de *Pinus cembra* L.)

Determinarea capacităţii reducătoare. Valorile absorbanţelor obţinute pentru extractul EC şi cvercetol în domeniul de concentraţii 0,24 - 4 μg/ml sunt reprezentate grafic în fig. 6. Extractul EC prezintă o capacitate reducătoare mai mică decât martorul pozitiv, cvercetolul. Astfel, la o concentraţie de 0,24 μg/ml, valoarea absorbanţei amestecului de reacţie, în cazul cvercetolului, este de 0,06 ± 0,00; la concentraţia de 4 μg/ml valoarea absorbanţei creşte la 0,71 ± 0,01. În acelaşi domeniu de concentraţii, extractul EC este mai puţin activ. Astfel, la concentraţia de 4 μg/ml, absorbanţa amestecului de reacţie este de numai 0,06 ± 0,00. În vederea calculării CE_{50} a extractului EC, au fost realizate diluţii suplimentare (8,25 – 41,25 μg/ml). Capacitatea reducătoare scăzută a extractului EC este dovedită şi de valoarea CE_{50} a acestuia, mult mai mare decât cea a cvercetolului (118,60 ± 1,89 şi respectiv, 2,58 ± 0,00 μg/ml) (tab. I).

Determinarea capacităţii de chelatare a ionilor feroşi. Rezultatele obţinute sunt reprezentate grafic în fig. 7. Deşi extractul EC este slab activ ca eliminator de radicali liberi şi agent reducător, acesta prezintă o bună activitate de chelatare a ionilor feroşi (CE_{50} =1,2 ± 0,0 mg/ml) (tab. I). La concentraţia de 0,5 mg/ml activitatea de chelatare a extractului EC este 6,27 ± 1,77%; la concentraţia de 4 mg/ml extractul EC chelatează ionii feroşi în proporţie de 98,07 ± 0,21%. În aceleaşi condiţii experimentale, cvercetolul este inactiv.

Ionii feroşi joacă un rol important în formarea de specii reactive ale oxigenului *in vivo*. Prin reducerea oxigenului molecular de către ionii feroşi ia naştere radicalul anion superoxid. În prezenţa ionilor feroşi peroxidul de hidrogen este convertit în radicali hidroxil (reacţia Fenton). Ionii feroşi se pot lega de oxigenul molecular cu formarea ionului perferil, deosebit de reactiv (13). În consecinţă, chelatarea ionilor feroşi reprezintă un mecanism important de reducere a stresului oxidativ. Având în vedere conţinutul polifenolic scăzut al extractului EC, este posibil ca, pe lângă polifenoli, şi alţi compuşi (oleorezine, saponine) să joace un rol important în activitatea de chelatare a ionilor feroşi.

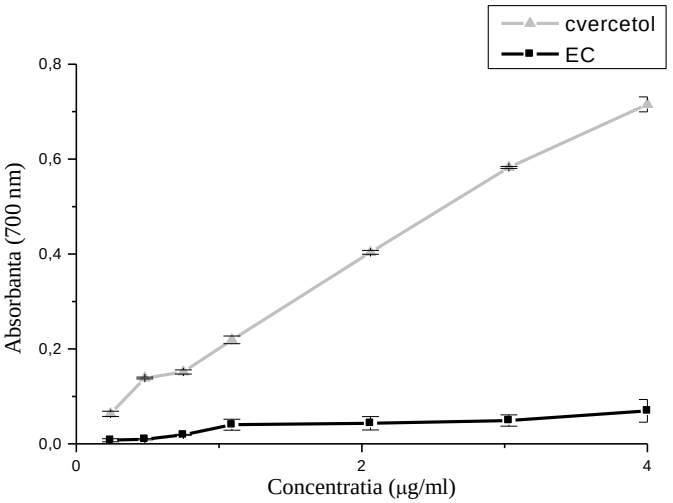

Fig. 6. Activitatea de reducere a ionilor ferici la ioni feroşi (EC = extract din conuri de *Pinus cembra* L.)

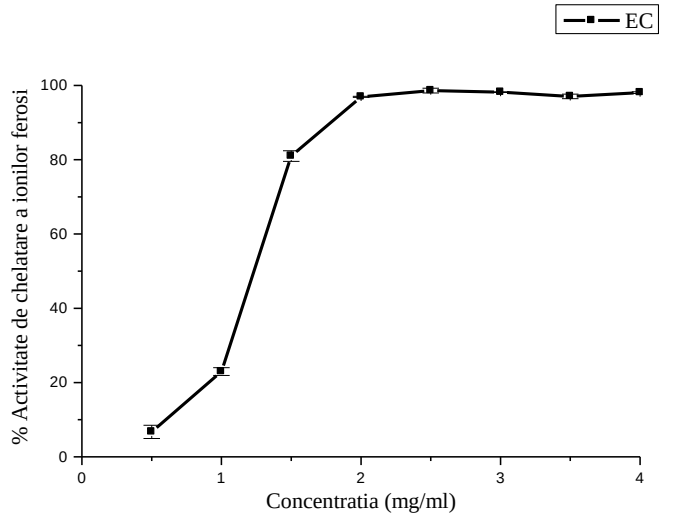

Fig. 7. Activitatea de chelatare a ionilor feroşi (EC = extract din conuri de *Pinus cembra* L.)

TABELUL I

Activitatea antioxidantă a extractului din conuri de *Pinus cembra* L.

Probă	Capacitatea de eliminare față de radicalul cation ABTS		Capacitatea reducătoare	Capacitatea de chelatare a ionilor feroşi
	CE_{50} [1]	TEAC [2]	CE_{50} [1]	CE_{50} [3]
Extract EC	64,6 ± 0,8	0,15 ± 0,00	118,60 ± 1,89	1,2 ± 0,0
Cvercetol	1,16 ± 0,05	7,93 ± 0,05	2,58 ± 0,00	inactiv

[1]μg/ml; [2]μM Trolox echivalent la 1 μg/ml extract EC/cvercetol; [3]mg/ml

CONCLUZII

Studiul histo-anatomic la nivelul solzului carpelar a indicat prezența unui număr mare de canale secretoare, iar secțiunea transversală prin ovulul tânăr a permis observarea perioadei de început a dezvoltării arhegoanelor. Studiul chimic a evidențiat un conținut redus de polifenoli totali, flavonoide şi proantocianidoli în extractul din conuri. Studiul acțiunii antioxidante a extractului a indicat o activitate redusă ca eliminator de radicali liberi şi agent reducător. În schimb, extractul a fost activ în testul de chelatare a ionilor feroşi. Rezultatele obținute justifică continuarea cercetărilor pentru identificarea compuşilor activi şi evaluarea capacității protectoare față de stresul oxidativ indus de ionii feroşi.

Cristina Lungu Apetrei mulțumeşte Domnului Profesor Dr. Constantin Toma (Facultatea de Biologie, Universitatea "Al. I. Cuza"-Iaşi) şi personalului Administrației Parcului Național Călimani R. A. pentru sprijinul acordat în achiziționarea materialului vegetal.

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FEATURES OF PUBERTAL MATURATION IN ADOLESCENTS FROM TIMIS COUNTY

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FEATURES OF PUBERTAL MATURATION IN ADOLESCENTS FROM TIMIS COUNTY (Abstract): **Aim.** Puberty, by content and finality, mean all the events of somatic and mental maturation, with the transformation of the child into an adult. It is the period in which it is acquired the reproductive capacity. **Material and methods**. Pubertal maturation was studied on a sample of secondary school adolescents from Timis County, 800 students from schools and high schools in the urban area aged 11-14 years, with uniform distribution by sex and age. The pubertal evaluation was made with reference to Tanner stages. The result was an earlier and stronger pubertal maturation in girls compared with boys. **Results**. Pubertal maturation in adolescents from Timis County is higher compared to the national level (step 1999), and is less elevated compared with the local level of maturation (1998). **Conclusions**. The study contributes to maintain actual the health condition in adolescence.
Keywords: ADOLESCENCE, PUBERTY, AVERAGE PUBERTAL AGE

World Health Organization defines with the term adolescence the period of 10-19 years of the human life in which a person passes from the appearance of secondary sexual characteristics stage to the sexual maturity stage; psychological and identification characteristics ceases to be of the child and become of the adult, the transition is gradual, from the stage of total socio-economic dependence to relative independence stage.

Chronological ongoing of the maturation varies from one child to another, for genetic reasons and under the environment influence. Adolescence is characterized by the onset of some major events in terms of maturation: somatic growth leap and concomitant occurrence of secondary sexual characteristics, as well as the first menstruation and first ejaculation. Therefore, there is variability in the chronology of these events in the sense that an evaluation of growth based exclusively on chronological age may be inaccurate or wrong, particularly at the individual level (1,2).

Puberty, by content and finality, mean all the events of somatic and mental maturation, with the transformation of the child into an adult. Puberty is defined as the period in which it is acquired the reproductive capacity. Puberty is different than adolescence which means the entire period of growth from childhood to an adult and extends more than puberty, between 10-19 years (3-,4,5).

There is a wide range of normality in puberty. Normal puberty begins around 10

years, girls tending to start puberty a little earlier than boys. Physical manifestations of the puberty, especially occurrence of secondary sexual characteristics begin to appear at 11 years to 50% of girls and 11 ½ years to 50% of boys. Common manifestation at female puberty, breast enlargement, it is more visible than the first manifestations of male puberty, testicular growth. The increase in height during puberty occurs earlier in girls, while in boys installs later, with a two-year difference between the two genders in the sequence of events in time. Puberty lasts 3-4 years, with the passage from one stage to another, almost every year. Puberty varies widely and not only as matching in time but also in duration (6,7).

The study allows to maintain actual the health condition in adolescence at national level in Romania, started in 1950 with anthropometric measurements carried out every seven years to assess growth and development of children and adolescents between 0-18 years.

MATERIAL AND METHODS

The sample of secondary school students from Timis County totaled 800 students in schools and high schools from urban area. In the sample, the gender distribution was equal, 50% for boys and girls. Age of the students enrolled in the study was between 11-14 years, age and gender groups consisting of 100 adolescents each.

Pubertal evaluation was made with reference to Tanner stages (8, 9, 10).

Global score of pubertal development (Tanner J.M.) in boys:

- P1 - Prepuberty (absence of any development);

- P2 - Onset of puberty: rare pigmented hairs at the root of the penis or on the scrotum, which is slightly pigmented; testicular size between 2.5 - 3.2 cm (IVT slightly over 4 cm^3);

- P3 - Testicular development at 3.3 - 4 cm in the great axis, beginning of penis lengthening, development of pubic hair, possibly few axillary hairs;

- P4 - Progress of testicular development at 4.1 - 4.5 cm in the great axis, sharp increase in penis length and thickness, an adult type pubic pilosity without extending to the thighs or on the midline, body and axillary hair growth in progression; characteristic body odor, change of voice, acne, ejaculations;

- P5 - Adult testicular waist, over 4.5 cm in the great axis; adult type genitalia, pilosity that interest the midline and front of thighs roots, complete spermatogenesis.

Global score of pubertal development (Tanner J.M.) in girls:

- P1 - Prepuberty (absence of any development);

- P2 - Onset of puberty: subareolar breast, enlargement of the areolas, possibly some pigmented hairs on the outer labia or axillary;

- P3 - Development of palpable breast volume exceeding the areola contour, increased pubic and axillary pilosity; occurrence of characteristic body odor;

- P4 - The mammary gland continues to grow, taking pyriform aspect, adult type axillary and pubic pilosity, acne, imminent menarche;

- P5 - Adult-sized breasts, an adult-type pilosity that interest and the inner upper thigh root; menarche.

RESULTS AND DISCUSSIONS

1. The degree of pubertal maturation

Pubertal development of adolescents from the studied lot, by age and sex groups, have the following characteristics (table I).

TABLE I

Distribution of boys and girls of 11 - 14 years in the pubertal maturation stages

Age years	Sex	No.	Pubertal maturation stages									
			I		II		III		IV		V	
			No.	%	No.	%	No.	%	No.	%	No.	%
11	Boys	100	24	24.0	66	66.0	10	10.0	-	-	-	-
	Girls	100	21	21.0	38	38.0	25	25.0	11	11.0	5	5.0
12	Boys	100	11	11.0	37	37.0	32	32.0	20	20.0	-	-
	Girls	100	10	10.0	10	10.0	21	21.0	40	40.0	19	19.0
13	Boys	100	9	9.0	14	14.0	48	48.0	26	26.0	3	3.0
	Girls	100	9	9.0	12	12.0	17	17.0	29	29.0	33	33.0
14	Boys	100	5	5.0	7	7.0	49	49.0	2	32.0	7	7.0
	Girls	100	-	-	2	2.0	5	5.0	25	25.0	68	68.0
Total 11-14 years	**Boys**	**400**	**49**	**12.2**	**124**	**31.0**	**139**	**34.8**	**78**	**19.5**	**10**	**2.5**
	Girls	**400**	**40**	**10.0**	**62**	**15.5**	**68**	**17.0**	**105**	**26.2**	**125**	**31.3**
	Boys and girls	**800**	**89**	**11.1**	**186**	**23.2**	**207**	**28.9**	**183**	**22.9**	**135**	**16.9**

At 11 years predominates the adolescents from stage II, 66.0% (66) among boys and 38.0% (38) among girls. Boys are more numerous in stage I, 24.0% (24) than girls, 21.0% (21). In stage III, the representation is more important for girls, 25.0% (25) than boys, 10.0% (10). In stage IV and V are located only girls: 11.0% (11) and 5.0% (5). Representatives of the boys are missing in stages IV and V.

At 12 years, two thirds of the boys are in stages II and III, 37.0% (37) respectively 32.0% (32). Two thirds of the girls are in stages II, III and IV, with increasing percentages from 10.0% (10) to 21.0% (21) and 40.0% (40). The lowest percentages were recorded in stage I, 11.0% (11) for boys and 10.0% (10) for girls. Only girls have representatives in the stage V of puberty, 19.0% (19).

At 13 years we meet adolescents in all five stages of pubertal development. Three quarters among boys are classified in the stages III - 48.0% (48), IV - 26.0% (26) and V-3.0% (3). Over three quarters of the girls are in the last three stages, except that the percentages are increasing: in stage III - 17.0% (17), IV - 29.0% (29), V - 33.0% (33).

At age 14, boys are present in all five stages: lower rates of 5.0% (5) and 7.0% (7), in the first two; the highest in stage III - 49.0% (49) and IV - 32.0% (32); 7.0% (7) in the stage V. Girls are missing in the first stage and are poorly represented in the following two: 2.0% (2) in stage II and 5.0% (5) in stage III. One quarter of the girls are in stage IV, 25.0% (25), and two thirds in the stage V of puberty, 68.0% (68).

In the studied age range, between 11-12 years it is observed a progressive increase of subjects in higher stages of pubertal development, boys missing in stages IV and V. At 13-14 years, the distribution is in all 5 stages, with a significant decrease of the ones from the lower stages and increasing the proportion of pubescents.

Pubescent girls appear at 11 years, 5.0% (5), and the first pubescent boys, at 13, 3.0% (3).

2. The average pubertal age

Pubertal mean age (calculated as a weighted arithmetic average of ages of first menstruation in girls and first ejaculations in boys) is probably close to ideal maturation indicator: has a high level of validity, is easily appreciated and is comparable to the reference average. The only possible dilemma remains the possibility of questioning in some cultural contexts.

2. 1. The average pubertal age in girls

The first menstruation age to the 125 pubescent girls from the studied lot, ranged between 9-14 years with a peak frequency at 12 years (68). The pubertal mean age was 11 years and 9 months (11) (table II).

TABLE II

Distribution of girls aged 11-14 years according to the first menstruation

No. of cases	**Age of first menstruation (years)** / No. of cases						**Average pubertal age**
	9	**10**	**11**	**12**	**13**	**14**	
125	1	13	27	68	7	9	11years, 9 months
Romania, girls, urban area, 1999							12 years, 9 months
Banat Region, girls, urban area, 1998							11 years, 8 months

Compared with the average pubertal age to girls from Romania, urban area, 1999 (12), girls from Timis County had a mean pubertal less than 1 year.

Compared with the average pubertal age of girls from Banat Region, urban area, 1998 (13,14), the girls from Timis County had a mean pubertal age higher with one month.

2. 2. The average pubertal age in boys

First ejaculation age to the 10 pubescent boys in the investigated group, ranged between 13-14 years, the number of cases was 2.5 times higher in 14 years compared to 13 years. Pubertal mean age was 14 years (table III).

TABLE III

The distribution of boys aged 11-14 years according to the first ejaculation

No. of cases	**Age of first ejaculation /** No. of cases		**Average pubertal age**
	13	**14**	
10	3	7	14 years
Romania, boys, urban area, 1999			14 years, 4 months
Banat Region, boys, urban area, 1998			14 years, 2 months

Compared with the average pubertal age to boys from Romania, urban area, 1999, boys from Timis County have an average pubertal age of lower than 4 months. Compared with the average pubertal age in boys from Banat Region, urban area, 1998, the boys from Timis County have an average pubertal age lower than 2 months.

CONCLUSIONS

Pubertal maturation is earlier and more pronounced in girls than boys, and with age, the number of adolescents in Tanner stages is growing, and decreases in the lower stages. Boys prevail in Tanner stages I-III, with 2.2-15.5-17.8 % more than girls. Girls prevail in prepubertal stage IV with 6.7 % and pubescent stage V with 28.8 %. The first pubescent girls have 11 years and first pubescent boys have 13 years. At age 14, the ratio of pubescent girls / boys is 12.5 / 1.

The age of the first menstruation in girls is between 9-14 years, resulting a pubertal mean age of 11 years and 9 months. The age of the first ejaculation in boys is between 13-14 years and the pubertal mean age is 14 years. The average pubertal age is lower in girls compared with boys with 2 years and 3 months.

Pubertal maturation at the studied adolescents from Timis County, is higher than the national maturity level (stage 1999), and is less elevated than the regional level of maturity (1998).

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O ABORDARE INTERDISCIPLINARĂ A UNOR SITUAȚII CLINICE COMPLEXE ORTODONTICO-PARODONTALE LA PACIENȚII ADULȚI

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AN INTERDISCIPLINARY APPROACH TO COMPLEX ORTHODONTIC-PERIODONTAL CLINICAL SITUATIONS IN ADULT PATIENTS (Abstract): Between orthodontics and periodontology there are many correlations, especially in the treatment of adults with already-damaged periodontal tissues and malocclusions. Aim of this paper was to emphasize that an interdisciplinary periodontal-orthodontic treatment could be beneficial even in a case that seemed hopelessly. A typical case of an adult patient with extensive bone loss due to periodontal disease, occlusal trauma caused by dental malpositions, spacing due to early loss of teeth in the lateral segments and pathologic tooth migration will be presented. The orthodontic treatment was initiated at 3 months after the stabilization of the periodontal therapy and was done with a fixed appliance. During the orthodontic stage periodontal maintenance visits continued at 2-month intervals. The therapy of “black triangles” occurred due to the loss of interdental gingiva was done by stripping followed by a space-closure procedure. A permanent retainer was applied. Benefits and problems of the comprehensive periodontal-orthodontic therapy and treatment protocols will be presented. In conclusion the interdisciplinary therapy of this patient, with a good compliance, was beneficial, improved esthetics, the periodontal status, the ability of the patient to clean the teeth and also led to best position of the abutments for optimal placement of prosthetic reconstructions. **Key words**: INTERDISCIPLINARY TREATMENT, ORTHODONTIC THERAPY, ADULT ORTHODONTICS, PERIODONTAL THERAPY, TREATMENT PROTOCOLS

Tratamentele ortodontice la pacienții adulți sunt parte a unei viziuni terapeutice globale, care presupune o abordare interdisciplinară ce implică o întregă echipă de specialişti în ortodonție, parodontologie, protetică, chirurgie orală şi maxilo-facială, echipă care să asigure astfel rezultate din ce în ce mai performante (1, 2).

Conform definiției lui Chester S. Handelman pacienții adulți care sunt susceptibili la boală parodontală şi au în antecedente pierderi avansate ale osului alveolar şi a unor unități dentare pot fi definiți ca şi pacienți ”compromişi parodontal”. Aceş-

tia constituie o categorie aparte deoarece: sunt mai predispuşi la pierderi osoase ulterioare, parodonţiul redus nu poate compensa pierderea în continuare de os alveolar existând riscul de a pierde dinţi, deseori prezintă şi o malocluzie dobândită iar parodonţiul redus obligă la un tratament ortodontic, o mecanică şi o contenţie specială (3).

Deseori adulţii prezintă anomalii dentomaxilare ”secundare” care nu au fost prezente odată cu erupţia dinţilor ci au apărut pe parcursul vieţii.

Tratamentul ortodontic la adulţii cu afecţiuni parodontale active este un dezastru deoarece deplasarea dentară poate accentua evoluţia bolii parodontale. De aceea se recomandă ca la aceşti pacienţi să se facă în primul rând terapia parodontală şi abia apoi, după ce se stabilizează statusul parodontal şi pacientul dovedeşte abilitatea de a menţine o igienă orală corectă se poate iniţia un tratament ortodontic.

Singura excepţie de la regula de a nu iniţia un tratament ortodontic în prezenţa inflamaţiei parodontale o constituie situaţiile clinice în care inflamaţia nu poate fi complet eliminată datorită malpoziţiilor dentare. În acest context situaţia poate fi îmbunătăţită prin terapie ortodontică (4).

Scopul acestei lucrări a fost de a evidenţia că o terapie interdisciplinară ortodontico-parodontală corect gândită şi aplicată poate fi benefică chiar şi în unele cazuri clinice ce par a fi iremediabil compromise.

PREZENTAREA CAZULUI

Pacientă prezentată este de sex feminin, cu vârsta de 32 de ani, compromisă parodontal, cu pierdere osoasă accentuată datorită bolii parodontale, traumă ocluzală cauzată de malpoziţiile dentare, spaţieri datorate pierderilor dentare precoce în zonele laterale cât şi datorită migrărilor dentare patologice (fig. 1).

Fig. 1. Status clinic intraoral înainte de iniţierea tratamentului ortodontic cu evidenţierea numeroaselor anomalii dento-maxilare, a edentaţiilor cât şi a retracţiilor gingivale datorate bolii parodontale.

Înainte de efectuarea terapiei parodontale pacienta prezenta adâncimi de sondaj mai mari de 5 mm, sângerarea gingiilor, retracţii gingivale cât şi mobilitate dentară. După etapa de terapie iniţială şi de conştientizare şi educare a pacientei în privinţa măsurilor de igienă orală s-a trecut la etapa de chirurgie parodontală pentru eliminarea pungilor parodontale şi reducerea inflamaţiei acute (fig. 2).

Fig. 2. Etapa de chirurgie parodontală

La 3 luni după stabilizarea tratamentului parodontal, în absența unei inflamații acute şi având în vedere existența atât a înghesuirilor dentare care îngreunează igienizarea optimă a interfeței dinte-gingie cât şi a spațierilor ce favorizează traumatizarea papilei interdentare s-a decis inițierea tratamentului ortodontic. Acesta s-a realizat cu aparat ortodontic fix, brackets metalici cu slot de 0,022. Pe parcursul tratamentului ortodontic fix care a durat 20 de luni, pacienta a fost odată la 2 luni la o evaluare parodontală de specialitate şi igienizare profesională. Pe tot parcursul tratamentului medicul ortodont a urmărit evoluția statusului parodontal pentru a semnala colegului parodontolog orice acutizare a afecțiunii parodontale. Pacienta a reuşit să mențină o igienă orală satisfăcătoare neexistând acutizări şi nefiind necesară întreruperea terapiei ortodontice.

Odată cu alinierea arcadelor dentare ambrazurile gingivale ajung la dimensiuni normale însă datorită papilelor interdentare reduse ca urmare a afecțiunii parodontale apare o entitate clinică inestetică cea a ”triunghiurilor negre” (black triangles, engl.). Terapia acestora se poate face fie prin augmentarea fiecărei papile interdentare fie printr-o ”mascare” a defectului. La acest caz datorită numeroaselor triunghiuri negre cât şi din dorința de a poziționa rădăcinile dentare cât mai în interiorul osului alveolar noi am ales a doua variantă prin care am redus proximal dinții implicați (stripping, engl.) şi apoi am închis spațiile. Prin aceasta ambrazurile gingivale s-au redus ca şi dimensiune, aria de contact a migrat spre gingival şi papila a fost ”comprimată” spre incizal ducând la camuflarea defectului (fig. 3).

Fig. 3. După realizarea stripping-ului am închis spațiile rezultate (săgeți) camuflând aspectul inestetic, caracteristic al ”triunghiurilor negre”.

La sfârşitul etapei de tratament ortodontic se observă o îmbunătățire considerabilă a esteticii dento-faciale, acesta fiind şi motivul principal pentru care pacienta s-a adresat medicilor dentişti (fig. 4).

Pentru menținerea alinierii arcadei dentare inferioare s-a aplicat o contenție fixă colată canin-canin. Pacienta a fost apoi îndrumată spre colegii parodontologi pentru inserarea implantelor dentare în zonele edentate şi refacerea ulterioară a continuității arcadelor dentare.

Fig. 4. Aspectul final după îndepărtarea aparatului ortodontic fix.

Pacienta a fost ulterior examinată periodic atât din punct de vedere parodontal cât şi din punct de vedere ortodontic constatându-se stabilitatea rezultatelor şi succesul terapiei interdisciplinare ortodontico-parodontale.

Din punct de vedere radiologic evoluția cazului a fost favorabilă cu menținerea nivelului osului alveolar şi obținerea unei topografii mai favorabile prin lărgirea septurilor interradiculare între dinții cu înghesuiri dento-alveolare ce au fost corectate (fig. 5).

Fig. 5. Evoluția radiologică a cazului, pe baza ortopantomografiilor, cu îmbunătățirea conturului osului alveolar

DISCUȚII

Existența unui protocol clar de colaborare în cadrul acestor echipe terapeutice interdisciplinare care să prevadă soluțiile la problemele ce pot apărea pe parcurs cât şi responsabilitățile fiecărui membru al echipei a dus la obținerea unor rezultate bune în situații care păreau compromise. Comunicarea permanentă între membrii echipei, facilitată şi de către tehnologia actuală digitală este esențială.

Din punct de vedere ortodontic terapia acestor pacienți adulți trebuie individualizată şi diferențiată de tratamentul ortodontic clasic. Datorită pierderii osoase generalizate implantarea dentară se reduce şi scade automat şi capacitatea de ancoraj. Astfel dinții au fost împărțiți în două categorii: cei care au o poziție relativ corectă (cei din zonele laterale) şi care au fost solidarizați pentru a constitui unități de ancoraj viabile şi dinții ce trebuiesc deplasați asupra cărora s-au aplicat forțe de intensitate redusă.

Având în vedere că prin deplasarea spre apical a centrului de rezistență al dinților (datorită reducerii înălțimii osului alveolar) tendința spre versie creşte am preferat să aplicăm brackets mai aplical (pentru a redu-

ce brațul forței și consecutiv și momentul) și am utilizat arcuri rectangulare de beta titanium ce permit un control mai eficient al deplasărilor dentare (5, 2). Arcurile de beta titanium au o combinație excelentă între rezistență și flexibilitate permițând o aplicare diferențiată a forțelor ortodontice cât și o individualizare a deplasărilor dentare.

CONCLUZII

Terapia interdisciplinară a acestei paciente, cu o bună complianță, a fost benefică îmbunătățind estetica, statusul parodontal, posibilitatea pacientei de a-și igieniza dinții și ducând la o amplasare optimă a unităților dentare în vederea unei reconstituiri protetice.

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UTILIZAREA REAL TIME PCR PENTRU TESTAREA IZOLATELOR CLINICE DE *STAPHYLOCOCCUS AUREUS* DIN SPITALUL CLINIC DE BOLI INFECŢIOASE IAŞI

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USE OF REAL TIME PCR FOR TESTING *STAPHYLOCOCCUS AUREUS* ISOLATES FROM THE IAŞI INFECTIOUS DISEASES HOSPITAL (Abstract): *S. aureus* is capable of producing a wide spectrum of diseases and can quickly develop resistance to antibiotics. These features require a careful monitoring of these organisms, by detection of resistance genes and virulence factors, such as Panton-Valentine leukocidin (PVL). **Aim:** To determine the presence of *mec*A and *pvl* genes in *S. aureus* isolates by a Real Time-PCR technique (RT-PCR) in order to shorten the detection time. **Materials and methods:** We tested 119 strains isolated from pus, using phenotypic methods for methicillin resistance characterization, according to CLSI 2008-2010 guidelines. Detection of *mec*A and *pvl* genes was done with hydrolysis probes. **Results:** The prevalence rate of methicilin resistant *S. aureus* (MRSA) was 40,33%, and *pvl* was detected in 52,08% of those strains. The results of the conventional methods for methicillin resistance detection were validated by those obtained by RT-PCR **Conclusions:** RT-PCR is useful in epidemiological surveillance of MRSA and PVL-producing strains and validation of test results for phenotypic resistance to oxacillin.
Key words: MRSA, *mec*A, PVL, PCR

Staphylococcus aureus este capabil să producă un spectru foarte larg de afecţiuni, de la infecţii minore, la infecţii cu potenţial letal. Apariţia tulpinilor rezistente la meticilină, care asociază frecvent rezistenţă multiplă la alte antibiotice, a dus la o creştere a mortalităţii şi a duratei de spitalizare. Cel mai frecvent, rezistenţa la meticilină este cauzată de achiziţia genei *mec*A, care codifică sinteza unei noi proteine de legare a penicilinei (PLP2a). Multă vreme, MRSA a fost asociat infecţiilor de spital, dar în ultimii ani a fost tot mai frecvent implicat în infecţii comunitare (*community aquired* MRSA: CA-MRSA), apărute la persoane tinere şi fără factori de risc cunoscuţi pentru infecţiile nosocomiale (1). Spectrul de boli cauzate de CA-MRSA variază de la

infecţii minore ale pielii şi ţesuturilor moi, la infecţii cu evoluţie severă rapidă şi adesea letale, precum: pneumonie necrozantă, osteomielită, abcese renale şi pulmonare, fasciită necrozantă, endocardită sau artrită septică. Virulenţa crescută a tulpinilor comunitare a fost asociată cu sinteza PVL, o exotoxină citolitică, prezentă în special la izolatele din leziuni dermonecrotice. Toxina este compusă din 2 proteine care acţionează sinergic, LukS PVL şi LukF-PVL, codificate de gene fagice şi are drept ţintă leucocitele polimorfonucleare, determinând formarea de pori la nivelul membranei citoplasmatice şi lizozomale, ceea ce duce la degranularea citoplasmei şi liză celulară (2). Prezenţa genelor care codifică PVL este considerată marker genetic stabil pentru infecţiile comunitare (1). Capacitatea tulpinilor CA-MRSA de a se răspândi rapid nu doar în comunitate, ci şi în mediul de spital, face necesară depistarea lor corectă, în timp scurt, în vederea instituirii măsurilor de prevenţie nespecifică. Metodele convenţionale de testare a sensibilităţii la antibiotice nu reuşesc sa evidenţieze întotdeauna meticilino-rezistenţa, în cazul tulpinilor heterorezistente şi, prin obligativitatea izolării bacteriei în cultură pură, presupun un timp mai indelungat pentru obţinerea profilului de sensibilitate la antibiotice. Tehnicile moleculare de detecţie a genei *mec*A sunt folosite, împreună cu metodele de evidenţiere a PLP2a, drept teste standard de confirmare pentru MRSA în cazul infecţiilor stafilococice severe (3). În acest studiu ne-am propus aplicarea tehnicii RT-PCR, utilizând infrastructura existentă în cadrul Disciplinei de Microbiologie a Facultăţii de Medicină a UMF „Gr. T. Popa” Iaşi, unde a fost pusă la punct relativ recent această metodă pentru detecţia genelor *mec*A şi *pvl* la izolate clinice de *S. aureus* din Spitalul Clinic de Boli Infecţioase „Sf. Parascheva” Iaşi.

MATERIAL ŞI METODĂ

Au fost testate 119 tulpini de *S.aureus* izolate din exsudate purulente, de la pacienţi internaţi în Spitalul Clinic de Boli Infecţioase „Sf. Parascheva” Iaşi, în perioada ianuarie 2008-decembrie 2010. Depistarea MRSA s-a făcut prin metoda difuzimetrică cu disc de 30 µg cefoxitin şi determinarea CMI a oxacilinei cu benzi E-test (bioMerieux, Franţa). Rezultatele testării fenotipice au fost verificate prin *singleplex* RT-PCR pentru amplificarea genei *mec*A. Depistarea tulpinilor producătoare de PVL s-a făcut prin *singleplex* RT-PCR pentru detecţia genelor *pvl*. În paralel, au fost testate tulpinile de referinţă *S. aureus* ATCC 25923 şi 49775 (*pvl* prezent, *mec*A absent) şi *S. aureus* ATCC 33592 (*mec*A prezent, *pvl* absent), drept martori pozitivi şi negativi. Pentru extracţia ADN stafilococic am folosit kitul *GenEluteTM Bacterial Genomic* DNA, lizostafin şi lizozim (Sigma Aldrich, Germania). Amplificarea şi detecţia cu sonde de hidroliză TaqMan a genelor *mec*A şi *pvl* s-a făcut utilizând instrumentul Stratagene Mx3005P, *Brilliant II QPCR Master Mix* (Agilent Technologies, SUA), primeri şi sonde de hidroliză mecA şi pvl (Biosearch Technologies, Inc., SUA) (tab. I).

Protocolul de lucru este descris în tab. II - V. Am realizat fiecare reacţie în triplicat, pentru verificarea reproductibilităţii rezultatelor.

TABELUL I

Secvenţele primerilor şi sondelor (după McDonald *et al*., 2005)(4)

Tip primeri/ sondă	**Secvenţă (5'-3')**	**GC (%)**
*mec*A Fwd	GGCAATATTACCGCACCTCA	50
*mec*A Rev	GTCTGCCACTTTCTCCTTGT	50
sondă *mec*A 5'FAM 3'TAMRA	AGATCTTATGCAAACTTAATTGGCAAATCC	33,3
pvl Fwd	ACACACTATGGCAATAGTTATTT	30,4
pvl Rev	AAAGCAATGCAATTGATGTA	30
sondă *pvl* 5'$Quasar_{670}$ 3'BHQ3	ATTTGTAAACAGAAATTACACAGTTAAATATGA	21,2

Legendă: Fwd = primer *forward*, Rev = primer *reverse*

TABELUL II

Amestecul de reacţie realizat pentru detectarea genei *mec*A.

Reactivi	**Concentraţia iniţială (µM)**	**Volumul utilizat (µl)**	**Concentraţia în reacţie (µM)**
Master Mix	2x	10	1x
primer *Fwd*	30	0,2	0,3
primer *Rev*	30	0,2	0,3
sondă de hidroliză	100	0,02	0,1
apă *nuclease-free*		7,58	
ADN ţintă		2	
Volumul final		20	

TABELUL III

Profilul termic utilizat pentru detecţia genei *mec*A

Nr cicluri	**Etapă**	**Temperatură**	**Timp**
1	predenaturare (activarea *Taq* polimerazei *Hot start*)	95ºC	7 minute
40	denaturare	95ºC	15 secunde
	hibridizare primeri şi elongare	60ºC	60 secunde

TABELUL IV

Amestecul de reacţie realizat pentru detectarea genei *pvl*

Reactivi	Concentraţia iniţială (µM)	Volumul utilizat (µl)	Concentraţia în reacţie (µM)
Master Mix	2x	10	1x
primer mix (*Fwd* şi *Rev*)	10	0,8	0,4
sondă de hidroliză	100	0,02	0,1
apă *nuclease-free*		7,18	
ADN ţintă		2	
Volumul final		20	

TABELUL V

Profilul termic utilizat pentru detecţia genei *pvl*

Nr cicluri	Etapă	Temperatură	Timp
1	predenaturare	95ºC	10 minute
40	denaturare	95ºC	15 secunde
	hibridizare primeri şi elongare	55ºC	60 secunde

REZULTATE

Prezenţa genelor amplificate a fost indicată prin creşterea fluorescenţei fluorocromului cu care a fost marcată sonda de detectare. Canalele de fluorescenţă utilizate au fost FAM şi Cy5, pentru detectarea sondelor marcate cu FAM şi respectiv Quasar 670, Gena *mec*A a fost detectată la 48 de izolate, reprezentând 40,33% din totalul tulpinilor testate. Numărul tulpinilor *pvl* pozitive a fost de 37, respectiv 31,09% din total şi 52,08% din tulpinile MRSA (tab. VI). Rezultatele testărilor fenotipice au fost interpretate conform standardelor CLSI (*Clinical Laboratory Standards Institute*) 2008–2010 şi s-au corelat cu detectarea meticilino-rezistenţei prin biologie moleculară. Curbele de amplificare a genelor *mec*A şi *pvl* sunt prezentate în fig. 1 şi 2.

TABELUL VI

Rezultatele determinării genelor *mec*A şi *pvl* la izolate clinice

	MRSA		MSSA	
An	Nr total tulpini	Nr tulpini *pvl* +	Nr total tulpini	Nr tulpini *pvl* +
2008	14	5	22	5
2009	14	6	16	3
2010	24	14	29	4

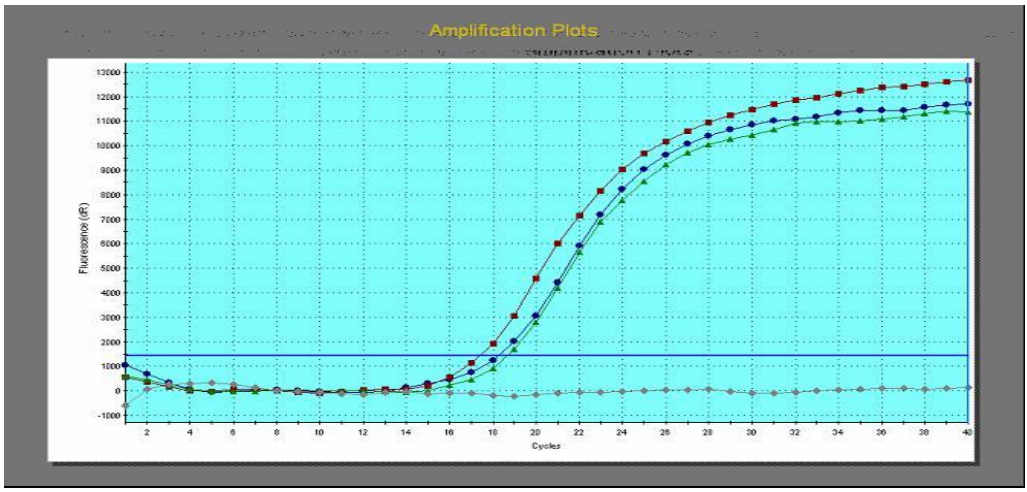

Fig. 1. Amplificarea genei *mec*A la izolate clinice de *S.aureus*

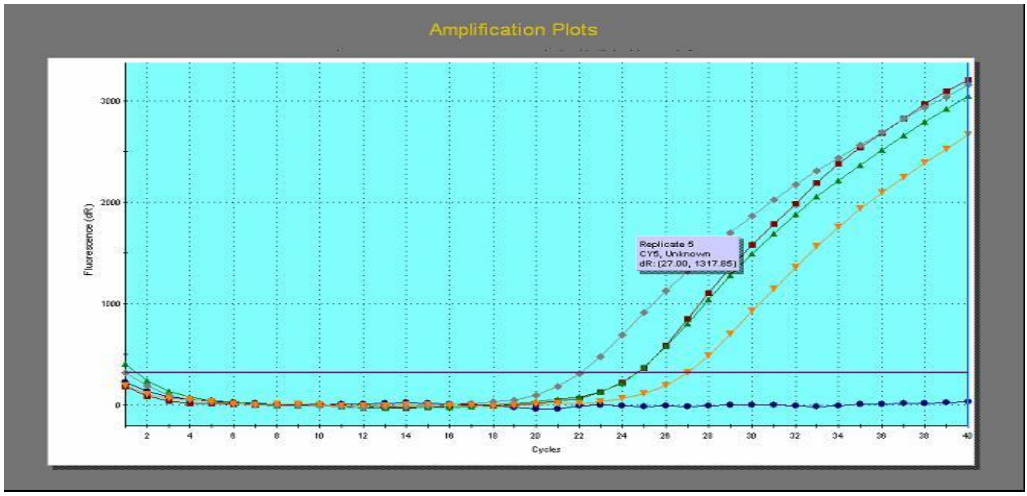

Fig. 2. Amplificarea genelor *pvl* la izolate clinice de *S. aureus*

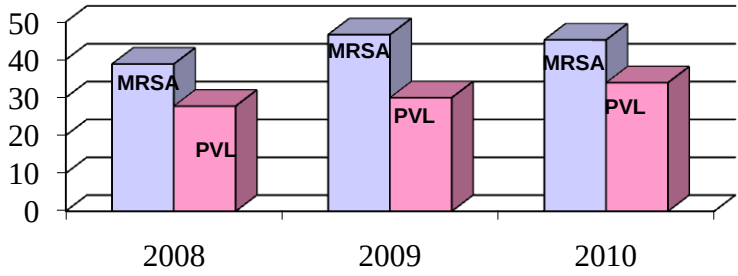

Fig. 3. Prevalenţa tulpinilor MRSA şi a tulpinilor PVL+

DISCUŢII

Detecţia genei *mec*A prin RT-PCR s-a corelat cu determinarea meticilino-rezistenţei prin metode fenotipice. Rezultatele noastre sunt similare cu cele publicate de Anand *et al*. (2009) şi Pottumarthya *et al*. (2005), care raportează o sensibilitate a testării cu discul de 30 µg cefoxitin de 100% şi respectiv 99% (5,6). Rata infecţiilor cu tulpini MRSA şi PVL pozitive a variat nesemnificativ pe parcursul celor 3 ani ($p > 0,05$) (fig. 3). Prevalenţa MRSA remarcată în cursul studiului (40,33%) este în acord cu date publicate anterior de Dorneanu *et al* (8) în 2006 (47%) şi cu raportările *European Antimicrobial Resistance Surveillance Network* (EARS-Net), care plasează România pe unul dintre primele locuri privind incidenţa MRSA izolat din infecţii invazive (35.6%) (9). Menţinerea prevalenţei MRSA la un nivel redus presupune un program riguros de prevenţie, prin *screening*-ul la internare al pacienţilor cu risc înalt de a fi colonizaţi. Folosirea RT-PCR oferă în perspectivă posibilitatea depistării MRSA direct în probă şi instituirea rapidă a măsurilor de prevenţie a răspândirii acestor tulpini în spital, ceea ce reprezintă un avantaj evident al folosirii tehnicilor de biologie moleculară (3, 4). Conform datelor din literatură, MRSA poartă mai frecvent, comparativ cu tulpinile sensibile la meticilină, gene care codifică sinteza unor toxine (85,5% versus 53,8%)(10), iar unele tulpini comunitare sunt mai virulente decât cele de spital (2). Expresia PVL este puternic asociată cu CA-MRSA, 96% dintre izolatele PVL pozitive fiind CA-MRSA (11). Studiul nostru a evidenţiat o rată crescută a infecţiilor cu tulpini MRSA, PVL pozitive (21%). Procentul de tulpini PVL pozitive raportat în literatură variază considerabil de la un autor la altul (între 2-66%), probabil datorită circulaţiei diferite a acestor tulpini în anumite regiuni. Într-un studiu realizat de Fang *et al.* (2008) dintre 104 tulpini CA-MRSA izolate în Stockholm în perioada 2000-2005, 56 % au prezentat genele *pvl* (12). Berglund *et al* (2011) a detectat genele *pvl* la 66% dintre tulpinile CA-MRSA (13), în timp ce Holmes *et al* (2005) a remarcat un procent redus de izolate de *S. aureus* purtătoare ale acestor gene (1,6%) (14). S-a observat asocierea tulpinilor producătoare de PVL cu o severitate crescută a leziunilor localizate şi un răspuns inflamator sistemic accentuat (15). Unii autori remarcă o infiltrare a tulpinilor comunitare în mediul de spital şi prezic înlocuirea tulpinilor clasice de spital de către tulpinile CA-MRSA, dotate cu proprietăţi deosebite de colonizare (16).

CONCLUZII

Creşterea fenomenului de rezistenţă la antibiotice impune o strictă monitorizare a tulpinilor de *S. aureus* circulante, cu identificarea izolatelor purtătoare ale genelor de rezistenţă. Dezvoltarea tehnicilor moleculare, mai rapide, specifice şi sensibile comparativ cu cele clasice reprezintă o perspectivă atrăgătoare. Detectarea genei *mec*A în cadrul laboratorului nostru a permis confirmarea rezultatelor testării fenotipice pentru rezistenţa la oxacilină, scurtând timpul de detecţie. În pofida unor costuri mai mari, tehnica face posibilă instituirea precoce a măsurilor preventive, cu economisirea fondurilor aferente diagnosticării şi tratării cazuilor secundare răspândiri acestor utilpini în comunitate.

În cadrul acestui studiu a fost evaluată pentru prima dată prevalenţa tulpinilor de *S. aureus* producătoare de PVL pentru izolate clinice din exsudate purulente din Spitalul Clinic de Boli Infecţioase „Sf. Parascheva” Iaşi. Prevalenţa crescută a tulpinilor de *S. aureus* producătoare de PVL (31,09%) şi

implicaţia lor în patologia infecţioasă face necesară detectarea acestora, datele putând fi utilizate, în asociere cu tehnicile de tipare, pentru identificarea tulpinilor CA-MRSA şi supravegherea epidemiologică a circulaţiei lor.

MULŢUMIRI

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CHISTUL DERMOID AL GLANDEI PAROTIDE

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DERMOID CYST OF THE PAROTID GLAND (Abstract): Dermoid cyst of the parotid gland is a rare clinical entity. Definitive clinical diagnosis is often difficult to determine preoperatively because of the lack of pathognomonic features. The most frequent location of a parotid dermoid cyst is a triangular area lying above the pinna. Imaging studies do not definitely diagnose a parotid dermoid cyst. Although parotid dermoid cyst is (generally) well-encapsulated, complete removal of the cyst wall is not sufficient to cure it, so it is mandatory to perform careful excision of the cyst by parotidectomy, in terms of preserving facial nerve integrity. Histopathology of the parotid gland tumor removed by parotidectomy makes a diagnosis of certainty, by revealing a cyst wall with keratinization of the squamous epithelium and the presence of skin annexes (hair follicles, sweat glands, sebaceous glands). We present a rare case of parotid dermoid cyst in a 21-year-old male patient with symptoms and imaging rather suggestive of arch I branchial cyst and a brief review of data in the medical literature of the last 20 years. **Key words:** DERMOID CYST, PAROTID GLAND, TRUE HAMARTOMAS

Chistul dermoid este o formă de teratom cu diferenţiere ectodermică, fiind rezultatul incluziei unor celule epiteliale de-a lungul liniilor de sutură embrionare (1,2). La nivelul segmentelor capului şi gâtului, această entitate clinică este mai puţin întâlnită, reprezentând aproximativ 7% dintre toate chisturile dermoide ale corpului (3, 4). În aria cap-gât, chisturile dermoide sunt decelate predominant la nivelul regiunilor: orbitară, orală şi nazală (peste 80%), cu următoarele frecvenţe raportate: 49,5% (pentru orbite), 12,6% (nas), 23,3% (regiunile submentală şi submandibulară) şi respectiv 14,6% - alte regiuni (occipitală, frontală, labială, cervicală, văl palatin) (4). Pentru teritoriul oro-maxilo-facial, cele mai multe cazuri de chisturi dermoide descrise în literatura medicală internaţională sunt cele localizate în planşeul bucal, situarea parotidiană a unui chist dermoid fiind considerată foarte rară, atât la adulţi cât şi la copii (1,2,5,6).

Dintre toate leziunile glandei parotide,

cele chistice reprezintă 2-5% şi pot fi: chisturi parotidiene congenitale sau chisturi parotidiene dobândite (obstrucţie, tumori, calculi, traumatisme, paraziţi) (4). Leziunile chistice congenitale ale glandelor parotide sunt clasificate în: chisturi dermoide, chisturi de arc branhial, chisturi de diverticul branhial, chisturi ductale. Un chist parotidian se poate dezvolta în orice porţiune a glandei, diagnosticul clinic preoperator fiind dificil de stabilit, în special în cazurile localizărilor profunde faţă de nervul facial. Explorările imagistice precum: tomografia computerizată, imagistica prin rezonanţă magnetică sau ultrasonografia nu pot furniza un diagnostic decisiv preoperator (6). În multe situaţii, diagnosticul definitiv se stabileşte după excizia leziunii.

Prezentăm un caz clinic de chist dermoid parotidian, la care suspiciunea diagnostică iniţială (preoperator şi chiar intraoperator) a fost de chist de arc I branhial cu localizare parotidiană, motivată de: vârsta pacientului, localizarea formaţiunii tumorale, evoluţia clinică cu numeroase episoade inflamatorii locale, aspectul imagistic, consistenţa lichidiană precum şi de recunoaşterea statistică a frecvenţei mai crescute a chisturilor branhiale între tumorile chistice parotidiene.

PREZENTAREA CAZULUI

În Clinica de Chirurgie Orală şi Maxilo-Facială Iaşi, s-a prezentat în mai 2010 un pacient de sex masculin, în vârstă de 21 ani, cu domiciliul în judeţul Bacău, motivul internării fiind deformarea regiunii parotidiene stângi (anterior şi inferior de lobulul urechii) printr-o formaţiune tumorală rotundă, de aproximativ 4 cm în diametru, renitent-elastică, nedureroasă, mobilă odată cu glanda parotidă, evoluţia clinică declarată fiind de un an (fig. 1 şi 2). S-a remarcat totodată că tegumentul acoperitor corespondent formaţiunii prezenta uşoară congestie şi era relativ aderent la tumora subiacentă.

Fig. 1. Tumoră parotida stângă (aspect faţă preoperator)

Fig. 2. Tumoră parotida stângă (aspect semiprofil stâng preoperator)

Antecedentele personale şi heredo-colaterale ale pacientului erau nesemnificative. Examenul clinic obiectiv pe aparate şi sisteme nu a decelat alte elemente patologice.

S-a efectuat o *tomografie computerizată*

pentru segmentul „cap”, evidențiindu-se prin explorare nativă la nivelul porțiunii posterioare a glandei parotide stângi *o imagine cu densitate lichidiană de 42 mm/48 mm, bine delimitată*, care nu infiltrează țesuturile adiacente, se insinuează posterior de ramul ascendent al mandibulei şi are efect de masă asupra venei retromandibulare pe care o deplasează medial (fig. 3).

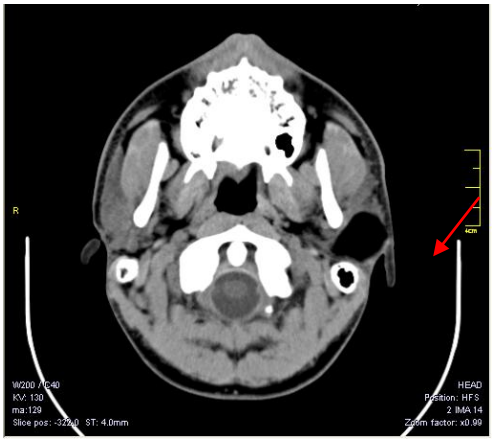

Fig. 3. Aspect computertomografic al formațiunii tumorale parotidiene stângi

Intraoperator, după expunerea glandei parotide stângi, s-a constatat la nivelul lobului superficial o *formațiune chistică*, cu un perete inegal, relativ aderentă la țesutul glandular adiacent. S-a efectuat *extirparea tumorii chistice parotidiene* respective prin *parotidectomie superficială stângă cu conservarea nervului facial* (fig. 4). În timpul manevrelor de disecție a lobului superficial parotidian, s-a întrerupt accidental continuitatea peretelui formațiunii chistice, eliberându-se un *conținut lichidian cu cristale de colesterină*. Intervenția chirurgicală a mai notat o particularitate, şi anume: *extinderea peretelui chistic până la nivelul conductului auditiv extern stâng*, aderența moderată stabilită între cele două elemente îngreunând disecția propriu-zisă. Toate aceste aspecte intraoperatorii au motivat orientarea de moment către diagnosticul de *chist congenital de arc I branhial*. Datorită împrăştierii conținutului chistului către țesuturile şi structurile anatomice vecine, după extirparea formațiunii menționate odată cu țesutul parotidian „gazdă” s-a procedat la toaletarea plăgii operatorii cu soluție de betadină 1%, pentru a evita o eventuală reacție de corp străin sau o potențială complicație infecțioasă.

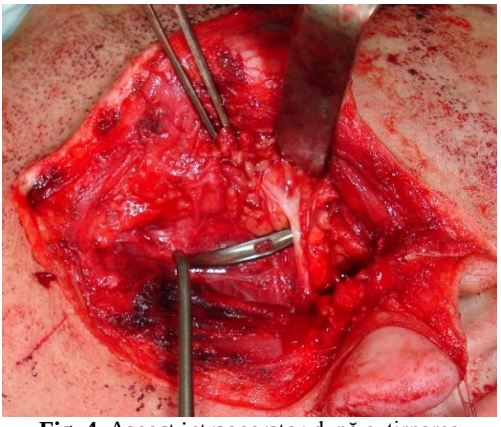

Fig. 4. Aspect intraoperator după extirparea tumorii prin parotidectomie superficială stângă cu conservarea nervului facial

Examenul histopatologic definitiv al piesei operatorii (A.P. nr. 78286/07.05.2010) a descris: formațiune *chistică*, cu perete sclerohialin gros, ce îngloba în unele zone numeroase *fire de păr*; s-au mai remarcat numeroase *chisturi lipidice* şi aspecte de *lipogranulomatoză*, cu evidențierea fragmentelor parotidiene pe versantul extern al chistului. Concluzia examenului morfopatologic al specimenului operator fixat a fost: *chist dermoid cu remaniere şi organizare conjunctivă* (fig. 5,6,7 şi 8).

Evoluția postoperatorie a fost obişnuită, favorabilă, cu cicatrizare corectă a plăgii şi fără semne ale paraliziei de nerv facial stâng (fig. 9 şi 10).

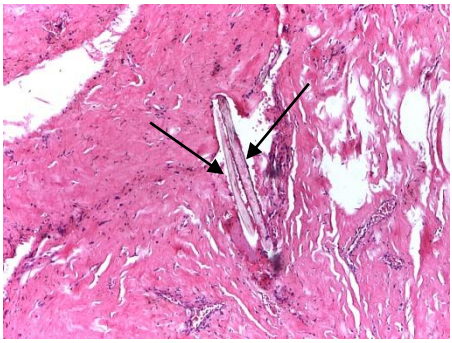

Fig. 5. Aspect microscopic al piesei operatorii (colorația hematoxilină-eozină, mărire obiectiv x4) cu evidențierea firelor de păr secționate longitudinal, la nivelul peretelui chistic sclerohialin: chist dermoid

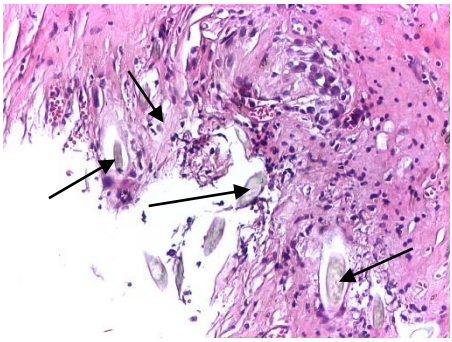

Fig. 6. Aspect microscopic detaliat al piesei operatorii (colorația hematoxilină-eozină, mărire obiectiv x10) cu evidențierea a numeroase fire de păr la nivelul peretelui chistic: chist dermoid

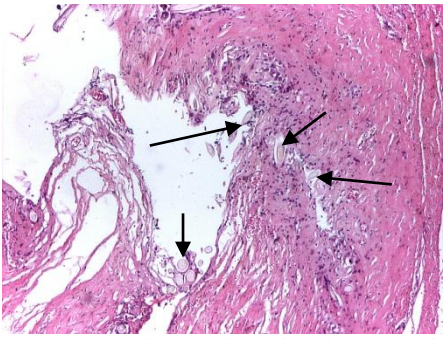

Fig. 7. Aspect microscopic (colorația HE, mărire obiectiv x4): fire de păr la nivelul peretelui chistic şi fragmente parotidiene pe versantul extern al chistului – chist dermoid parotidian

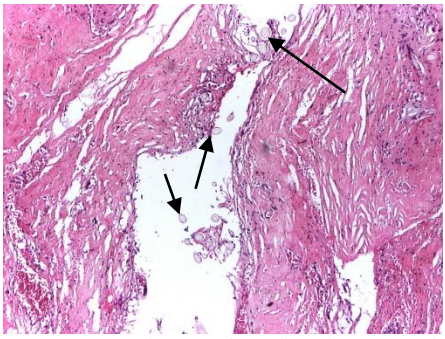

Fig. 8. Aspect microscopic (colorația HE, mărire obiectiv x4): numeroase fire de păr incluse în sclerohialinoză (remaniere conjunctivă) la nivelul peretelui chistic parotidian: chist dermoid

Fig. 9. Aspect profil stâng postoperator, după 2 săptămâni (plagă postoperatorie cicatrizată corect)

Fig. 10. Aspect semiprofil stâng postoperator, după 2 săptămâni (absența paraliziei de nerv facial stâng)

DISCUȚII

Chisturile dermoide ale capului şi gâtului sunt relativ rare, constituind doar 7% din totalul chisturilor dermoide ale corpului. Frecvența chisturilor dermoide este şi mai mică pentru regiunea parotidiană, aceste cazuri fiind sporadic decelate şi raportate (5). În Clinica de Chirurgie Orală şi Maxilo-Facială Iaşi, în perioada 2006-2010, au fost diagnosticate şi tratate 4 cazuri de chisturi dermoide cu localizare parotidiană, unul dintre acestea fiind cel prezentat mai sus.

Termenul de „chist dermoid" este utilizat actualmente pentru a descrie formațiuni care pot fi: chist epidermoid, chist dermoid sau chist teratoid (7). Din punct de vedere histologic, chisturile dermoide adevărate sunt căptuşite de epiderm şi prezintă anexe ale pielii precum: glande sudoripare, glande de sebacee, peri şi foliculi piloşi. Dacă aceste anexe lipsesc din alcătuirea peretelui chistului, termenul folosit este de „chist epidermoid". Dacă peretele chistic prezintă structuri derivate din toate cele trei straturi germinale (ectoderm, mezoderm şi endoderm), entitatea respectivă se defineşte ca fiind un „teratom" sau „chist teratoid" (8). Conținutul chistului dermoid poate fi reprezentat de: cheratină, cazeum, sebum, puroi, peri, unghii, celule adipoase, cristale de colesterină, cartilaj. Prin prisma acestor mențiuni histopatologice, cazul raportat de noi s-a dovedit a fi un chist dermoid adevărat, cu localizare în glanda parotidă, cu toate că lichidul intrachistic observat intraoperator avea un aspect mai sugestiv pentru chistul branhial.

Chisturile dermoide sunt leziuni congenitale rare, derivate din celule pluripotente. Cea mai credibilă teorie embriologică privind dezvoltarea acestor formațiuni este cea care susține incluzia eronată a unor celule pluripotente, în timpul primelor săptămâni de viață intrauterină, care conduce la dezvoltarea ulterioară a structurilor din unul sau din toate cele trei straturi germinale: ectoderm, mezoderm, endoderm (9). Diferențierea acestor straturi germinale produce: anexe ale pielii (păr, glande sebacee şi sudoripare), muşchi, oase, cartilaj, dinți, membrane mucoase. Cauzele acestor modificări pot fi: traumatisme, infecții sau creşteri autonome spontane. Astfel se explică cele trei varietăți de chisturi descrise anterior, clasificate după caracteristicile histologice: chistul epidermoid este căptuşit de un epiteliu scuamos stratificat şi este alcătuit dintr-un singur strat germinal (ectoderm); chistul dermoid este căptuşit de un epiteliu stratificat, prezentând şi anexe ale pielii (origine ectodermică şi mezodermică); chistul teratoid este alcătuit din structuri cu triplă origine: ectodermică, mezodermică şi endodermică.

Chisturile dermoide sunt frecvent situate în regiunile unde se produc fuziuni între mugurii embrionari. Majoritatea cazurilor raportate sunt pe linia mediană a corpului. Pentru teritoriul oro-maxilo-facial sunt descrise următoarele localizări: linia mediană a planşeului bucal (sublingual sau submental), palatul (dur sau moale), limba, regiunile geniene, glandele parotide, maxilarele şi mandibula (10,11). Chisturile dermoide parotidiene prezentate în literatura medicală de specialitate interesează toate vârstele, inclusiv copiii (1,2,3,4,5). Cazul nostru aparține unui adult tânăr, încadrându-se de altfel în cea mai frecventă categorie de vârstă afectată de această patologie.

Diagnosticul clinic definitiv de chist dermoid parotidian este dificil de stabilit. Examenul fizic nu poate decela elemente caracteristice acestei leziuni parotidiene. Diagnosticul diferențial al unei tumori chistice parotidiene aduce în discuție (în

afara chistului dermoid): chistul branhial, chistul unui diverticul branhial, chistul ductal congenital sau un chist dobândit al glandei parotide. Consistența predominant lichidiană a conținutului chistului parotidian descris de noi a motivat orientarea intraoperatorie mai curând către un chist branhial şi nu către un chist dermoid, acesta din urmă având adesea o consistență mai păstoasă (asemănătoare „grişului cu lapte") şi o culoare alb-gălbuie. Mai mult, în cazul relatat, localizarea profundă, intraparenchimatoasă, sub nivelul lobului urechii ar fi pledat de asemenea pentru un chist cu originea în arcul I branhial.

Sialografia poate releva un sistem ductal parotidian normal sau o uşoară compresiune la nivelul său. Explorările imagistice mai performante precum ecografia, examenul prin tomografie computerizată sau imagistica prin rezonanță magnetică nucleară sunt utile pentru stabilirea densității şi extinderilor tumorale dar nu pot concluziona definitiv existența unui chist dermoid parotidian. În cazul nostru, explorarea prin tomografie computerizată a fost orientativă, evidențiind o densitate lichidiană pentru tumora parotidiană. De menționat că, au fost raportate în literatură confuzii preoperatorii ale unor chisturi dermoide parotidiene cu lipoamele parotidiene (3,4, 6). Studiul imagistic prin rezonanță magnetică nucleară are însă o acuratețe mai mare în evaluarea diseminărilor picăturilor grăsoase într-un chist dermoid, acestea fiind caracteristice pentru diagnosticul unui chist dermoid cervical.

Evaluarea completă a unui chist dermoid parotidian ar trebui să includă şi examenul citologic prin puncția-aspirație cu ac fin, după examenul fizic minuțios şi investigația imagistică. Această explorare poate pune în evidență resturi de epiteliu scuamos cu sau fără nuclei precum şi resturi de keratină, trăsături considerate sugestive pentru un chist dermoid. Aspirația cu ac fin este o metodă care poate îmbunătăți în mod real diagnosticul preoperator de chist dermoid parotidian (5,12).

Examenul histopatologic al unui chist dermoid parotidian relevă un perete chistic cu cheratinizarea epiteliului scuamos precum şi prezența anexelor pielii (foliculi piloşi, glande sudoripare, glande sebacee) (4, 13). Diagnosticul definitiv în cazul descris mai sus s-a stabilit în urma detaliilor caracteristice oferite de studiul de microscopie optică al piesei operatorii.

Un chist dermoid al glandei parotide este, în general, relativ bine încapsulat, astfel încât extirparea sa este oarecum mai facilă față de chisturile dermoide cu localizare în regiunile orbitară sau nazală. Dar simpla excizie a chistului poate conduce la persistența unor resturi microscopice de țesut, astfel încât leziunea va recidiva mai târziu. Pentru vindecarea completă a unui chist dermoid parotidian este obligatorie extirparea sa împreună cu țesutul parotidian în care s-a dezvoltat (parotidectomie). În cazul relatat în prezentul material, decizia extirpării tumorii parotidiene prin parotidectomie superficială s-a luat preoperator şi din considerentul evoluției profunde, intraparenchimatoase a leziunii. Evoluția postoperatorie a fost favorabilă, fără a se consemna recidivă după un an de la intervenția chirurgicală. Un chist dermoid al glandei parotide este, în general, relativ bine încapsulat, astfel încât extirparea sa este oarecum mai facilă față de chisturile dermoide cu localizare în regiunile orbitară sau nazală. Dar simpla excizie a chistului poate conduce la persistența unor resturi microscopice de țesut, astfel încât leziunea va recidiva mai târziu. Pentru vindecarea com-

pletă a unui chist dermoid parotidian este obligatorie extirparea sa împreună cu țesutul parotidian în care s-a dezvoltat (parotidectomie). În cazul relatat în prezentul material, decizia extirpării tumorii parotidiene prin parotidectomie superficială s-a luat preoperator şi din considerentul evoluției profunde, intraparenchimatoase a leziunii. Evoluția postoperatorie a fost favorabilă, fără a se consemna recidivă după un an de la intervenția chirurgicală.

CONCLUZII

Deşi chistul dermoid al glandei parotide este o entitate clinică rar întâlnită şi nu prezintă aspecte clinice sau imagistice patognomonice trebuie luat în discuție în diagnosticul diferențial al formațiunilor chistice parotidiene.

Particularitatea chistului dermoid parotidian raportat de noi o constituie consistența neobişnuită, lichidiană a conținutului acestuia.

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ASPECTE CORELAŢIONALE PRIVIND OSTEOMIELITA HEMATOGENĂ LA ADULŢI ŞI COPII

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CORRELATIVE ASPECTS INVOLVING HEMATOGENOUS OSTEOMYELITIS AT CHILDREN AND ADULTS (Abstract) : Osteomyelitis is an inflammation of the bone caused by an infecting organism. The infection may be limited to a single portion of the bone or may involve a number of regions such as the marrow, cortex, periostium and even the surrounding soft tissue. Material and method : This retrospective study included a number of 256 cases made by 152 children (56%) and 115 adults (44%). Results : Hematogenous osteomyelitis apears at any age, most frequently in growth period, 85% cases until 16 years. This study relieve a high frequency of subacute (40,1%) and acute (31,1%) hematogenous osteomyelitis compare with chronic form (28,8%). Regarding the distribution of cases by sex, there are no differences beetwen man and woman in chronic osteomyelitis, but subacute form is most frequently at men. The analysis of the cases distribution by location and sex relieve that at womans metaphysis and diaphysis are most interested (70,09%) but only 53,7% at men. Regarding the treatment, the distribution of cases relieve that chronic osteomyelitis generally cannot be eradicated without surgical treatment(sequestrectomy and resection followed by reconstruction with material. Those others forms, acute and subacute hematogenous osteomyelitis, were resolved with medical and surgical treatment, equaly. Conclusion : Prompt diagnosis and early treatment are required to prevent further destruction and growth disturbance. Generally, a multidisciplinary approach is requred, involving an orthopaedic surgeon, an infectious disease specialist and a plastic surgeon in complex cases with soft tissue loss. **Key words** : HEMATOGENOUS OSTEOMYELITIS, EARLY DIAGNOSIS, TREATMENT

Înainte de a defini osteomielita, trebuie să precizăm că datorită polimorfismului acestei afecţiuni este greu de sintetizat într-o singură frază tabloul clinic şi anatomo-patologic, motiv pentru care au existat de-a lungul timpului dificultăţi în elaborarea unei definiţii care să redea cât mai exact această maladie. Actualmente osteomielita acută este definită ca o infecţie acută hematogenă a osului, frecvent consecinţa unei septicemii stafilococice ; afectează cel mai adesea metafizele oaselor lungi şi se întâlneşte cu maximum de incidenţă în perioada de creştere a organismului, începând cu sugarii mici până la 14-15 ani, utilizându-se mai frecvent denumirea de "*osteomielita acută a perioadei de creştere*".

Osteomielita acută a reprezentat la copil una dintre bolile cele mai grave, până la utilizarea în practica medicală a antibioticelor mortalitatea ajungând de la 14% la 34% (Congresul Francez de Ortopedie-1932). Odată cu introducerea pe scară largă a antibioticelor s-a înregistrat o scădere reduta-

TABELUL I
Repartiţia cazurilor în funcţie de diagnostic în loturile de studiu

Forma clinică	Lot A Copii	Lot B Adulţi	Total
Osteomelită acută hematogenă	43	40	**83**
%	28.3%	34.8%	
Osteomielită subacută hematogenă	94	13	**107**
%	61.8%	11.3%	
Osteomielită cronică	15	62	**77**
%	9.9%	53.9%	
Total	**152**	**115**	267

TABELUL II
Repartiţia cazurilor în funcţie de sexul pacienţilor şi forma clinică în loturile de studiu

	Sexul pacienţilor (total lot)		
Forma clinică	**Feminin**	**Masculin**	**Total**
Osteomelita acuta hematogena	35	48	**83**
%	42.17%	57.83%	
Osteomielită subacută hematogenă	29	78	**107**
%	27.10%	72.90%	
Osteomielită cronică	43	34	**77**
%	55.84%	44.16%	
Total	**107**	**160**	267

bilă a mortalităţii, pentru o scurtă perioada de timp, deoarece apariţia rezistenţei la antibiotice a stafilococului auriu în special a dus progresiv la creşterea numărului de cazuri de osteomielită.

Osteomielita hematogenă (OH) are sediul preponderent în metafizele fertile ale oaselor şi din această cauză în literatură se menţionează faptul că în 75% din cazuri este interesată o metafiză „aproape de genunchi" şi în 12% de cazuri „departe de cot". Restul localizărilor se împart între extremitatea femurală superioară, extremitatea inferioară a tibiei, bazin, rar oasele scurte şi plate (1-2%). Vertebrele pot fi afectate la adult. Studii recente menţionează faptul că traumatismul este întâlnit la o treime din cazuri în istoricul bolii.

Depistarea precoce a osteomielitei hematogene duce la eliminarea erorilor de diagnostic şi tratament ce pot determina progresarea procesului patologic local la bolnavi şi asocierea complicaţiilor locale şi generale osoase, viscerale.

Deseori se constată faptul că o mare parte a copiilor s-au adresat la 2-4 zile de la debut (conform datelor de anamneză) şi au urmat tratament ambulatoriu sau staţionar la domiciliu cu diagnosticul de viroze, fracturi, entorse, artrite etc., fiind internaţi ulterior în stare extrem de gravă.

MATERIAL ŞI METODĂ

Datele de anamneză au pus în evidenţă preexistenţa frecventă a unui traumatism, pacienţii fiind trataţi ambulatoriu şi internaţi la 7–10 zile de la debut cu stare septică, leziuni osoase multiple, diseminări pulmo-

TABELUL III
Repartiţia cazurilor în funcţie de mediul de proveniență al pacienţilor şi forma clinică în lotul de studiu

	Mediul de proveniență pacienților (total lot)		
Forma clinică	**Rural**	**Urban**	**Total**
Osteomelita acuta hematogena	61	22	**83**
%	73.49%	26.51%	
Osteomielită subacută hematogenă	54	53	**107**
%	50.47%	49.53%	
Osteomielită cronică	52	25	**77**
%	67.53%	32.47%	
Total	**167**	**100**	**267**

TABELUL IV
Repartiţia cazurilor în funcţie de localizare şi forma clinică a OS în lotul de studiu

	Localizare (total lot)		
Forma clinică	**Diafiză/metafiza**	**Epifiză**	**Total**
Osteomelita acuta hematogena	78	5	**83**
%	93.98%	6.02%	
Osteomielită subacută hematogenă	21	86	**107**
%	19.63%	80.37%	
Osteomielită cronică	62	15	**77**
%	80.52%	19.48%	
Total	**161**	**106**	**267**

nare şi cardiace. În alte cazuri s-au constatat leziuni stafilococice cutanate netratate, focare cronice de infecţie (carie dentară, amigdalită cronică etc.), care au şi fost puncte de plecare cu determinări osoase.

Debutul bolii la toţi bolnavii a fost brusc, cu stare febrilă, agitaţie, inapetenţă etc. Concomitent copiii prezentau dureri, instalate brusc, progresante cu localizare metafizară iniţial (100% cazuri), ulterior cu răspândire în regiunile învecinate, dereglarea funcţiei membrului afectat.

La momentul internării în Clinica Chirurgie Pediatrică pacienţii prezentau dureri violente continui exacerbate de mişcări neînsemnate, poziţie antalgică a membrului afectat – membrul afectat semiflectat în articulaţia de şold şi/sau genunchi în abducţie, mişcările active în articulaţiile învecinate erau reduse sau lipseau, mişcările pasive produceau dureri insuportabile. Semnele acestea au fost prezente la toţi pacienţii.

Semnele clinice locale: în primele 3–4 zile de la debut segmentul afectat a fost moderat edematiat, în următoarele zile - edem, tumefacţie circumferenţială progresantă cu răspândire în regiunile învecinate cu nuanţări de la caz la caz, cu circulaţia venoasă evidentă. La toţi pacienţii s-a probat mobilitatea membrului afectat iar palparea şi percuţia pe os produceau durere insuportabilă în 100% din cazuri.

Analiza rezultatelor examenelor de laborator a relevat, în toate cazurile, următoa-

TABELUL V
Repartiţia cazurilor în funcţie de localizare şi forma clinică a OS în lotul de studiu

	Localizare – la nivelul scheletului (total lot)		
Forma clinică	**Oase lungi**	**Oase scurte**	**Total**
Osteomelita acuta hematogena	71	12	**83**
%	39.44%	13.79%	
Osteomielită subacută hematogenă	74	33	**107**
%	41.11%	37.93%	
Osteomielită cronică	35	42	**77**
%	19.44%	48.28%	
Total	**180**	**87**	**267**

TABELUL VI
Repartiţia cazurilor în funcţie de localizare (oase lungi) şi forma clinică a OS în lotul de studiu

	Localizare – OASE LUNGI (total lot)				
Forma clinică	**femur**	**tibie**	**humerus**	**cubitus/ radius**	**Total**
Osteomelita acuta hematogena	31	21	10	9	
%	44.93%	26.25%	62.50%	60.00%	
Osteomielită subacută hematogenă	27	36	5	6	
%	39.13%	45.00%	31.25%	40.00%	
Osteomielită cronică	11	23	1	0	
%	15.94%	28.75%	6.25%	0.00%	
Total	**69**	**80**	**16**	**15**	**180**

rele schimbări : anemie de diverse grade, leucocitoză 15.000–25.000 mm^3 cu devierea formulei leucocitare spre stânga şi accelerarea VSH (30–75 mm/oră).

O parte din copiii au fost supuşi examenului radiologic. Radiografia segmentului afectat în 2 incidenţe s-a efectuat la internare la toţi bolnavii : Examenul radiologic în perioada iniţială a permis excluderea leziunilor traumatice şi diferenţierea de diverse procese patologice, iar la etapele de tratament – evoluarea procesului osteomielitic. Examenul sonografic a confirmat diagnosticul de artrită a articulaţiei învecinate segmentului osos afectat. Scintigrafia osoasă a evidenţiat modificări vasculare majore a segmentului osos afectat în toate cazurile.

Astfel, diagnosticul pozitiv s-a bazat pe : analiza detailată a datelor de anamneză, examenul clinic amănunţit al fiecărui pacient, investigaţiile de laborator şi imagistice.

Studul efectuat a evidenţiat că toţi pacienţii au fost supuşi unui tratament complex intensiv, care a constat în :

- tratament medical complex (antibiotice sub controlul antibiogramei, tratament intensiv de detoxicare cu utilizarea metodelor extracorporale de detoxicare şi reechilibrare volemică, terapie simptomatică, desensibilizantă) ;
- tratament chirurgical – intervenţie chirurgicală de urgenţă ;

TABELUL VII
Repartiţia cazurilor în funcţie de forma clinică a OS şi aspectele radiologice în lotul de studiu

	Aspect radiologic					
Forma clinică	Osteoliza metafizară	Abces subperiostic	Osteo-periostita	Abces Brodie	Sechestru osos	**Total**
Osteomelita acuta hematogena	49	20	12	0	2	**83**
%	59.04%	24.10%	14.46%	0.00%	2.41%	
Osteomielită subacută hematogenă	14	12	8	73	0	**107**
%	13.08%	11.21%	7.48%	68.22%	0.00%	
Osteomielită cronică	11	7	13	42	4	**77**
%	14.29%	9.09%	16.88%	54.55%	5.19%	
Total	**74**	**39**	**33**	**115**	**6**	**267**

- ortopedic.

Explorarea intraoperatorie a confirmat diagnosticul de OH în toate cazurile – decolarea periostului de la corticală, eliminări sub presiune din canalul osos medular, din ţesuturile moi.

Tehnica chirurgicală utilizată pentru rezolvarea procesului local a inclus paralel cu deschiderea flegmonului subperiostic, perforaţia osului, după indicaţii – puncţii a articulaţiilor învecinate (articulaţia de şold 1 –3 puncţii, genunchi 1 –2, deschiderea şi drenarea recesurilor superioare.

S-a atras mare atenţie la poziţionarea membrului afectat – poziţia fiziologică: în cazurile afectării segmentului superior – abducţie a membrului. La bolnavii cu implicarea în proces a articulaţiei de şold şi / sau a genunchiului – extensie cutanată a membrului.

Ca rezultat al aplicării tratamentului complex combinat medical, chirurgical şi ortopedic s-au obţinut rezultate favorabile cu însănătoşirea bolnavilor.

REZULTATE

Osteomielita hematogenă se întâlneşte la orice vârsta dar are maximum de frecvenţă in perioadele de creştere intensa a organismului. Acest fapt explică de ce în literatură se prezintă faptul că 85% din îmbolnăviri apar până la 16 ani (un vârf de frecvenţă sub 2 ani si al doilea intre 8 si 12 ani). Este remarcat faptul că afecţiunea prezintă o frecvenţă mai mare în rândul copiilor cu vârste până în 16 ani (56,9%) comparativ cu frecvenţă la adulţi (43,07%).

Repartiţia cazurilor din lotul de studiu în funcţie de vârstă şi forma clinică de osteomielită hematogenă

În lotul de studiu s-a remarcat frecvenţa ridicată a osteomielitei subacute hematogene (40.1%) şi a osteomielitei acute (31,1%). Aşa cum era de aşteptat, o frecvenţă mai scăzută a fost întâlnită pentru osteomielita cronică (28,8%).

În cazul osteomielitei cronice hematogene ponderea sexului masculin este comparabilă cu cea a sexului feminin, însă diferenţe semnificative apar pentru osteomielită subacută hematogenă situaţie în care cel mai frecvent este intâlnit sexul masculin (72.9%).

Distribuţia cazurilor cu osteomielită hematogenă funcţie de mediul de provenienţă şi forma clinică

Distribuţia cazurilor în funcţie de forma clinică şi mediul de provenienţă al pacienţilor cu osteomielită hematogenă evidenţiază frecvenţa diferită a cazurilor cu osteomielită hematogenă acută şi osteomielită hematogenă cronică. În aceste grupe de

pacienţi este semnificativ mai mare frecvenţa cazurilor din mediul rural (73,5% respectiv 67,5%). Pentru pacienţii cu osteomielită subacută hematogenă nu se constată o diferenţa semnificativă între numărul cazurilor din mediul urban faţă de cele din mediul rural.

Distribuţia în funcţie de forma clinică a osteomielitei hematogene şi de localizarea la nivelul osului (epifizar, diafizar, metafizar)

Pentru evidenţierea asocierii dintre forma clinică şi localizarea osteomielitei hematogene s-a realizat tabelul de contingenţă al celor două aspecte studiate (tab. IV).

Rezultatele studiului sunt elocvente evidenţiindu-se în mod clar diferenţele între localizările osteomielitei hematogene în funcţie se forma clinică a acesteia. În cazul osteomielitei acute hematogene localizarea la nivelul epifizei este foarte rar întâlnită (6,02% din cazuri), epifizar cel mai des se întâlneşte osteomielita subacută hematogenă (80,37% din cazuri). Osteomielita cronică şi cea acută se întâlnesc cel mai frecvent la nivelul diafizei sau metafizei (80,5% respectiv 93,98%).

Distribuţia în funcţie de sexul pacienţilor cu osteomielită hematogene şi de localizarea la nivelul osului (epifizar, diafizar, metafizar)

Studiul comparativ al localizării în funcţie de sexul pacienţilor cu osteomielită hematogenă a fost posibil prin realizare tabelului de contingenţă.

Analiza distribuţiei cazurilor în funcţie de localizare şi sexul pacienţilor evidenţiază prezenţa localizării pe diafiză sau metafază mai frecventă la sexul feminin 70,09% în timp ce la pacienţii de sex masculin această localizare este regăsită la 53,7% din cazuri.

Studiul formelor clinice raportate la localizarea osteomielitei hematogene la nivelul scheletului impune ca referinţă numărul total de cazuri pentru fiecare din părţile afectate ale scheletului. În acest fel s-a putut aprecia obiectiv prezenţa formelor clinice la nivelul fiecărui tip de os. Concluzia studiului constă în faptul că la nivelul oaselor lungi se regăsesc cel mai frecvent formele subacute (41,1%) în timp ce la nivelul oaselor scurte se regăsesc formele cronice (48,28%).

Rezultatele analizei indică ponderea mare a cazurilor cu osteomielită acută hematogenă localizată la nivelul humerusului (62,5%) sau cubitus/radius (60%). La nivelul tibiei cel mai frecvent a fost întâlnită forma de osteomielită hematogenă subacută (45%) în timp ce la nivelul femurului forma acută a avut o pondere asemănătoare de 44,93%.

Osteomielita cronică cel mai frecvent apare la nivelul tibiei (28,75%), la femur regăsindu-de într-o proporţie de 15,9%. Nu au fost înregistrate cazuri cu osteomielită cronică la nivelul cubitus/radius, iar pentru humerus prevalenţa a fost foarte mică de 6,25%.

Un studiu similar celui efectuat pentru oasele lungi ale scheletului a fost realizat şi la nivelul oaselor scurte, rezultatele demonstrând aşa cum era de aşteptat că şi la această grupă apar diferenţe semnificative între repartiţia cazurilor în funcţie de forma clinică şi localizare.

Referindu-ne la oasele scurte ale scheletului am constatat că cel mai frecvent osteomielita subacută hematogenă este localizată la nivelul calcaneului, toate cazurile localizate la acest nivel au fost de formă subacută (100%). Trebuie avut în vedere însă numărul mic de cazuri cu această localizare (1 caz).

Osteomielita subacută hematogenă este regăsită de asemenea frecvent la nivelul pelvisului şi al coloanei vertebrale (53,8% respectiv 54,3%).

În osteomielita cronică radiologia a evidenţiat în 54,55% din cazuri abces Brodie, în 16,8% din cazuri osteoperiostită şi în 14,29% din cazuri osteoliză metafizară. Pen-

tru această formă clinică cu o frecvenţă mai scăzută se regăsesc abcesul subperiostic (9,09%) şi sechestrul osos (2,41%). În osteomielita acută hematogenă rezultatele examenului radiologic a evidenţiat o frecvenţă mare a cazurilor cu osteoliză metafizară (59,04%) şi abces subperiostic (24,1%). Osteomielita subacută hematogenă prezintă radiologic în 68,2% din cazuri abces Brodie.

DISCUŢII ŞI CONCLUZII

Osteomielită hematogenă până în prezent rămâne una dintre cele mai grave şi frecvente infecţii chirurgicale la copil şi adult cu menţinerea la un nivel ridicat al afecţiunii, cea ce este confirmat şi în studiul nostru. Erorile de diagnostic şi tratament cel mai frecvent se produc în etapa iniţială din cauza necunoaşterii afecţiunii. Pentru un diagnostic precoce a OH, reducerea la minimum a riscului erorilor de diagnostic şi tratament în scopul îmbunătăţirii rezultatelor tratamentului copiilor cu osteomielită hematogenă este necesară elaborarea unui algoritm de diagnostic şi tratament adecvat chiar de la debutul acestei afecţiuni grave.

Procesul osteomielitic cel mai frecvent afectează femurul, cu prevalenţă segmentul superior. Pe grupe de vârstă mai afectată este vârsta 8 – 12 ani, şi cu predilecţie sexul masculin.

În studiul prezentat la etapa iniţială s-au analizat traumatismul suportat, leziunile stafilococice cutanate (focare primitive) netratate, precum şi debutul afecţiunii. Semnele locale şi generale ale OH au fost destul de caracteristice pentru OH în toate cazurile analizate: debutul brusc cu dureri locale metafizare continui progresante pe fundalul stării generale alterate şi simptomele unei infecţii grave (febră cu caracter intermitent, leucocitoză cu devierea formulei leucocitare spre stânga şi accelerarea VSH). Aceste semne s-au înregistrat la toţi copiii cu OHA din studiul nostru. Remarcăm că tabloul clinic al OHA are o specificare clasică şi permite diagnosticarea afecţiunii la debut. Analiza datelor paraclinice (imagistice) a permis confirmarea diagnosticului de OH.

Diagnosticul OH s-a realizat prin cumularea datelor anamnestice, clinice, imagistice. Pentru realizarea scopului propus printre metodele utilizate unele sau folosit ca scrining (spre exemplu: analiza datelor de anamneză, acuzelor prezentate de bolnav, manifestărilor clinice, palparea, percuţia pe os, probelor biologice - leucocitoză cu devierea formulei leucocitare spre stânga, accelerarea VSH).

Studiul efectuat a evidenţiat că toţi pacienţii au fost supuşi unui tratament complex intensiv, care a constat în tratament: medical, chirurgical de urgenţă, ortopedic. Studiul a contribuit la optimizarea diagnosticului şi tratamentului în baza utilizării în practică a algoritmilor corespunzători cu selectarea metodelor optime a tacticii curative.

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THE STUDY OF AMINOPHENAZONE RADICAL CATION AND ITS INTERACTION WITH SOME ANTIOXIDANTS

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THE STUDY OF AMINOPHENAZONE RADICAL CATION AND ITS INTERACTION WITH SOME ANTIOXIDANTS (Abstract): The purpose of this study was to identify both the possibilities of *in vitro* generation of 1–phenyl–2,3–dimethyl–4–dimethylamino–5–pyrazolone (aminophenazone) radical cation (abbreviated $APH^{\bullet+}$) and the mechanisms of interaction with different antioxidants. **Material and method**: The chromophore radical cation $APH^{\bullet+}$ was obtained by oxidizing APH with ammonium persulfate. The stages of the oxidation reaction and the production of the intermediate compounds were studied by spectrophotometrical and potentiometric titration. As standard antioxidant there was used a catechin solution in distilled water with concentrations ranging between 50–250 μg/mL, in weak alkaline environment. **Results**: The oxidation of APH with ammonium persulfate developed in four stages, involving the production of some intermediate compounds with different colorations. A mechanism for this reaction has been proposed. The radical cation forms to an APH – ammonium persulfate molar ratio of 2 : 1. In the presence of an antioxidant (catechin) the radical cation $APH^{\bullet+}$ forms to a molar ratio APH–ammonium persulfate of 1 : 1, in weak alkaline environment. It has an absorbtion maxima at λ = 540 nm. The molar extinction coefficient of $APH^{\bullet+}$ at λ = 540 nm has been calculated. Its absorbance was proportional with catechin concentration in the range 50–250 μg/mL (r^2 = 0.9988). **Conclusion**: The APH–ammonium persulfate system can be used for spectrophotometric determination of total polyphenols in different systems. **Key words**: AMINOPHENAZONE, RADICAL CATION, ANTIOXIDANT, CATECHIN

The methods used for measurement of *in vitro* antioxidant activity are based on direct quantification of antioxidants in different systems or on measuring the ability of an antioxidant to scavenge free radicals generated through controlled chemical oxidation of some easily oxidable compounds.

As stable chromophore radicals there are widely used 2,2'–azinobis–(3–ethylbenzo– thiazoline–6–sulfonic acid) (ABTS) and 2,2'–diphenyl–1–picrylhydrazyl (DPPH).

The spectrophotometric methods using these radicals measure the reduction of the radicals by an antioxidant, calculated as percentage inhibition of absorbance.

Trolox, a synthetic derivative of vitamin E, is the most often used antioxidant; the antioxidant activity is expressed as TEAC (Trolox Equivalent Antioxidant Capacity), term that reflects the ability of the antioxidant to donate protons or electrons to scavenge the $ABTS^{\bullet+}$ radical, relative to the reactivity of Trolox as a standard (1–5).

These methods were used to determine antioxidant activity of foods, cosmetic products, biological liquids (6–9).

The aim of our research was to generate a new chromophore radical and to study the parameters that influence its stability in order to use oxidation intermediates as de-

TABLE I
Maximum absorbtion of the main oxidation products of APH with $(NH_4)S_2O_8$

Product	Reaction time (minutes)	Colour	$ë_{max}$ (nm)
P_1	1	Blue	600
P_2	3	Violet	580
P_3	15	Red	540
P_4	30	Yellow	355

tection system for spectrophotometric assay of total phenolic content.

MATERIAL AND METHOD

The reagents used in the study of oxidation reaction of aminophenazone were: 1–phenyl–2,3–dimethyl–4–dimethylamino–5–pyrazolone (aminophena– zone – APH), ammonium persulfate – $(NH_4)_2S_2O_8$, catechin (trans–3,3',4',5,7–pentahydroxy flavan) with analytical purity and $NaHCO_3$ solution 5%.

The stoichiometry of APH with $(NH_4)_2S_2O_8$ oxidation reaction was carried out by spectrophotometric and potentiometric titration. The spectrophotometric titration was performed with a Hewlett Packard 8453 series UV–VIS spectrophotometer and the potentiometric titration was performed with an electronic pH–meter Seibold Wien, using the platinum electrode as the indicator electrode and the calomel electrode as the reference electrode.

RESULTS

The oxidation of APH with $(NH_4)S_2O_8$ developed in several stages, involving the production of intermediate compounds with different colorations: blue, violet, red, yellow.

The absorbtion spectrum showed the values of wavelengths where the absorbance of the intermediate products was highest (tab. I).

It has been determined the molar combination ratio APH : $(NH_4)S_2O_8$ through spectrophotometric titration for three of the main products (tab. II).

Through potentiometric titration of 0.1 M APH with 0.1 M $(NH_4)S_2O_8$ there has been established that the molar ratio APH : $(NH_4)S_2O_8$ in red compound is 2 : 1.

There has been studied APH interaction with some antioxidants, in order to obtain a detection system for the antioxidant activity. It has been found that polyphenols (catechins) react with APH in the presence of $(NH_4)S_2O_8$ [APH : $(NH_4)S_2O_8$ molar ratio of 1 : 1] in weak alkaline medium (pH=8–9), forming a red–orange compound, with maximum absorbance at λ = 540 nm.

A linear increase of the absorbance with increasing amounts of catechin was observed in the range 50–250 μg catechin/mL, respectively 0.216–1.080 μmols; correlation coefficient R^2=0.9988 (fig. 1).

Standard error of regression line (SE) = 0,009343; detection limit (DL) = 0,1884 μg/mL and limit of quantification (LOQ) = 0.6281 μg/mL

DISCUSSIONS

Previous researches have established that the intermediate product with red colour (P_3) is a radical cation. Its radicalic structure was determined by isolating the compound under the form of spin aduct through its precipitation with silicotungstic acid and analyzed through electron spin resonance (ESR).

From the experimental data we can conclude that the oxidation reaction of APH develops in several successive stages (fig. 2). In these reactions APH appeared as Ant. $N(CH_3)_2$.

In the first step APH is oxidized with

TABLE II
Molar ratio APH : $(NH_4)S_2O_8$

Volume of 0.01 M APH (mL)	ë (nm)	Volume of 0.01 M $(NH_4)S_2O_8$ at equivalence point (mL)	Molar ratio APH:$(NH_4)S_2O_8$
5.00	600 (P_1)	5.00	1:1
5.00	540 (P_3)	2.50	2:1
5.00	355 (P_4)	2.50	2:1

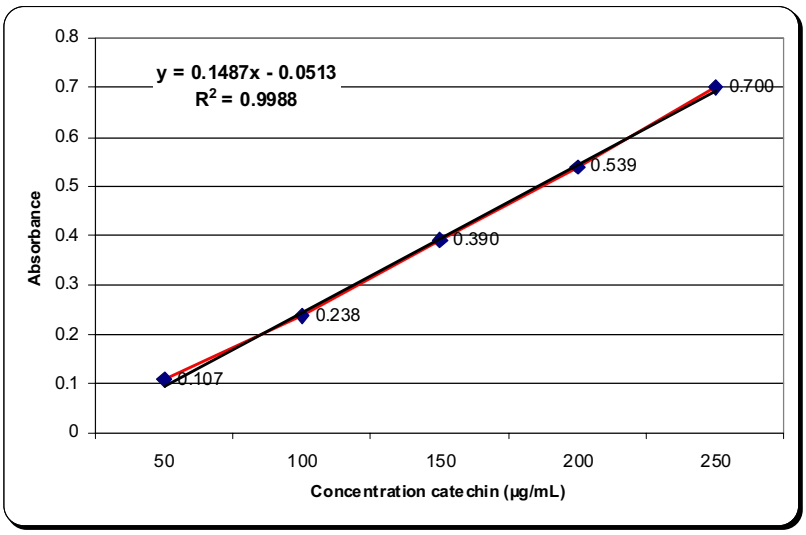


Fig. 1. The calibration curve

$$Ant.N(CH_3)_2 \overset{-2e}{\rightleftharpoons} Ant.\overset{+}{N}(CH_3)_2$$
$$P_1$$

$$Ant.\overset{+}{N}(CH_3)_2 + Ant.N(CH_3)_2 \rightleftharpoons 2Ant.\overset{\bullet+}{N}(CH_3)_2$$
$$P_3$$

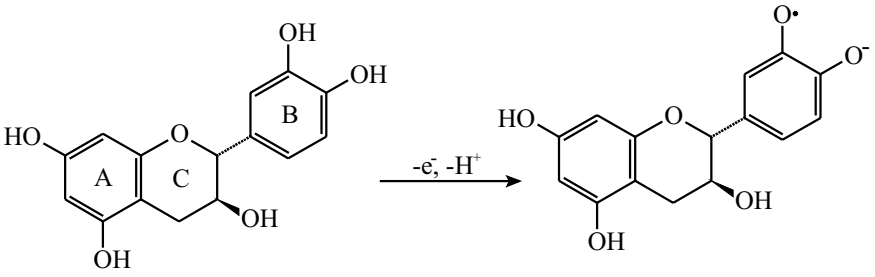
Fig. 2. Mechanism of oxidation of APH

the two electron change (P_1 – blue compound).

In the second stage, another molecule of APH reduced P_1 compound, forming a radical cation $APH^{\bullet+}$ (P_3 – red compound).

In the third stage, probably, a dimerization of cationic radical (P_4 – yellow compound, stable) took place.

(+)–Catechin is involved in the second stage of the reaction, reducing APH^+ cation formed within the first stage to the red colored radical cation $APH^{\bullet+}$. (+)–Catechin is oxidized to semiquinone anion radical with donation of one electron and one



(+)-catechin semiquinone

Fig. 3. Mechanism of oxidation of catechin

proton from B ring (10,11) (fig. 3).

The reaction between catechin and APH is sensible ; the molar extinction coefficient $\varepsilon = 7.32 \cdot 10^2$ $mol^{-1} \cdot L \cdot cm^{-1}$.

The colored compound is very stable. The proposed method is applicable for quantification of total polyphenols, expressed in catechin, from different environments.

CONCLUSIONS

In this study we suggest a method for generating $APH^{\bullet +}$ radical cation, with the purpose of using it for the quantitative determination of several antioxidants. The reaction that takes place between (+)–catechin and APH in the presence of $(NH_4)_2S_2O_8$ in alkaline media generates a red colored radical cation. The absorbance of the solution is proportional to the concentration of catechin.

The optimal conditions for applying this reaction to spectro–photometrically quantification of total polyphenolic content in different products have been established.

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CARIA SEVERĂ TIMPURIE CONSIDERAŢII EPIDEMIOLOGICE ÎN ZONA IAŞI

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EARLY CHILDHOOD CARIES. EPIDEMIOLOGICAL CONSIDERATIONS (Abstract): **Aim**: To asses the current demographic tendencies of the carioactivity, in Iaşi area, especially ECC and S-ECC. **Material and methods**: Study group included 500 subjects (276 girls and 224 boys), 3-6 years of age, 70% urban and 30% rural. The oro-dental evaluation was achieved through clinical investigation, based on criteria and markers recommended by WHO, the estimation of the indices of the caries prevalence: caod, cao, SIC, ICDAS, pshychological assesment and sociological investigation using interviews and questionnaires. Recorded data were used for statistical analysis in SPSS 17 trial, in visual FOX-PRO9. **Results**: The hole assessment showed a low caries risk (83.3%), compared to the values of the medium caries risk (9.2%), and of the high caries risk (7.5%). In term of clinic variability, the normal carial pattern is frequent, followed by atypical carial patterns, ECC and S-ECC, with differences between genders (boys – 1.91, girls – 1.99). In relation to the socio-econimic level of the family, the cao index varies: cao-1 - high socio-economic level, cao-1.46 - medium socio-economic level, cao-2.87 - low socio-economic level. **Conclusions**: The values of the epidemiologic indices, showed a variability of the carioactivity of the temporary dentition, with growth of the atipical carial patterns, ECC and SECC. The above mentioned aspects require to reconsider the view points on temporary dentition and the promotion of an educational and prevention programme.
Key words: CHILD, DENTAL CARIES, TEMPORARY DENTITION, ECC, SECC, EDUCATION, PREVENTION

Caria dinţilor temporari reprezintă o problema majoră de sănătate oro-dentară cu care se confruntă copilul preşcolar.

În acest cadru de referinţă, caria timpurie (ECC), dar mai ales caria severă timpurie (SECC), au o semnificaţie aparte, prefigurându-se ca o problemă de sănătate publică pe plan mondial, cu particularizări pe plan zonal, ale prevalenţei şi variabilităţii clinice, mai ales în ţările în curs de dezvoltare (1).

În 1994, European Centres for Diseases Control and Prevention, delimitează prin termenul de carie timpurie, ''orice formă de carie la copii, ce reflectă un proces etiologic, multifactorial implicat în boală'' (2).

Caracterul, relativ restrâns, al informaţiilor existente în aceasta direcţie se motivează, după cum menţionează Horowitz în 1998, prin accesibilitatea de examinare limitată şi dificultatea de evaluare clinică stomatologică al copilului din comunităţile preşcolare şi îndeosebi din comunităţile ante-preşcolare (3,4,5,6,7).

Majoritatea studiilor de referinţa sunt focalizate preponderent pe etapa de vârsta 5 ani, pe grupuri populaţionale relativ mici şi pe tiparele dentare maxilare anterioare

(4,6,7). Marthaler et al. (1996), Peterson şi Brathall (1996), Tinanoff et al. (2002) semnalează o creştere polarizată a experienţei cariale la nivelul populaţiilor preşcolare în ţările vest europene (prevalenţa globală fiind sub 5%) (7).

Poulsen în 2002, în contextualitatea ţărilor nordice, afirmă că 5-10% dintre copiii sub vârsta de 3 ani, au carii dentare şi o mare proporţie a acestor leziuni, chiar şi cele necavitare, reprezintă un risc înalt de progresie carială cavitară la vârsta şcolară.

În SUA, Horowitz semnalează încă din 1998 o prevalenţă a ECC de 3-6% (8,9,10).

În România, primele studii referitoare la ECC sunt efectuate de Zarnea şi Luca în 1987 (4). Ca relevanţă zonală reţin în mod deosebit atenţia studiile efectuate după 1990 : Cocarla et al. (Cluj-1993), Dorobăţ et al. (Iaşi, 1994), Luca et al. (Bucureşti, 2001), Dănilă et al. (Iaşi, 2004-2007).

Obiectivul acestui studiu urmăreşte evaluarea în dinamică la nivel zonal Iaşi a tendinţelor demografice (epidemiologice) actuale a carioactivităţii dentaţiei temporare, în mod deosebit a ECC şi a SECC, precum şi interrelaţia cu modelul familial şi comunitar.

Scopul studiului este de a socializa şi modela comportamental copilul preşcolar privind sănătatea oro-dentară, bazat pe un concept social şi un model medical de management stomatologic pediatric.

MATERIAL ŞI METODĂ

În argumentarea scopului propus am efectuat un studiu complex, „cross-sectional", pe un lot de 500 de subiecţi (276 fete şi 224 băieţi), cu limita de vârstă între 3 şi 6 ani, cu un procentaj de 70% din mediul urban şi 30% din mediul rural, cu apartenenţă familială şi comunitară variate.

În strategia de studiu s-a urmărit standardizarea metodologiei de lucru, prefigurarea şi derularea studiului pe parte integrativă a componentei zonale promovată de Disciplina de Dentistică Preventivă în cadrul Programului Naţional actual de Prevenire a afecţiunilor oro-dentare la copil, identificarea necesarului de resurse şi infrastructură, evaluarea interdisciplinară a subiecţilor, organizarea la nivelul comunităţilor preşcolare monitorizate a cursului interactiv ''teaching the teachers'', aplicarea flexibilă a programului educaţional preventiv şi interceptiv, atât la nivel comunitar cât şi la nivelul bazei clinice.

Evaluarea oro-dentară s-a realizat prin investigarea clinică bazată pe criterii şi markeri, recomandaţi de O.M.S., estimarea indicilor de prevalenţă carială : caod, caos, SIC, ICDAS, evaluare psihologică şi anchetă sociologică - prin interviu şi chestionare cu întrebări deschise şi închise.

În prelucrarea datelor statistice s-a utilizat programul SPSS-17 în Visual FOX PRO 9, teste statistice : Anova, Chi-patrat, Fischer, Curba ROC şi regresia linear multiplă R1 şi R2.

REZULTATE ŞI DISCUŢII

Pentru a cuantifica interrelaţia între riscul carios şi variabilele de clasificare am calculat coeficientul de contingenţă. Global, am remarcat preponderenta unui risc carios mic (83,3%), comparativ cu valorile scăzute ale riscului carios mediu (9,2%) şi ale riscului carios mare (7,5%), cu o reprezentare graduală în raport cu etapa de vârstă (fig. 1). În ceea ce priveşte riscul carios, comparativ pe sexe, am putut re-

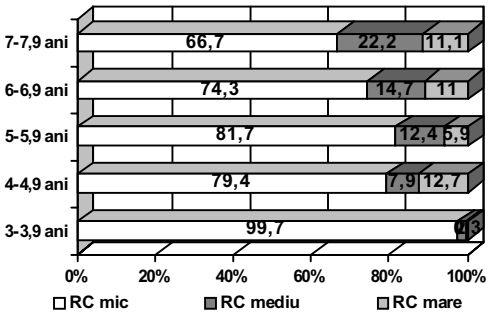

Fig. 1. Riscul carios pe grupe de vârstă

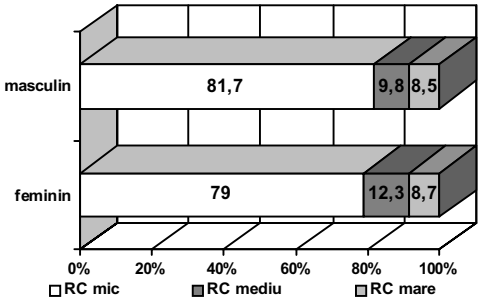

Fig. 2. Valorile riscului carios comparativ pe sexe

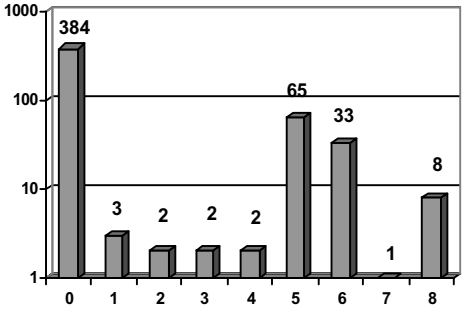

Fig. 3. Evoluţia indicelui cao în raport cu statutul socio-economic

TABELUL I

Valorile indicelui cao şi a indicelui sic pe grupe de vârstă

	C+A+O	Nr. cazuri	Valoare	Indice SIC
Indicele CAO global	979	500	**1.958**	**0.250**
Valorile pe intervale de vârstă				
între 3 – 3,9 ani	37	43	**0.860**	**0.100**
între 4 – 4,9 ani	271	126	**2.151**	**0.280**
între 5 – 5,9 ani	354	186	**1.903**	**0.240**

marca, existenţa unui risc carios mic cu un nivel mai ridicat la băieţi, 81,7%, în comparaţie cu fetele, 79%, un risc carios mediu cu un nivel mai ridicat la fete, 12,3%, în comparaţie cu băieţii, 9,8%, şi un risc carios mare cu un nivel mai ridicat la fete, 8,7%, în comparaţie cu băieţii, 8,5% (fig. 2.)

În mediul rural predomină un risc carios mic, iar în mediul urban riscul carios este moderat.

În raport cu dinamica etapelor de vârstă, am remarcat o evoluţie vizibil ascendentă a carioactivităţii dentaţiei temporare, aspect ce se prefigurează ca potenţial predictor carial pentru dentaţia permanentă.

Pentru grupa de vârsta 3-4 ani am găsit un indice cao de 0,86, iar la grupa de vârstă 4-5 ani un indice cao de 2,15 comparativ cu indicele cao de 1,34 semnalat de Dănila et al. în perioada 2004-2007, la nivel zonal Iaşi pentru etapele de vârstă 2,5-5 ani, şi un indice mai mic decât indicele cao surprins în 2001 de Luca et al. la nivel zonal Bucureşti, respectiv 3,98.

Sub aspectul variabilităţii clinice, se prefigurează alături de tiparele cariale convenţionale tot mai des tiparele atipice, cu predominanţa carie timpurie şi carie severă timpurie, cu uşoare diferenţieri nesemnificative statistic între cele două sexe (băieţi - 1,91, fete - 1,99).

În raport cu nivelul socio-economic al familiei aparţinătoare indicele cao se nuanţează astfel : cao 1 - în contextul nivelului socio-economic ridicat, cao 1,46 - nivel socio-economic mediu, cao 2,87 - nivel socio-economic scăzut (fig. 3).

Valorile indicelui sic pe etape de vârstă atesta trendul epidemiologic actual al carioactivităţii dentaţiei temporare la nivel

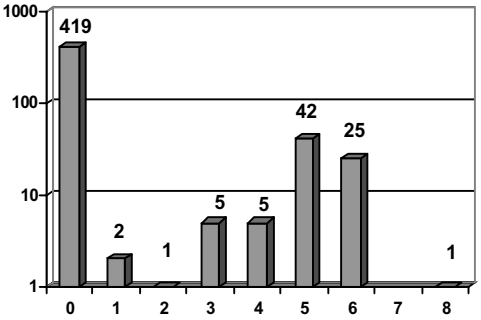

Fig. 4. Valoarea indicelui ICDAS pentru molarul doi temporar 65

zonal Iaşi.

Cu o semnificaţie deosebită, atât retrospectiv cât mai ales ca predictibilitate dento-dentară, cât şi dento-alveolară, ne apar valorile indicelui ICDAS (International Caries Dental Assesment) pentru molarii doi temporari, cu variabile diferenţieri, atât între cele doua maxilare, cât şi între cele patru cadrane (fig. 4).

CONCLUZII

Trendul epidemiologic actual al carioactivităţii dentaţiei temporare şi îndeosebi al ECC constatat de noi, în zona Iaşi, ilustrează „omniprezenţa factorului de risc major" (11) asupra stării de sănătate orodentară şi psiho-somatică, implicit asupra calităţii vieţii copilului.

Valorile indicilor epidemiologici relevă o variabilitate a carioactivităţii dentaţiei temporare, cu emblemarea ascendentă a ECC şi SECC.

Departajările valorice ale indicelui ICDAS sunt edificatoare în ceea ce priveşte dinamica epidemiologică şi clinică a ECC şi SECC.

Aspectele menţionate impun: reconsiderarea opticii privind dentaţia temporară, promovarea unui program educaţional şi de prevenţie stomatologică pediatrică primară, realist, efectiv şi flexibil, coparticipativ, accesibil tuturor copiilor la nivel comunitar.

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PREVENŢIA AFECŢIUNILOR COLOANEI VERTEBRALE LA MEDICII DENTIŞTI

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PREVENTION OF SPINAL DISORDERS IN DENTISTRY (Abstract): Professional activity in dentistry account for excessive request of the spine and peripheral joints. **Material and method**: We performed an observational prospective study on ten physicians (six women, four men) aiming to evaluate the time used in vicious positions during a working day and to establish a specific kinetic program directed to relaxation and rebalancing lumbo-abdomino-pelvic muscles. The working time in a typical day either using orthostatic or sitting position was assessed according to a standard protocol. **Results**: Left dorsal scoliosis with rotation lasting over 97 minutes of daily working time, sinistro-concave dorsal scoliosis with a typical duration of 42.5 minutes, dextro-concave kyphoscoliosis with a mean time of 37 minutes and dorsal scoliosis with right rotation lasting 31 minutes were reported. Moreover, both left and right rotations were demonstrated during the working time accounting for about 22.5 minutes and 29 minutes respectively, while a lesser time deal with the side of right-left uncombined movement. **Conclusion**: Almost three quarters of working time (71%) takes place in vicious positions, suggesting that, unfortunately, the ergonomic position is seldom maintained in current dentistry practice. A complex rehabilitation program is therefore mandatory in order to prevent muscle imbalance. **Key words**: DENTIST POSTURE, BACK PAIN, ERGONOMIC POSTURE

SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME HYDRAZONES WITH XANTHINE STRUCTURE

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SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME HYDRAZONES WITH XANTHINE STRUCTURE (Abstract): **Aim:** To synthesize some hydrazones with xanthine structure and to evaluate their antioxidant activity. **Material and methods:** The synthesis started from theophylline sodium salt, prepared from theophylline and sodium methoxide, which was transformed into its ethyl ester and then in hydrazide. The hydrazone derivatives were obtained by the condensation of hydrazide with different aromatic aldehydes (benzaldehyde, 4-nitro/3-nitro/3-hydroxy/4-chloro/4-bromo-benzaldehyde). The antioxidant potential was evaluated *in vitro* by using the DPPH radical scavenging assay and was compared with theophylline activity used as parent compound. **Results:** Six hydrazones with xanthine structure were obtained. They were characterized in terms of their physical properties and their structures were confirmed by IR and ^{1}H-NMR spectroscopy. Some of these compounds have an antioxidant effect more pronounced than theophylline at higher concentrations. The most active compound was 3-nitro-benzylidene-hydrazino-acetyl-theophylline (compound g), with an inhibition rate of 65% ± 0.99 at 30 min and 79% ± 0.81 at 60 min. **Conclusions:** The antioxidant activity of synthesized hydrazones depends on the substituents used in structural modulation. The substitution of aromatic ring with nitro group in *meta* position had the most favorable influence. **Keywords:** THEOPHYLLINE, HYDRAZONE, ANTIOXIDANT ACTIVITY, DPPH.

Xanthine structure is known for its important biological effects and could be found in the structure of compounds with vasodilator, bronchodilator, anticancer, anti-inflammatory and hypoglycemic action (1). The most important compounds with xanthine structure are caffeine, theophylline and theobromine (2). Theophylline is used for the treatment of essential asthma, asthmatic dyspnea and bronchopulmonary disease. The xanthine moiety is present also in the structure of Linagliptin (Tradjenta®, Trajenta®), a DPP-4 inhibitor used for the treatment of diabetes mellitus type 2 (3).

On the other hand, there is increasing evidence supporting the involvement of oxidative stress in such diseases as atherosclerosis, diabetes, cancer, glaucoma, rheumatoid arthritis, psoriasis and Alzheimer disease (4). Oxidative stress is an imbalance between production of harmful reactive oxygen species such as free radi-

cals or peroxides named oxidants and the ability of the body to fight with their effects using different endogenous antioxidants (5).

The main objective of this study was the synthesis of some hydrazones with xanthine structure and the evaluation of their potential antioxidant activity.

MATERIAL AND METHODS

Reagents. Theophylline, sodium, ethyl chloroacetate, hydrazine hydrate 64%, aromatic aldehydes (benzaldehyde, 4-nitro/3-nitro/3-hydroxy/4-chloro/4-bromo-benzaldehyde), organic solvents (methanol, ethanol, dimethyl formamide - *p.a.* quality) and standard reagents for antioxidant assay were purchased from Sigma Aldrich Company. All reagents and solvents were used without previous purification. For monitoring the reactions Thin Layer Chromatography (TLC) Silica gel 60 F_{254} plates produced by Merck Company were used. The Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra were obtained in dimethyl sulfoxide deuterated (DMSO-d_6) produced by Sigma Aldrich Company.

Chemistry. The synthesis of compounds started from theophylline sodium salt (b), which was obtained from theophylline (a) and sodium methoxide, prepared from sodium and dry methanol. In the next step, the sodium salt reacted with ethyl chloroacetate when theophylline-ethyl acetate (c) was formed. Then, the resulted ester was treated with an excess of hydrazine hydrate (64%) (6-8) to obtain hydrazide (d), which, by condensation with different aromatic aldehydes (benz-aldehyde, 4-nitro/3-nitro/3-hydroxy/ 4-chloro/ 4-bromo-benzaldehyde), formed the corresponding hydrazones (e-j) (fig. 1).

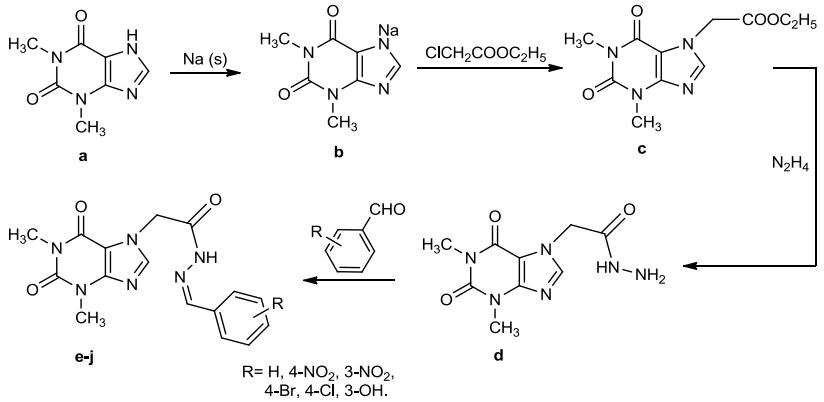

Fig. 1. The synthesis of hydrazone derivatives with xanthine structure.

General procedure for synthesis of hydrazones with xanthine structure

Theophylline-ethyl acetate (c). To a solution of sodium methoxide freshly prepared from sodium (0.1 mol) and dry methanol (200 mL), theophylline (0.1 mol) was added in small parts. The reaction was carried out at room temperature with stirring until all the solvent was evaporated. The resulted theophylline sodium salt (0.1 mol) was added to a mixture of ethanol: dimethyl formamide (DMFA) (260 mL: 100 mL). The mixture was heated under reflux and stirred for 1 h and after that

ethyl chloroacetate (0.1 mol, 10.71 mL) was added dropwise and left refluxing overnight. The ethyl ester was recrystallized from ethanol. The reaction was monitored by TLC using dichloromethane: methanol = 9.6: 0.4 as eluent system.

Theophylline-hydrazide (d). The ethyl ester of theophylline (0.035 mol) in ethanol (50 mL) was heated under reflux and stirred until solubilization and then hydrazine hydrate 64% (0.11 mol, 5.52 mL) was added dropwise. The extra amount of ethanol (150 mL) was added and the mixture was heated under reflux for 6 h. The reaction was monitored by TLC using ethyl acetate: acetone =7.5:3 as eluent system. The product was separated by filtration and washed two times with diethyl ether.

Theophylline-hydrazones (e-j). A mixture of acetyl-theophylline-hydrazine (0.006 mol) and ethanol (200 mL) was refluxed for 1 h. Some drops of glacial acetic acid and the aromatic aldehyde (benzaldehyde, 4-nitro/ 3-nitro/ 3-hydroxy/ 4-chloro/ 4-bromo-benzaldehyde) (0.009 mol) were added and the mixture was refluxed for 4-24 h with continuous stirring. The reaction was monitored by TLC using ethyl acetate: acetone =7.5:3 as eluent system. The resulted hydrazones were purified by filtration and washed two times with diethyl ether and n-pentane.

Spectral characterization. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet iS50 FT-IR Spectrometer, at a resolution of 4 cm^{-1} after 16 scans in the 4000-600 cm^{-1} range. The spectra were interpreted using Omnic Spectra Software. ^{1}H-NMR spectra were recorded using a NMR Bruker 400 MHz spectrometer (Germany).

Antioxidant assay

DPPH radical scavenging activity. In order to evaluate the antiradical capacity against DPPH radical, the hydrazone derivatives (e-j) were dissolved in DMSO to obtain a stock solution with a concentration of 25 mg/mL. From the stock solution different volumes (50 µL, 100 µL, 150 µL, 200 µL, 250 µL, 300 µL) were taken and diluted with DMSO in order to obtain 400 µL. The sample solution (400 µL) was added to 2.5 mL solution of DPPH in methanol 0.1 mM and the mixture was left in the dark. After 30 min and 60 min, respectively, the absorbance was measured at 517 nm against a blank (methanol) using a UV-VIS spectrophotometer (9, 10). The DPPH radical scavenging activity was expressed as percentage according to the following equation: % Inhibition = $(A_0 - A_1)/A_0 \times 100$, where

A_0= absorbance of the control (solution of DPPH in methanol);

A_1= absorbance of the sample at 30 min and 60 min respectively.

Statistical analysis. All experiments were performed in triplicate and the data were analyzed using the analysis of variance and were expressed as arithmetic mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemistry

Theophylline-ethyl acetate (c) is colorless or white needle crystals, with melting point (m.p.) = 137-140 ºC, soluble in ethanol on heating.

The hydrazide of theophylline (d) is a white powder, very electrostatic, with m.p.=272-275 ºC, soluble on heating in DMSO and a mixture of acetone and few drops of water.

The hydrazones with xanthine structure (e-j) are fluffy solid compounds, with small density and cotton structure. They have electrostatic properties. The compounds are white or yellow in color. They are soluble in a mixture of acetone and few drops of

water, DMSO and DMFA on heating, partially soluble in methanol on heating, slightly soluble in chloroform, insoluble in absolute ethanol (tab. I).

TABLE I

Physical characteristics of hydrazones with xanthine structure (e-j)

Comp.	R	Formula	Molecular weight	Appearance (solid)	M.p. (°C)	Yield (%)
e	H	$C_{16}H_{16}N_6O_3$	340.34	white	270-272	74 %
f	4-NO_2	$C_{16}H_{15}N_7O_5$	385.34	yellow	305-308	87 %
g	3-NO_2	$C_{16}H_{15}N_7O_5$	385.34	white	295-296	77 %
h	4-Cl	$C_{16}H_{15}ClN_6O_3$	374.79	white	294-296	95 %
i	4-Br	$C_{16}H_{15}BrN_6O_3$	419.24	white	301-303	95 %
j	3-OH	$C_{16}H_{16}N_6O_4$	356.34	yellowish	295-296	94 %

TABLE II

Spectral characteristics for hydrazones with xanthine structure

Comp.	R	FT-IR characteristic band (cm^{-1})	^{1}H-NMR (400 MHz, DMSO-d6, δ ppm)
e	H	3078 (-NH-), 2966 (CH_{AR}), 1671 (-CO-NH-), 1608 (-CH=N)	11.77 (s, 1H, CO-NH-), 8.06 (d, 2H, CH=N), 7.69-7.74 (m, 2H, Ar-H), 7.43-7.46 (m, 3H, Ar-H), 5.55 (s, 2H, CH_2-CO), 3.46 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); tautomer form (20%): 11.77 (s, 1H, CO-NH), 8.22 (s, 1H, CH=N), 8.07 (s, 1H, CH=N), 7.69-7.74 (m, 2H, Ar-H), 7.43-7.46 (m, 3H, Ar-H), 5.12 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3)
f	4-NO_2	3068 (-NH-), 2948 (CH_{AR}), 1653 (-CO-NH-), 1597 (-CH=N), 1551 (-C-N)	12.07 (s, 1H, CO-NH), 8.29 (d, 2H, Ar-H), 8.16 (s, 1H, CH=N,), 8.06 (s, 1H, CH=N), 7.99 (dd, 2H, Ar-H), 5.59 (s, 2H, CH_2-CO), 3.46 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); tautomer form (23%): 12.07 (s, 1H, CO-NH), 8.33 (s, 1H, CH=N), 8.29 (d, 2H, Ar-H), 8.08 (s, 1H, CH=N), 7.99 (dd, 2H, Ar-H), 5.16 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3)
g	3-NO_2	3079 (-NH-), 2953 (CH_{AR}), 1653 (-CO-NH-), 1610 (-CH=N), 1551 (-C-N)	12.01 (s, 1H, CO-NH), 8.52 (s, 1H, CH=N), 8.25 (d, 1H, Ar-H), 8.18 (d, 2H Ar-H), 8.05 (s, 1H, CH=N), 7.74 (t, 1H, Ar-H), 5.59 (s, 2H, CH_2-CO), 3.45 (s 3H, N-CH_3), 3.18 (s, 3H, N-CH_3); tautomer form (25%): 12.01 (s, 1H, CO-NH), 8.52 (s, 1H, CH=N), 8.34 (s, 1H, Ar-H), 8.25 (d, 1H, Ar-H), 8.14 (d, 1H, Ar-H), 8.07 (s, 1H, CH=N), 7.74 (t, 1H, Ar-H), 5.15 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.18 (s, 3H, N-CH_3)
h	4-Cl	3109 (-NH-), 2974 (CH_{AR}), 1635 (-CO-NH-), 1594 (-CH=N), 826 (-C-Cl)	11.83 (s, 1H, CO-NH), 8.05 (s, 1H, CH=N), 8.04 (s, 1H, CH=N), 7.76 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H), 5.55 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); tautomer form (23%): 11.83 (s, 1H, CO-NH), 8.21 (s, 1H, CH=N), 8.07 (s, 1H, CH=N), 7.72 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H), 5.12 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3)
i	4-Br	3109 (-NH-), 2974 (CH_{AR}), 1635 (-CO-NH-), 1609 (-CH=N), 824 (-C-Br)	11.83 (s, 1H, CO-NH), 8.04 (d, 2H, CH=N), 7.67 (dd, 4H, Ar-H), 5.54 (s, 2H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); tautomer form (22%): 11.83 (s, 1H, CO-NH), 8.19 (s, 1H, CH=N), 8.07 (s, 1H, CH=N), 7.67 (dd, 4H, Ar-H), 5.12 (s, 1H, CH_2-CO), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3)
j	3-OH	3135 (-NH-), 3014 (-OH), 2987 (CH_{AR}), 1652 (-CO-NH-), 1588 (-CH=N)	11.69 (s, 1H, CO-NH), 9.62 (s, 1H, C-OH), 8.06 (s, 1H, CH=N), 7.96 (s, 1H, CH=N), 7.25 (t, 1H, Ar-H), 7.18 – 7.05 (m, 2H, Ar-H), 6.84 (d, 1H, Ar-H), 5.53 (s, 2H, CH_2-CO), 3.46 (s, 3H, N-CH_3), 3.20 (s, 3H, N-CH_3); tautomer form (24%): 11.69 (s, 1H, CO-NH), 9.62 (s, 1H, C-OH), 8.12 (s, 1H, CH=N), 8.06 (s, 1H, CH=N), 7.25 (t, 1H, Ar-H), 7.18 – 7.05 (m, 2H, Ar-H), 6.84 (d, 1H, Ar-H), 5.11 (s, 1H, CH_2-CO), 3.46 (s, 3H, N-CH_3), 3.20 (s, 3H, N-CH_3)

The structure of the final compounds was confirmed by FT-IR and ^{1}H-NMR spectroscopy (tab. II). Hydrazone derivatives (e-j) were obtained in two tautomer forms, the second form being in the ratio of 20-25%. The presence of the tautomer

forms was demonstrated using ^{1}H-NMR-temperature at 80 ºC.

Antioxidant assay

DPPH radical scavenging activity. The evaluation of antioxidant activity was performed by measurement of radical scavenging ability of the compounds against free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide anion radical, hydroxyl radical or 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical ($ABTS^{+\cdot}$) (11). The DPPH assay is the most used method to evaluate the antioxidant activity. This method evaluates the hydrogen atom donating activity using the spectrophotometric measurement of the radical concentration. DPPH is a purple-colored organic nitrogen radical, which changes its color to pale yellow upon a reduction reaction with hydrogen donating compounds (fig.2). The decrease in optical density expresses a measure of antioxidant activity (12) (tab. III, IV).

It can be observed that the most active compound is compound g, which has as substituent on aromatic ring the *nitro* group in meta position. For this compound the inhibition rate (%) ranged from 31% to 65% (at 30 min) and from 44% to 79% (at 60 min), depending on sample concentration (0.431 mg/mL, 0.862 mg/mL; 1.293 mg/mL; 1.724 mg/mL; 2.155 mg/mL; 2.586 mg/mL). The antioxidant activity of compound g was 2-3 times higher than that of theophylline, used as parent compound (fig. 3, 4). These results support the favorable influence of chemical modulation on radical scavenging ability. The other compounds have an inhibition percentage almost equal to that of theophylline at small concentrations (0.431 mg/mL, 0.862 mg/mL, 1.293 mg/mL), but at higher concentrations the antioxidant effect was more pronounced.

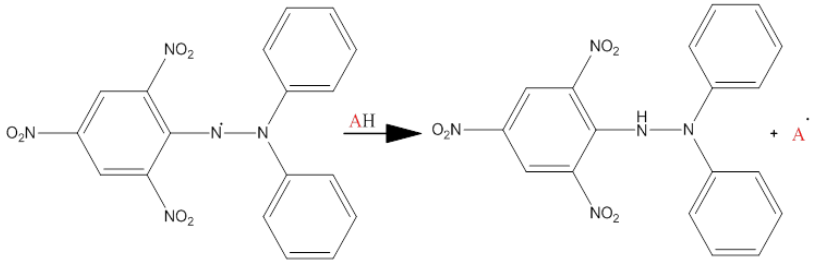

Fig. 2. The reduction reaction of the DPPH.

TABLE III

DPPH radical scavenging activity (inhibition rate - %) of theophylline and its hydrazone derivatives at 30 min

Comp.	R	Inhibition rate (%) at different concentrations					
		C_1	C_2	C_3	C_4	C_5	C_6
a	-	19.85±0.17	22.05±0.43	23.42±0.89	21.66±0.87	22.55±0.15	23.05±0.14
e	H	18.58±0.43	22.25±0.51	25.42±0.33	26.76±0.41	29.84±0.23	33.11±0.25
f	4-NO_2	19.21±0.22	21.17±0.76	23.76±0.81	25.42±0.65	26.57±0.37	27.03±0.35
g	3-NO_2	31.45±0.19	41.35±0.15	47.38±0.82	58.71±0.56	62.98±0.85	65.14±0.99
h	4-Cl	18.67±0.18	20.33±0.52	21.60±0.06	24.61±0.84	23.80±1.89	26.42±1.44
i	4-Br	18.06±0.44	19.88±0.52	21.15±0.19	22.18±0.46	23.07±0.20	24.02±0.07
j	3-OH	21.61±1.07	21.36±0.16	23.41±0.18	23.80±0.60	25.27±0.60	24.47±0.20

a: theophylline; C_1: 0.431 mg/mL; C_2: 0.862 mg/mL; C_3: 1.293 mg/mL; C_4: 1.724 mg/mL; C_5: 2.155 mg/mL;C_6: 2.586 mg/mL

TABLE IV

DPPH radical scavenging activity (inhibition rate - %) of theophylline and its hydrazone derivatives at 60 min

Comp.	R	Inhibition rate (%) at different concentrations					
		C_1	C_2	C_3	C_4	C_5	C_6
a	-	22.88±0.05	25.89±0.46	27.77±0.80	26.03±0.20	26.28±0.37	26.55±0.27
e	H	19.49±0.07	23.69±0.63	27.41±1.25	30.23±0.27	31.86±0.41	34.27±0.26
f	4-NO_2	20.23±0.34	22.87±0.85	26.52±0.4	27.02±0.91	30.76±0.54	31.32±0.67
g	3-NO_2	43.94±0.27	57.20±0.71	63.93±0.91	72.42±0.83	75.83±0.66	79.26±0.81
h	4-Cl	20.70±0.80	23.91±0.48	25.72±1.02	27.13±1.69	29.35±0.42	31.05±0.70
i	4-Br	19.96±0.34	22.92±1.73	25.01±0.22	26.27±1.44	28.44±0.96	27.66±0.38
j	3-OH	21.67±0.46	22.12±0.39	25.08±0.24	27.33±0.79	26.29±0.49	25.85±0.22

a: theophylline; C_1: 0.431 mg/mL; C_2: 0.862 mg/mL; C_3: 1.293 mg/mL;C_4: 1.724 mg/mL; C_5: 2.155 mg/mL; C_6: 2.586 mg/mL

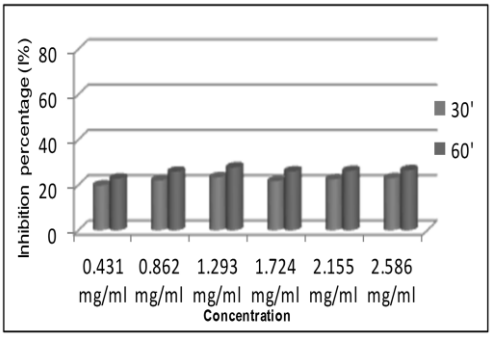

Fig. 3. The antiradical effect of theophylline at different concentrations

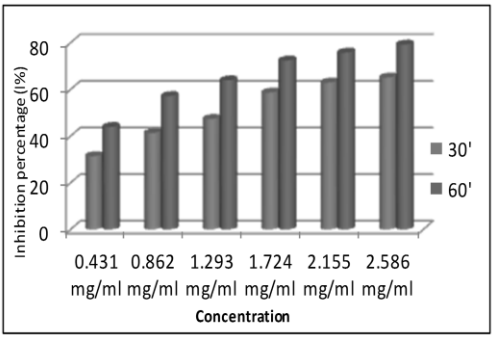

Fig. 4. The antiradical effect of compound g at different concentrations

CONCLUSIONS

In our studies, six hydrazones with xanthine structure have been synthesized starting from theophylline, a substance known for its beneficial effects, especially as bronchodilator agent. The resulted compounds were characterized in terms of their physical properties (chemical formula, molecular weight, yield, melting point, solubility in different organic solvents, etc.). Their structure was confirmed by FT-IR and ^{1}H-NMR spectroscopy. The antioxidant activity of the synthesized compounds was evaluated *in vitro* using the DPPH radical scavenging assay. Compound g (3-nitrobenzylidene-hydrazino-acetyltheophylline) was the most active compound, with the highest inhibition rate compared with theophylline.

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ISOLATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF THE CRUDE POLYSACCHARIDE FROM *PHYLLOPHORA PSEUDOCERANOIDES*

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ISOLATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF THE CRUDE POLYSACCHARIDE FROM *PHYLLOPHORA PSEUDOCERANOIDES* (Abstract): The red seaweed *Phyllophora pseudoceranoides* (Gmelin) New. et Tayl, commonly found in the Romanian Black Sea coastal waters, has not been studied regarding its chemical composition and biological activities. **Aims**: The aim of the present study was to isolate, characterize and assess the *in vitro* antioxidant activity of the crude polysaccharide from *P. pseudoceranoides*. **Materials and Methods**: The water soluble polysaccharide was characterized by Fourier transform infrared spectroscopy (FT-IR). Total phenolic content was determined by Folin-Ciocalteu method. Antioxidant activity was evaluated by ABTS radical cation scavenging and reducing power assays. Commercial-grade carrageenan was used as positive control. **Results and Discussion**: FT-IR analysis of the crude polysaccharide showed characteristic bands of carrageenan-type structure. A total phenolic content of 25.08 ± 1.00 mg GAE/g was determined in the crude polysaccharide. At 1.5 mg/mL, the polysaccharide exhibited important ABTS scavenging activity (49.59 ± 0.03%) and showed a good reducing power (0.4060 ± 0.002), when compared with the positive control (ABTS scavenging activity: 30.30 ± 0.03% and reducing power: 0.2494 ± 0.002, at a concentration of 1.5 mg/mL). **Conclusions**: The antioxidant activity of the sulfated polysaccharide suggests its possible use as an ingredient and antioxidant agent in the food and pharmaceutical industries. **Keywords**: *PHYLLOPHORA PSEUDOCERANOIDES*, POLYSACCHARIDES, ALGAE, ANTIOXIDANT ACTIVITY.

Over the past decades, marine macroalgae have attracted an emerging interest due mainly to their bioactive constituents with a broad spectrum of activities (1). Carrageenans are linear sulfated galactans extracted from red algae; these phycocolloids are classified according to the number and position of sulfated groups, the presence of

3,6-anhydro-D-galactose and the conformation of the pyranose ring, resulting six groups: iota (ι)-, kappa (κ)-, lambda (λ)-, mu (μ)-, nu (ν)- and theta (θ)- carrageenans, the first three carrageenans being of commercial interest (fig. 1). Carrageenans are used in the food industry as thickening and gelling agents, but also in the non-food industries such as the pharmaceutical, cosmetics, printing and textile industries. Furthermore, carrageenans might be used in the development of new therapeutic agents, as they are endowed with anticoagulant, antiviral, antioxidant, antitumor, immunomodulatory and cholesterol-lowering properties (2).

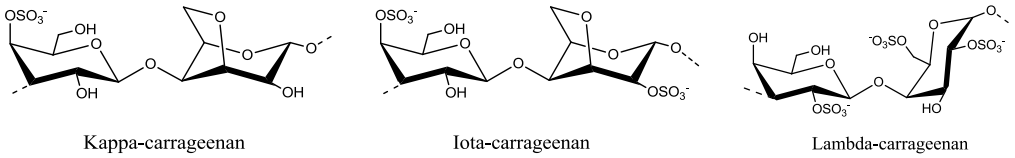

Fig. 1. Chemical structures of carrageenans

Research in the field of antioxidants from macroalgae has gained considerable attention, as these metabolites can delay or inhibit the effects of reactive oxygen species (ROS) in biological systems (3). It is known that increased ROS generation leads to DNA damage, lipid peroxidation and protein denaturation, playing a key role in the etiology and pathogenesis of a wide range of diseases and age-related disorders (4). Furthermore, as the extensive use of synthetic antioxidants (butylhydroxytoluene, butylhydroxyanisole, propyl gallate and tert-butylhydroquinone) has been associated with potential health risks (5), seaweed polysaccharides could offer a natural alternative to synthetic antioxidants by enhancing the safety and preserving the quality of foods (3).

Phyllophora pseudoceranoides (Phyllophoraceae) is a red seaweed, commonly found in the coastal areas of the Romanian Black Sea. The aim of this study was to isolate and characterize the crude polysaccharide from *P. pseudoceranoides*; in addition, the total phenolic content and *in vitro* antioxidant activity were investigated in order to assess its potential use in the field of food and pharmaceutical industries.

MATERIAL AND METHODS

Chemicals

Carrageenan (commercial grade, type I), gallic acid, potassium ferricyanide, iron (III) chloride and sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), disodium phosphate dodecahydrate and Folin-Ciocalteu's phenol reagent were supplied by Fluka (Steinheim, Germany). Monopotassium phosphate was purchased from Merck (Darmstadt, Germany). Trichloroacetic acid and potassium persulfate were purchased from Riedel-de Haën (Seelze, Germany). All other solvents and reagents were of analytical grade.

Algal material

Phyllophora pseudoceranoides was collected along the Black Sea coastal areas of Romania in June 2014 (location: Constanta - 44°11'44"N, 28°39'24"E). A voucher specimen was deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy

Grigore T. Popa - Iasi. Fresh seaweed material was washed thoroughly with distilled water to remove salt and sand; the air dried sample (40 ºC) was powdered and kept at -4 ºC until analysis.

Isolation of the crude polysaccharide

Algal material was first extracted with ethanol to remove pigments and polyphenols; then, the residue was dried and extracted with ultrapure water (1.5% w/v) for 2 h at 100 ºC. After filtration and concentration of the aqueous extract, the polysaccharide was isolated by precipitation with ethanol (1:3 v/v); the precipitate was washed with acetone and air dried. Then, the polysaccharide was re-dissolved in ultrapure water, followed by the same protocol of precipitation, washing and drying (6). The final precipitate was lyophilized, giving a white-brownish solid (21% of the seaweed dry weight) which was used for further analysis.

Chemical characterization

FT-IR spectroscopic analysis. FT-IR spectrum was recorded on ABB MB3000 FT-IR spectrometer (Québec, QC, Canada). FT-IR spectrum was acquired over a range of 4000–650 cm^{-1} with 16 scans at a spectrum resolution of 4 cm^{-1}. Spectrum processing was carried out using the Horizon MB^{TM} FTIR software.

Total phenolic content. Phenolic content of the crude polysaccharide and commercial-grade carrageenan were determined according to the Folin-Ciocalteu method (7). A standard curve was plotted using gallic acid and the results were expressed in mg gallic acid equivalents (GAE)/ g as mean ± SD standard deviation from triplicate determination.

In vitro antioxidant activity

ABTS radical cation scavenging assay. The assay was based on the method of Re et al. (8), with minor modifications.

Reducing power assay. The assay was carried out using the method of Sellimi et al. (9), with slight modifications. In both antioxidant assays, a series of polysaccharide concentrations (1-10 mg/mL) was prepared; commercial-grade carrageenan was used as positive control. Results were expressed as mean ± SD standard deviation from triplicate determination.

RESULTS AND DISCUSSION

Chemical characterization

FT-IR spectroscopic analysis. FT-IR spectroscopic analysis is a simple, fast, accurate and non-destructive technique that requires small amounts of sample; over the last decades, it has been extensively used to identify polysaccharide structures from various seaweeds, based on the analysis of their characteristic absorbtion bands (10).

Fig. 2 presents the FT-IR spectrum of the polysaccharide isolated from *P. pseudoceranoides.* The spectrum showed characteristic bands belonging to the stretching vibration of O-H (3741–3363 cm^{-1}) (I, II) and saturated hydrocarbon groups (2958 cm^{-1}, 2891 cm^{-1}) (III, IV). The polysaccharide fraction showed a strong absorption band at 1224 cm^{-1} (V) that could be assigned to the vibration of S=O group of the sulfate ester. Besides, the spectrum had a characteristic band at 1026.05 cm^{-1} (VII) which could be assigned to the glycosidic linkage (C-O and C-C streching vibrations of the pyranose ring). The spectrum presented characteristic bands at 1068 cm^{-1} (VI) and 929 cm^{-1} (VIII) that belong to the stretching vibration of C-O group of 3,6-anhydro-D-galactose. The two strong absorption bands at 844.76 cm^{-1} (IX) and

802.33 cm^{-1} (X) could be attributed to C-O-SO_3 bond of galactose-4-sulfate and to C-O-SO_3 bond of 3,6-anhydro-D-galactose-2-sulfate, respectively (10-12).

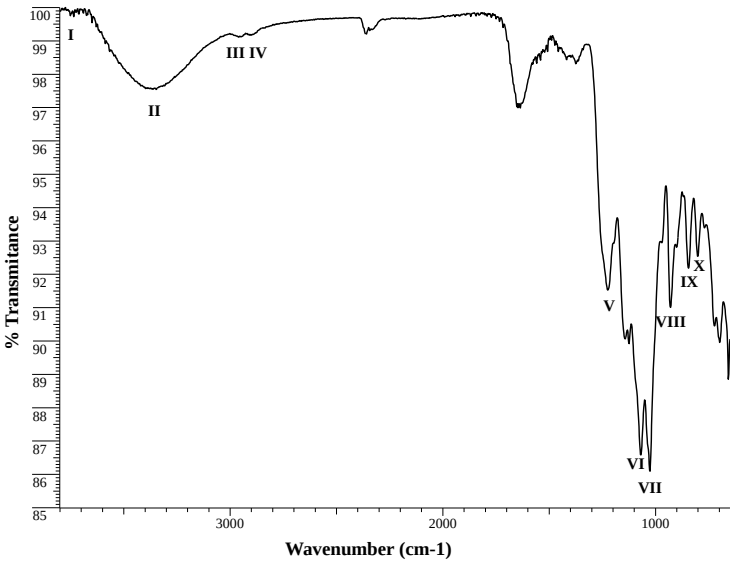

Fig. 2. FT-IR spectrum of the *P. pseudoceranoides* crude polysaccharide

The FT-IR spectrum of *P. pseudoceranoides* polysaccharide showed characteristic absorption bands of carrageenan type structure; FT-IR band assignment of carrageenans are presented in table I. Generally, the repeating disaccharide unit of carrageenans consists of alternating units of β-D-galactopyranose and α-D-galactopyranose or 3,6-anhydro-α-D-galactopyranose. As shown in table I, the FT-IR spectra of ι-carrageenan presents characteristic bands at 1210-1260 cm^{-1}, 840-850 cm^{-1} and 800-805 cm^{-1}, thus enabling us to tentatively identify the polysaccharide isolated from *P. pseudoceranoides* as ι-carrageenan (10-12).

TABLE I

FT-IR band assignment of carrageenans (adapted from Gomez-Ordonez et al. (12))

Wavenumber (cm^{-1})	Assignment	Carrageenan type
1210-1260	sulfate ester	ι, κ, λ, μ, ν, θ
928-933, 1070 (shoulder)	3,6-anhydro-D-galactose	κ
840-850	D-galactose-4-sulfate	ι, κ, μ, ν
830	D-galactose-2-sulfate	λ, θ
820, 825 (shoulder)	D-galactose-2,6-disulfate	λ, ν
810-820, 867 (shoulder)	D-galactose-6-sulfate	μ
800-805, 905 (shoulder)	3,6-anhydro-D-galactose-2-sulfate	ι, θ

Total phenolic content. The crude polysaccharide obtained from *P. pseudoceranoides* showed a higher amount of phenolics (25.08 ± 1.00 mg GAE/g), when compared with commercial-grade carrageenan (11.17 ± 0.73 mg GAE/g). Lim et al. reported lower total phenolic content (0.039 mg GAE/g) for a fucoidan fraction isolated from *Sargassum binderi*, native to Malaysia (13).

Antioxidant activity

It has been reported that algae polysaccharides exhibit significant antioxidant effects; the antioxidant activity depends on several chemical features of polysaccharides such as molecular weight, type of major sugar units, sulfate content and solubility (3).

ABTS radical cation scavenging activity. The assay is based on the ability of antioxidants to transform the preformed radical cation ABTS, a blue-green chromophore with characteristic absorbtion at 734 nm, into its reduced form with a consequent decolorization (8). The polysaccharide and the positive control showed good ABTS radical scavenging abilities which were dose-dependent (fig. 3). The scavenging activity of *P. pseudoceranoides* polysaccharide increased from 19.00 ± 0.30% (at 0.15 mg/mL) to 49.59 ± 0.03% (at 1.5 mg/mL). However, at all tested concentrations, the positive control, commercial-grade carrageenan, was less active than the crude polysaccharide, with activities ranging from 15.68 ± 0.45% to 30.30 ± 0.03%. Shao et al. reported higher ABTS radical cation quenching abilities (36.94%) for a sulfated polysaccharide isolated from Chinese green alga *Ulva fasciata*, tested in a concentration of 0.15 mg/mL (14). Additionally, a polysaccharide isolated from *Sargassum horneri* showed higher scavenging activity (94%) at a concentration of 1.5 mg/mL (15). Several studies reported linear concentration dependent reaction kinetics for algae polysaccharides (14, 16).

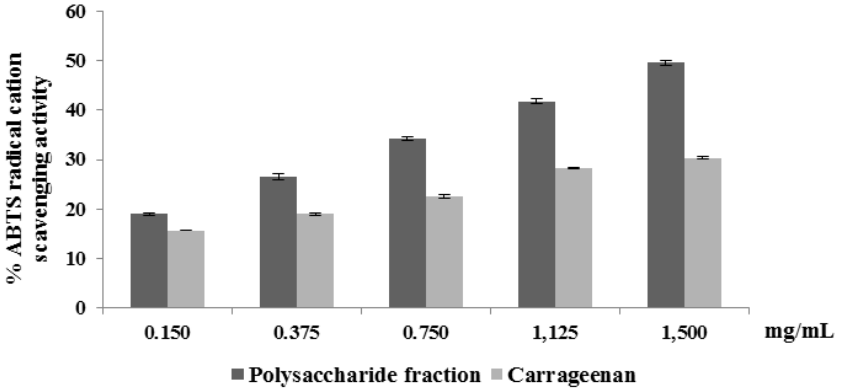

Fig. 3. ABTS radical cation scavenging effect of crude polysaccharide from *P. pseudoceranoides* and commercial-grade carrageenan

Reducing power. This method was used to evaluate the ability of the crude polysaccharide to donate an electron, thus reducing the Fe^{3+}/ferricyanide complex to the ferrous form (Perl's Prussian blue), quantified at 700 nm (17). As shown in fig. 4, the

polysaccharide reduced Fe^{3+} in a concentration-dependent manner. At 1.5 mg/mL, the crude polysaccharide exhibited higher reducing power (0.406 ± 0.002) than the positive control, commercial-grade carrageenan (0.249 ± 0.002). A liniarity of the response in the reducing power assay was observed for sodium alginate isolated from Tunisian *Cystoseira barbata;* however, according to the absorbance values at 700 nm, sodium alginate showed stronger reducing activity (the reducing power was 2 at a concentration of 1.2 mg/mL) (9).

On the contrary, a polysaccharide extracted from Chinese red alga *Porphyra haitanensis* was reported to possess lower reducing activity than the *P. pseudo ceranoides* polysaccharide; the reducing power of *P. haitanensis* polysaccharide was only 0.12 at a concentration of 1.84 mg/mL (18).

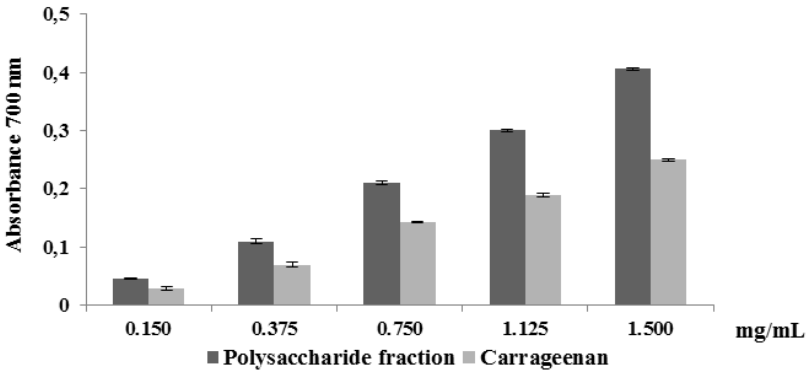

Fig. 4. Reducing power of crude polysaccharide from *P. pseudoceranoides* and commercial-grade carrageenan

CONCLUSIONS

The sulfated polysaccharide isolated from *P. pseudoceranoides* as characterized by FT-IR showed the characteristic bands of carrageenans. The crude polysaccharide, with a low phenolic content, was more active as free radical scavenger and reducing agent than the commercial-grade carrageenan. The crude polysaccharide isolated from *P. pseudoceranoides* might be used, in its present form or after purification, as an ingredient and potential antioxidant agent in the food and pharmaceutical industries.

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PRELIMINARY RESEARCH CONCERNING ANTINOCICEPTIVE AND ANTIINFLAMMATORY EFFECTS OF TWO EXTRACTS FROM *GLINUS OPPOSITIFOLIUS* (L.) AUG. DC.

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PRELIMINARY RESEARCH CONCERNING ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF TWO EXTRACTS FROM *GLINUS OPPOSITIFOLIUS* (L.) AUG. DC. (Abstract): In Mali *Glinus oppositifolius* (L.) Aug. DC. (*Aizoaceae*) is used to treat inflammations and joint pains. **Aim**: The present study was designed to evaluate the antinociceptive and anti-inflammatory effects of the aqueous and ethanol extracts obtained from the aerial parts of the plant. **Material and methods:** The aqueous and ethanol extracts (G-A1 and G-E1, respectively) were studied regarding their acute toxicity. The extracts were further evaluated in different models using nociceptive (chemical, thermal, mechanical) and inflammatory stimuli. Experimental protocol was implemented according to international regulations in animal experiments. **Results and discussions:** No acute toxicity was noticed for these two extracts. ED_{50} (mg/kg bw) values were determined in different nociception models: the constrictive abdominal response test (G-A1: 229.00 ± 53.30; G-E1: 146.38 ± 31.75), hot plate test (G-A1: 278.59 ± 73.00; G-E1: 383.52 ± 89.69), Randall-Selitto test (G-A1: 207.14 ± 26.15), evaluation of the inflammatory edema test (G-A1: 228.31 ± 13.84; G-E1: 62.55 ± 89.69). **Conclusions:** The results of the inflammatory nociception studies show that G-E1 extract had a higher potency than G-A1 extract in the constrictive abdominal response test. Also, for the same level of anti-inflammatory activity, G-E1 extract was more potent than G-A1 extract. These results encourage further studies to separate, isolate and identify the phytochemicals responsible for the analgesic and anti-inflammatory effects.
Keywords: *GLINUS OPPOSITIFOLIUS* (L.) AUG. DC., ANTINOCICEPTIVE ACTIVITY, RANDALL-SELITTO TEST, INFLAMMATORY EDEMA

Since ancient times, plants have been used to treat inflammatory conditions. Willow (*Salix* sp.) is probably the best known example; its bark and leaves have been used to alleviate pain and fever. The standardized willow bark extract STW 33I (23-26% salicin) and its water soluble fraction inhibit the expression of COX-2 and TNF-α, NO release and nuclear translocation of NF-kB. Turmeric (*Curcuma longa* L.) has been used in the Traditional Chinese Medicine and Ayurvedic Medicine in the treatment of rheumatism and other inflammation-associated disorders (fever, skin and hepatic diseases). The main constituent of turmeric, curcumin, has been intensively studied as anti-inflammatory agent. Curcumin has been shown to target enzymes (COX, LOX),

cytokines (IL-1, -6, -18, TNF-α), transcription factors (NF-kB) and other mediators (NO) involved in inflammation. The resin of *Boswellia serrata* L. has many uses in the Ayurvedic Medicine among which is the treatment of rheumatic conditions. *Boswellia* resin and its ethanol extracts down-regulate the expression of COX and matrix metalloproteinases (MMP-3) and inhibit NF-kB activation. Inhibitory effects on COX, MMP, NF-kB but also a reduction of primary T-cell response have been shown for stinging nettle (*Urtica dioica* L.) extracts; in Middle Ages the plant was used to ameliorate joint pains, arthritis and gout (1, 2).

In Mali *Glinus oppositifolius* (L.) Aug. DC. (*Aizoaceae*) is used to treat inflammations and joint pains (3). The present study was designed to evaluate the antinociceptive and antiinflammatory effects of the aqueous and ethanol extracts obtained from the aerial parts of the plant.

MATERIAL AND METHODS

Plant material. The aerial parts of *Glinus oppositifolius* (L.) Aug. DC. were provided by the Department of Traditional Medicine, Bamako, Mali.

Preparation of plant extracts. The aqueous and ethanol extracts were obtained as previously described (4).

Chemicals: sodium carboxymethyl cellulose (CMC-Na) (Sigma, St. Louis, Missouri, USA), zymosan A (Sigma, St. Louis, Missouri, USA), λ-carrageenan (Sigma, St. Louis, Missouri, USA), physiological saline solution (Sicomed S.A., Bucharest, Romania).

Animals. Experiments were carried out on Swiss white male mice (20-25 g) obtained from *Cantacuzino Institute* (Bucharest, Romania). Animals were housed under constant standard laboratory conditions (room temperature 21±2°C, dark/light cycle of 12 h). The animals were fed with standard diet and water *ad libitum*. Three hours before each experiment, mice were maintained without food and water. The investigations were performed on groups of mice in the *Experimental Pharmacodynamics Laboratory*, Department of Pharmacodynamics and Clinical Pharmacy, University of Medicine and Pharmacy *Grigore T. Popa* - Iasi and followed the international regulations in animal experiments and the university specific standards (5, 6).

Antinociceptive evaluation. The study was conducted using different nociceptive (chemical, thermal, mechanical) and inflammatory stimuli.

The constrictive abdominal response. When administered intraperitoneally, zymosan A suspension produces hyper-algesia in mice. It appears a characteristic response, the constrictive abdominal response, consisting in the elongation and constriction of the animal, abdominal retraction and *opistotonus* (twisting of the trunk followed by extension of the hind limbs). The response can be reduced or abolished by administration of some analgesic drugs (7). The experiment was conducted using the method modified by Bild *et al.* (8).

The drugs were administered orally in CMC-Na suspension. The groups of mice were treated with a series of doses in geometrical progression with ratio 2. For G-A1 and G-E1 doses between 25-1600 mg/kgbw and 50-1600 mg/kg bw, respectively were used. Zymosan A suspension (20 mg/kgbw) was administered intra-peritoneally 30-90 min. after extracts administration. The number of constrictive abdominal responses was recorded for 12 min. after administration of the irritant agent (7, 9). The maximal possible effect was calculated according to the following equation:

$$\%\ Inhibition\ (Antinociception) = \frac{no.\ of\ non-responders}{no.\ of\ animals\ /group} \times 100.$$

The hot plate test. The method was described by Woolfe and Mac Donald and modified by Eddie and Laborit. The experiment was conducted using the method modified by Bild *et al.*.

Mice were placed on a hot plate (*Ugo Basile* 7280) maintained at a constant temperature of 52,5°C (*cut-off* of 30 sec.). The latency period till the first antinociceptive behavioral response was measured (10-12). The analgesic effect was evaluated using the formula:

$$\%\ \text{Inhibition (Antinociception)} = \frac{T_x - T_0}{T_m - T_0} \times 100, \text{ where:}$$

- T_x – the latency of the response measured after the administration of the extracts;
- T_0 – the latency of the response measured before the administration of the extracts;
- T_m – maximal allowed time (*cut-off* time).

Tail immersion test. The experiment was conducted using the method modified by Bild *et al.*. The animals' tails were placed in a water bath heated to 52,5°C (*cut-off* of 15 sec.) (13). The latency period till the first antinociceptive behavioral response was measured. The maximal possible effect was calculated according to the following equation:

$$\%\ Inhibition\ (Antinociception) = \frac{no.\ of\ non-responders}{no.\ of\ animals/group} \times 100.$$

Randall-Selitto test. The experiment was conducted using the method modified by Bild *et al.*. This model evaluates pain that occurs in inflammatory conditions. Inflammation was induced by subplantar injection of 1% suspension of λ-carragenan in mice. The effects of extracts on carrageenan-induced inflammation were assessed using an analgesy-meter (*Ugo Basile* 7215). This device generated a mechanical force on the affected paw (*cut-off* of 250 g). The latency period till the first antinociceptive behavioral response was measured (10, 11, 14, 15). The analgesic effect was appreciated using the formula:

$$\%\ \text{Inhibition (Antinociception)} = \frac{g_x - g_0}{g_m - g_0} \times 100, \text{ where:}$$

- g_x – the latency of the response measured after the administration of the extracts;
- g_0 – the latency of the response measured before the administration of the extracts;
- g_m – maximal allowed time (*cut-off* time).

The evaluation of the inflammatory edema. The experiment was conducted using the method modified by Bild *et al.*. The anti-inflammatory potential of the extracts was assessed by evaluating their capacity to inhibit carrageenan-induced edema (10, 11, 16, 17). The paw volume was measured using a *Ugo Bazile* 7200 plethysmometer and the inflammatory edema inhibition was appreciated using the formula:

$$\%\ Inhibition = \frac{M - T}{M} \times 100, \text{ where:}$$

- M – value of the degree of inhibition of the control group;
- T – value of the degree of inhibition of the treated group.

RESULTS AND DISCUSSION

Antinociceptive evaluation. No acute toxicity was observed in animals treated with the extracts (data not shown). This aspect allowed further investigations concerning antinociceptive /anti-inflammatory activity.

The test of the constrictive abdominal response. In case of oral administration of extracts in doses between 37.5-300 mg/kgbw (G-A1) and 50-200 mg/kgbw (G-E1), ED_{50} values of 229.00±53.30 mg/kgbw (G-A1) and 146.38±31.75 mg/kgbw (G-E1) were determined (tab. I, fig. 1-a,b). Data analysis shows that G-E1 extract had a higher potency than G-A1 extract.

The hot plate test. In case of oral administration of extracts in doses between 100-400 mg/kgbw (G-A1) and 150-600 mg/kgbw (G-E1), ED_{50} values of 278.59±73.00 mg/kgbw (G-A1) and 383.52±89.69 mg/kgbw (G-E1) were determined (tab. I, fig. 2-a,b). Data analysis shows that G-A1 extract had a higher potency than G-E1 extract.

Tail immersion test. In case of oral administration of extracts in doses between 100-400 mg/kgbw (G-A1) and 150-600 mg/kgbw (G-E1), ED_{50} values could not be identified. The maximal possible effect was 30.80% (G-A1) and 24.00% (G-E1).

Randall-Selitto test. In case of oral administration of extracts in doses between 75-300 mg/kgbw (G-A1), an ED_{50} value of 207.14±26.15 mg/kgbw was determined for G-A1 extract (tab. I, fig. 3-a,b). Using a sequence of doses between 100-400 mg/kg bw, ED_{50} value could not be determined for G-E1 extract. The maximal possible effect was 30.80%.

The evaluation of the inflammatory paw edema. In case of oral administration of extracts in doses between 75-300 mg/kgbw (G-A1) and 50-400 mg/kgbw (G-E1), ED_{50} values of 228,31 ± 13,84 mg/kgbw (G-A1) and 62,55 ± 89,69 mg/kgbw (G-E1) were determined (tab. I, fig. 4-a,b). Data analysis shows that G-E1 extract had a higher potency than G-A1 extract.

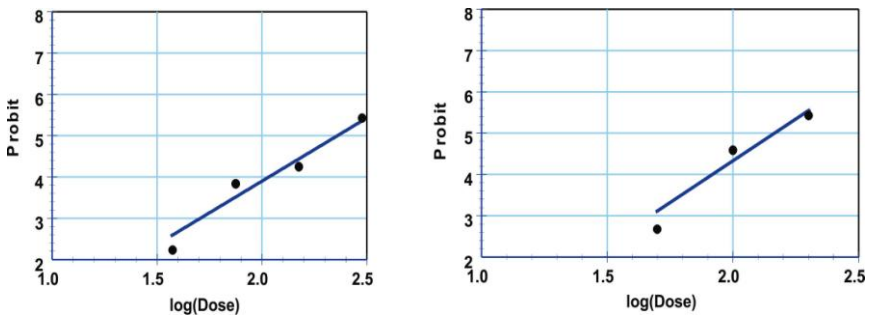
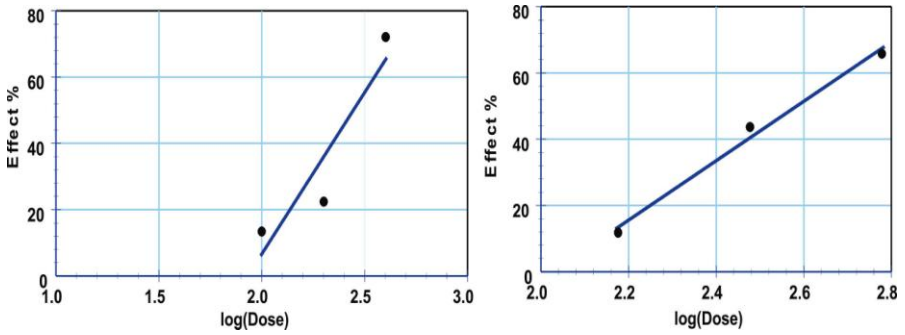


Fig. 1. The regression lines for calculation of ED_{50} of *Glinus oppositifolius* extracts in constrictive abdominal response test (**a**: G-A1, **b**: G-E1)

In case of G-A1 extract, except tail immersion test, ED_{50} values in nociception models are similar and the true confidence levels have a common overlap domain. In addition, the capacity to inhibit the inflammatory edema at 50% activity was similar with ED_{50} value for antinociceptive activity.

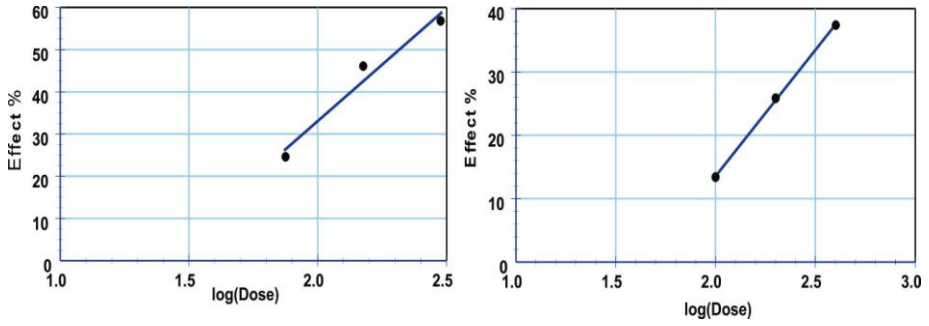
G-E1 extract had a different behavior in nociception models. In the tail immersion and Randall-Selitto tests, its ED_{50} values could not be determined using the established dose sequences. In the thermal stimulus nociception model, G-E1 extract was

less potent than in zymosan nociception model. The inhibitory activity on inflammatory edema at 50% activity was achieved at 62.55 ± 89.69 mg/kgbw (tab. I).



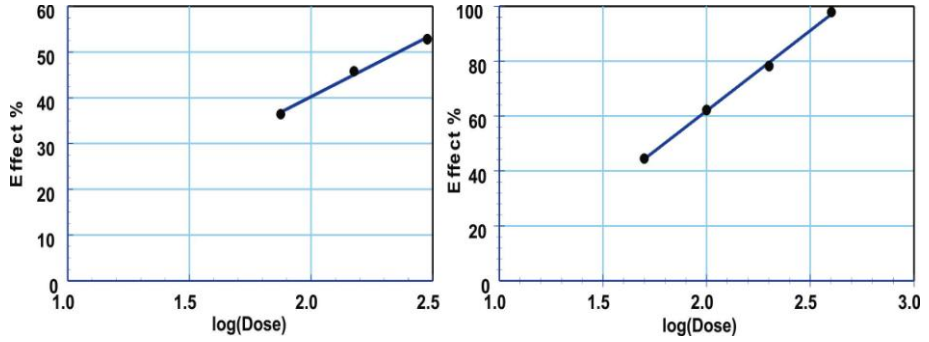
a ***b***

Fig. 2. The regression lines for calculation of ED_{50} of *Glinus oppositifolius* extracts in hot plate test (**a**: G-A1, **b**: G-E1)



a ***b***

Fig. 3. The regression lines for calculation of ED_{50} of *Glinus oppositifolius* extracts in Randall-Selitto test (**a**: G-A1, **b**: G-E1)



a ***b***

Fig. 4. The regression lines for calculation of ED_{50} of *Glinus oppositifolius* extracts in the evaluation of the inflammatory paw edema test (**a**: G-A1, **b**: G-E1)

TABLE I

ED_{50} values of *Glinus oppositifolius* extracts and some statistical parameters

Nociception model	G-A1 extract	G-E1 extract	Statistical parameters
Constrictive abdominal response test	229.00 ± 53.30	146.38 ± 31.75	ED_{50}
	Y = -2.22 + 3.06·X	Y = -3.67 + 4.04·X	Regression line equation
	0.970	0.960	R
	(152.96 ; 595.11)	(88.74 ; 553.01)	TCL
Hot plate test	278.59 ± 73.00	383.52 ± 89.69	ED_{50}
	Y = -188.38 + 97.49·X	Y = -181.74 + 97.49·X	Regression line equation
	0.928	0.995	R
	(111.25 ; 350.10)	(103.25 ; 460.05)	TCL
Tail immersion test	ED_{50} value could not be determined using the established dose sequence	ED_{50} value could not be determined using the established dose sequence	ED_{50}
			Regression line equation
			R
			TCL
Randall-Selitto test	207.14 ± 26.15	ED_{50} value could not be determined using the established dose sequence	ED_{50}
	Y = -73.88 + 53.48·X		Regression line equation
	0.982		R
	(77.326 ; 256.23)		TCL
Inflammatory paw edema	228.31 ± 13.84	62.55 ± 89.69	ED_{50}
	Y = -14.16 + 27.20·X	Y = -55.11 + 58.51·X	Regression line equation
	0.996	0.999	R
	(0.395 ; 286.96)	(53.99 ; 70.79)	TCL

The analysis of inflammatory nociception models shows that G-E1 extract had a higher potency than G-A1 extract in the constrictive abdominal response test. Also, for the same level of anti-inflammatory activity, G-E1 extract was more potent than G-A1 extract.

CONCLUSIONS

G-A1 extract showed antinociceptive activity in all tested nociception models except tail immersion test. Additionally, it had anti-inflammatory activity. Taking into consideration the ED_{50} values and the ability of the phytocomplex to inhibit the release of mediators of inflammation and pain by applying appropriate stimuli, it can be concluded that both activities are equally present. The extract has the characteristic profile of moderate analgesics and NSAIDs.

G-E1 extract showed a good anti-inflammatory activity. The extract has the characteristic profile of NSAIDs. In nociception models using thermal stimuli, the extract had a low potency and, in some situations, ED_{50} could not be determined. G-E1 extract has the ability to inhibit the inflammatory mediators release. The extract showed a high potency in inflammatory paw edema test (ED_{50}=62.55±89.69 mg/kg bw) and constrictive abdominal response test (ED_{50}=146.38±31.75 mg/kg bw).

The results in nociception models using thermal stimulus (working temperature of 52.5°C) invalidate the morphine-like behavior of the extracts. However, such a behavior cannot be excluded but in case it would be present, it is certainly moderate, similar to that of codeine.

These data confirm the validity of the working hypotheses and encourage further studies concerning the separation, isolation

and identification of groups of active phytochemicals with analgesic and anti-inflammatory effects.

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TAGUCHI METHOD FOR PARTIAL DIFFERENTIAL EQUATIONS WITH APPLICATION IN TUMOR GROWTH

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TAGUCHI METHOD FOR PARTIAL DIFFERENTIAL EQUATIONS WITH APPLICATION IN TUMOR GROWTH (Abstract): The growth of tumors is a highly complex process. To describe this process, mathematical models are needed. A variety of partial differential mathematical models for tumor growth have been developed and studied. Most of those models are based on the reaction–diffusion equations and mass conservation law. A variety of modeling strategies have been developed, each focusing on tumor growth. **Material and methods**: Systems of time-dependent partial differential equations occur in many branches of applied mathematics. The vast majority of mathematical models in tumor growth are formulated in terms of partial differential equations. We propose a mathematical model for the interactions between these three cancer cell populations. The Taguchi methods are widely used by quality engineering scientists to compare the effects of multiple variables, together with their interactions, with a simple and manageable experimental design. In Taguchi's design of experiments, variation is more interesting to study than the average. **Results:** First, Taguchi methods are utilized to search for the significant factors and the optimal level combination of parameters. Except the three parameters levels, other factors levels other factors levels would not be considered. Second, cutting parameters namely, cutting speed, depth of cut, and feed rate are designed using the Taguchi method. Finally, the adequacy of the developed mathematical model is proved by ANOVA. According to the results of ANOVA, since the percentage contribution of the combined error is as small. **Conclusions:** Many mathematical models can be quantitatively characterized by partial differential equations. The use of MATLAB and Taguchi method in this article illustrates the important role of informatics in research in mathematical modeling. The study of tumor growth cells is an exciting and important topic in cancer research and will profit considerably from theoretical input. Interpret these results to be a permanent collaboration between math's and medical oncologists. **Keywords:** TUMOR GROWTH, DIFFERENTIAL EQUATIONS, TAGUCHI METHOD, NUMERICAL MODELING

Cancer is a generic name given to a group of malignant cells that have lost their specialization and normal control growth. This group of malignant cells can be regarded as a nonlinear dynamic system self-organized in time and space. Mathematical modeling is a process by which a real world problem is described by a mathematical formulation. Mathematical modeling of avascular tumors can be seen as the first step in building models for tumors in later stages (1, 2). Taguchi methods consist of 3

phases: designing the experiment, running and analyzing, and confirming and validating the assumptions (3, 4).

MATERIAL AND METHODS

The model is based on a set of partial differential equations with form:

$$\begin{cases} \frac{\partial u}{\partial t} = \frac{\partial}{\partial x}[\frac{u}{u+v}\frac{\partial}{\partial x}(u+v)] + g(c)u(1-u-v) - f(c)u. \\ \frac{\partial v}{\partial t} = \frac{\partial}{\partial x}[\frac{v}{u+v}\frac{\partial}{\partial x}(u+v)] + f(c)u - h(c)v \\ \frac{\partial w}{\partial t} = h(c)v \\ c = \frac{c_1[1-\alpha(u+v+w)]}{\beta+u}, \beta = \frac{k_1}{k_2} \end{cases}$$

Where are denoted by:

$u(x,t), b(x,t), v(x,t), w(x,t)$ the cell densities for the proliferating, quiescent, necrotic cells and concentration of some nutrients (1),(2). In this stage, Taguchi methods were used to find the optimal parameters combination. Taguchi (4) has envisaged a new method of conducting the design of experiments which are based on well defined guidelines. This method uses a special set of arrays called orthogonal arrays. These standard arrays stipulate the way of conducting the minimal number of experiments which could give the full information of all the factors that affect the performance parameter. The table I shows an L^{16} orthogonal array (3). There are totally 16 experiments to be conducted and each experiment is based on the combination of level values as shown in the table. The third experiment is conducted by keeping the independent design variable 1 at level 1, variable 2 at level 3, and variable 3 at level 3.

TABLE I

Layout of L^{16} orthogonal array.

Taguchi's orthogonal arrays are highly fractional orthogonal designs

Exp. No.	Parameter 1	Parameter 2	Parameter 3
1	1	1	1
2	1	2	2
3	1	3	3
4	1	4	4
5	2	1	2
6	2	2	1
7	2	3	4
8	2	4	3
9	3	1	3
10	3	2	4
11	3	3	1
12	3	4	2
13	4	1	4
14	4	2	3
15	4	3	2
16	4	4	1

The relationship between one column and another is arranged so that factor for each level within one column, each level within any other column occur an equal number of times as well. These designs can be used to estimate main effects using only a few experimental runs.

There are totally 16 experiments to be conducted and each experiment is based on the combination of level values as shown in the table II. The third experiment is conducted by keeping the independent design variable 1 at level 1, variable 2 at level 3, and variable 3 at level 3.The additive assumption implies that the individual or main effects of the independent variables on performance parameter are separable, but the model assumes that there exist no cross product interactions among the individual factors (4). Furthermore, each factor can be assigned a significance weight to denote its importance in affecting the end result of the experiment.

TABLE II
Experimental data for Taguchi method

Tests	α	k_1	k_2
1	0.5	0.08	115
2	0.5	0.092	120.5
3	0.5	0.105	127
4	0.5	0.121	133
5	0.63	0.08	120.5
6	0.63	0.092	115
7	0.63	0.105	133
8	0.63	0.121	127
9	0.78	0.08	127
10	0.78	0.092	133
11	0.78	0.105	115
12	0.78	0.121	120.5
13	0.97	0.08	133
14	0.97	0.092	127
15	0.97	0.105	120.5
16	0.97	0.121	115

RESULTS

The design of the experiments has an effect on the number of experiments required. Taguchi's experimental approach was applied to design the experiments using the three parameters of this study .The results of the 16 experiments of Taguchi's L^{16} series, were analyzed in order to determine the mean effect of each factor. These results suggest that the method of Taguchi's optimization approach remarkable rapid tumor growth. A major finding of the study was that the Taguchi methods predicted the combination of factors that results in the lowest survival of the malignant cells. The experimental results indicate that the proposed mathematical models suggested could adequately describe the performance indicators within the limits of the factors that are being investigated. After the simulation model using different values of the three parameters α,k_1 and k_2 are obtained following fig. 1.

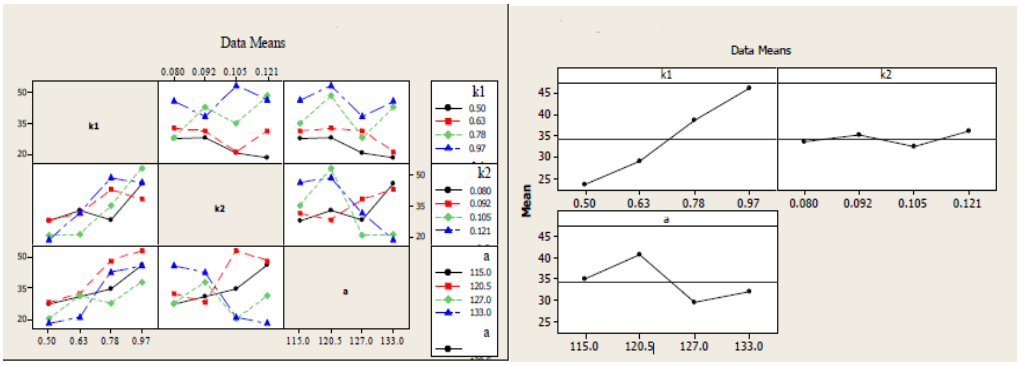

Fig. 1. The results of L^{16}.

Each experiment run was done in triplicate and repeated three times, the mean values were calculated and the results were expressed as mean. The adequacy of the predictive model was verified using ANOVA at 95% confidence level. The ANOVA revealed that feed is the most significant factor influencing the response variables investigated. The first step in the optimization process is to determine the ratio for the entire experiments test. The percentage contribution of each control factor is employed to measure the corresponding effect on the quality characteristic. In this experiment, Taguchi's graphical approach is used to plot the "marginal means" of each level of each factor and "pick the winner" to determine the best setting for each control factor. An advantage of the Taguchi method is that it emphasizes a mean performance characteristic value close to the target value rather than a value within certain specification limits.

CONCLUSIONS

In this paper we presented a partial differential equations model of avascular tumor growth which consists of proliferating, quiescent and necrotic cells and includes chemo taxis interactions and nutrient diffusion. The use of Taguchi method in this article illustrates the important role of informatics in research in mathematical modeling. A major finding of the study was that the Taguchi methods predicted the combination of factors that results in the lowest survival of the malignant cells. We have presented this method in systems of partial differential equations, which is integration based. For model systems have been chosen to demonstrate the validity of this method. We have seen that this method is relatively simple and therefore requires very little computational time. The study of avascular tumor growth cells is an exciting and important topic in cancer research and will profit considerably from theoretical input.

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SPECTROMETRIC METHOD FOR THE ASSAY OF RAMIPRIL USING MOLYBDOPHOSPHORIC ACID

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SPECTROMETRIC METHOD FOR THE ASSAY OF RAMIPRIL USING MOLYBDOPHOSPHORIC ACID (Abstract): Ramipril is a drug of the angiotensin converting enzyme inhibitor class. **Aim:** A new molecular absorbance spectrometric method was developed for the assay of ramipril using molybdophosphoric acid in acidic medium. **Material and methods:** The reaction product showed a maximum absorbance at 361 nm. The optimum conditions of the reaction were established. The developed method was validated. **Results:** The method showed a good linearity in the range of 8 - 36 μg/ml (correlation coefficient r = 0.9996). The detection limit (LD) was 0.86 μg/ml and the quantification limit (LQ) 2.88 μg/ml. Precision and accuracy were determined (RSD = 1.15%); mean recovery was 98.90% in the 97.13-101.03% concentration range. **Conclusions:** The proposed method is simple, easy to perform, sensitive, linear, precise, accurate and robust. **Keywords:** RAMIPRIL, MOLYBDOPHOSPHORIC ACID, SPECTROMETRIC METHOD, VALIDATION.

Ramipril is a drug of the angiotensin converting enzyme (ACE) inhibitor class that is primarily used in the treatment of high blood pressure and congestive heart failure. Ramipril, a prodrug, is converted to the active metabolite ramiprilat by liver esterase enzymes. Ramiprilat is mostly excreted by the kidneys. The half-life of ramiprilat is variable (3–16 hours), and is prolonged by heart and liver failure, as well as kidney failure (1).

This paper presents a UV-Vis molecular absorbance spectrometric method for the assay of ramipril using molybdophosphoric acid in acidic medium. When comparing it to other assay methods in the literature, the proposed method is simple, easy to perform, sensitive, linear, precise, accurate and robust (2-3). The developed method was validated using the following criteria: linearity, detection and quantification limit, precision, accuracy and robustness (4-7).

MATERIAL AND METHODS

Reagents and apparatus:

- ramipril (99.21% reference substance, produced by Lupin, India);
- 0.1 M - 1.0 M hydrochloric acid aqueous solutions;
- 0.1% - 3.0% molybdophosphoric acid aqueous solutions;
- 0.01% sodium lauryl sulphate aqueous solution;
- analytical balance (Kern 770);

- UV-Vis Spectrophotometer (Hewlett Packard 8453).

Except ramipril, all other reagents were produced by Merck, Germany.

Principle of the method: Ramipril forms with molybdophosphoric acid in hydrochloric acid medium an insoluble compound which can be spectrometrically measured at 361 nm.

In order to establish the optimum wavelength for the detection, 2 ml of the 10 µg/ml working solution were mixed with 1 ml 0.2 M hydrochloric acid and 1% molybdophosphoric acid solution; then, 1 ml 0.01% sodium lauryl sulphate was added in order to prevent precipitation. The UV-Vis absorption spectrum was recorded using 1 cm cell, after 20 minutes.

In order to establish the optimum working conditions, two solutions of 8 µg/ml and 32 µg/ml were used, while the parameters of the method were changed. The optimum concentration of the hydrochloric acid solution and molybdophosphoric acid solution was established and the stability of the reaction product was evaluated.

Procedure: 1 ml 1M hydrochloric acid and 1 ml of 2% molybdophosphoric acid solution were added to 1 ml ramipril solution and 1 ml of 0.02% lauril sodium sulphate. The absorbance was measured at 361 nm versus a blank solution prepared in identical conditions.

RESULTS AND DISCUSSION

The analysis of absorption spectra showed a maximum absorbance for the reaction product at 361 nm. This value was used for all the determinations (fig. 1).

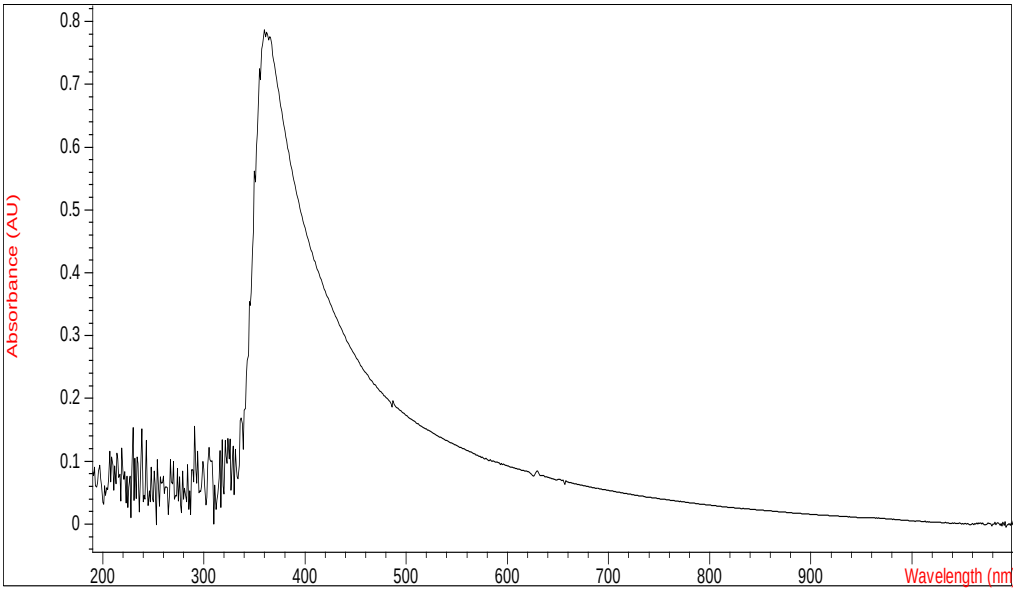

Fig. 1. Spectrum of the reaction product obtained for 32 µg/ml ramipril

The best concentration of the hydrochloric acid was established at 1 M, because when using that solution the maximum absorbance measured at 361 nm had the greatest value (tab. I). The same criterion was used when optimum concentration

of molybdophosphoric acid was set to 1% (tab. II). By studying the stability of the samples it was established that the chemical reaction between ramipril and molybdophosphoric acid was complete 20 minutes after the last reagent was added. The results proved that absorbance remained almost the same for at least another 20 minutes, sufficient time for the measurements to be performed (tab. III).

TABLE I
Influence of medium acidity upon absorbance

HCl mol/L	Ramipril (361 nm)	
	8 µg/ml	36 µg/ml
0.50	0.03965	0.28511
0.75	0.08156	0.68221
1.00	0.12535	0.87965
1.25	0.11741	0.86963
1.50	0.09642	0.85211
1.75	0.08952	0.75152
2.00	0.06965	0.62353

TABLE II
Influence of reagent concentration upon absorbance

Molybdo-phosphoric acid %	Ramipril (361 nm)	
	8 µg/ml	36 µg/ml
0.5	0.01965	0.29441
1.0	0.06743	0.49145
1.5	0.09021	0.77125
2.0	0.12742	0.88426
2.5	0.11096	0.85522
3.0	0.09832	0.78956
3.5	0.06153	0.68642
4.0	0.04123	0.57426

TABLE III
Stability of the reaction product

Time (minutes)	Ramipril (361 nm)	
	8 µg/ml	36 µg/ml
5	0.05221	0.36854
10	0.12459	0.59562
15	0.12632	0.85422
20	0.12642	0.85429
25	0.12742	0.84522
30	0.11821	0.85421
40	0.12742	0.84985
50	0.12655	0.83456
60	0.12502	0.79412

TABLE IV
Linearity of the method

Ramipril (µg/ml)	Absorbance (361 nm)				
	Ist Series	IInd Series	IIIrd Series	IVth Series	Average
4	0.07375	0.07155	0.06893	0.07011	0.07108
8	0.12189	0.12524	0.11961	0.11926	0.12150
12	0.24722	0.24149	0.25028	0.24383	0.24571
16	0.35117	0.35107	0.36097	0.31686	0.34502
20	0.45176	0.45650	0.45190	0.45005	0.45255
24	0.56135	0.57117	0.56974	0.56546	0.56693
28	0.69654	0.68455	0.69581	0.67996	0.68922
32	0.78080	0.81412	0.78145	0.81617	0.79814
36	0.88693	0.87143	0.91045	0.89763	0.89161
40	0.89181	0.89302	0.92068	0.90763	0.90329
Correlation and regression coefficients r = 0.9996, r^2 = 0.9992; Standard error = 0.0079; Intercept = – 0.0951; Slope = 0.0277					

Linearity was studied in the 4-40 µg/ml concentration range (fig. 2). The obtained data were statistically evaluated (tab. IV) and the calibration curve was obtained (fig. 3).

According to the experimental data, the developed method for ramipril determination was linear in the concentration range of 8 – 36 µg/ml.

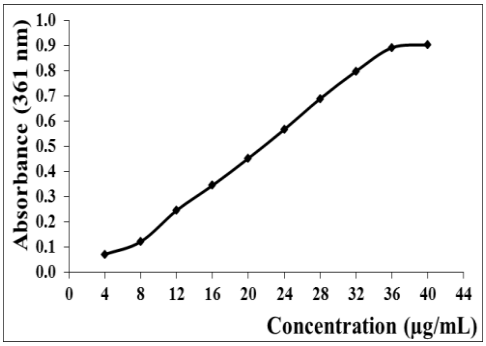

Fig. 2. Method linearity

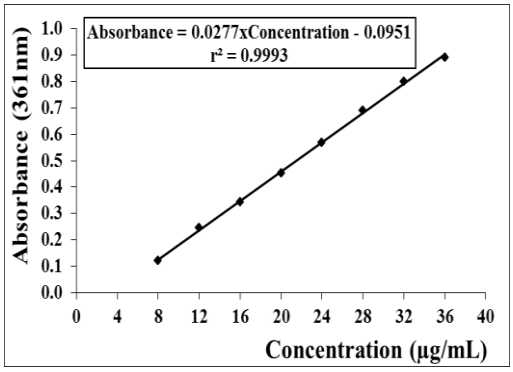

Fig. 3. Calibration curve

Detection and quantification limits were calculated using the following formulas (4-7):

LD = 3 x Standard error / Slope = 0.86 µg/ml

LQ = 10 x Standard error / Slope = 2.88 µg/ml

When we compared this concentration range with that of other published methods we found out that this new method has the following advantages: it does not involve rare or complex reagents, nor does it involve the use of toxic solvents. It is simple and easy to perform (2, 3). The calibration curve equation was established (fig. 3).

For precision determination, three solutions of 16, 20 and 24 µg/ml ramipril were used. Three assays were performed for each concentration. Two sets of assays were performed in different days in order to evaluate the intermediate precision.

Samples concentrations were calculated using the calibration curve equation (tab. V). We found that for each set of data and for both sets together the relative standard deviation was lower than 2%. This fact proved that the proposed method was precise.

In order to establish the accuracy of the method, ramipril solutions of 16, 20, and 24 µg/ml were analyzed. For each concentration, three determinations were performed (6).

The concentration of the samples was calculated based on the experimental absorbance values, using the regression curve equation (tab. V). We found that recovery was 100.59% for the studied concentration range, mean 98.90% (minimum 98.51% and maximum 102.39%), and the relative standard deviation was under 2% (RSD = 1.42 %). These values prove that the proposed method was accurate.

A robustness study was performed to establish system suitability in order to ensure the validity of the analytical procedure. This was done by varying the instrument, analyst, and time of study. The analysis was performed on Shimadzu UV-Visible spectrophotometer, model-1700. Interday and intraday analysis was performed by changing the analyst. Reproducibility of the results confirmed the robustness of the method.

TABLE V

Precision and accuracy of the method

Concentration (µg/ml)	Precision		Intermediate precision		Accuracy	
	Real concentration (µg/ml)	RSD %	Real concentration (µg/ml)	RSD %	Real concentration (µg/ml)	Recovery (%)
16	16.2282	0.72	16.2877	0.97	15.8040	98.78
	15.9959		16.0248		15.8947	99.34
	16.0851		16.3095		16.1650	101.03
20	19.8079	0.77	19.6981	0.16	19.4260	97.13
	19.7111		19.7602		19.8000	99.00
	20.0120		19.7133		19.7427	98.71
24	23.9530	0.76	23.6412	0.40	23.6228	98.43
	23.6286		23.8349		23.9794	99.91
	23.6503		23.7532		23.4675	97.78
					Mean	98.90

CONCLUSIONS

A UV-VIS molecular absorbance spectrophotometric method was developed for the assay of ramipril using molybdophosphoric acid in hydrochloric acid medium. The reaction product showed a maximum absorbance at 361 nm.

The analytical method was validated for the linearity range of 8.0 - 36 µg/ml, with a correlation coefficient of r = 0.9996, detection limit of 0.86 µg/ml, quantification limit of 2.88 µg/ml below the lowest concentration of the linearity range, method precision RSD% = 0.35% (4), and accuracy evaluated by recovery R = 98.90%.

In conclusion, the proposed method is simple, easy to perform, sensitive, linear, precise, accurate and robust.

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PHYTOTOXICITY ASSESSMENT OF METHANOLIC EXTRACTS FROM *CORIADRUM SATIVUM* L. FRUITS

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PHYTOTOXICITY ASSESSMENT OF METHANOLIC EXTRACTS FROM *CORIADRUM SATIVUM* L. FRUITS (Abstract): The fruits of *Coriandrum sativum* L. (Apiaceae) are used as a spicing and flavoring agent in food products and are also recommended as a drug in the treatment of digestive tract disorders. The safety of botanicals and botanical preparations used as food supplements is of great interest, as the market of such products is expanding worldwide. **Aim:** In this study, aimed at assessing the safety of coriander as food ingredient, we investigated the phytotoxicity of fruit extracts on *Triticum aestivum* L. **Material and methods:** We studied the effects of methanolic coriander fruit extracts, Omagiu cultivar (V1-V3; three dilutions: 1%, 2.5% and 5 % in distilled water), upon germination, elongation, and accumulation of fresh and dry biomass in the plants; also it was investigated the influence of the extracts on mythosis in root tissues. **Results and discussions:** The extracts did not influence the germination and growth of test plants and did not inhibit root and shoot elongation of wheat plants (a 20 % increase in root length over the control being registered for V2 1%). Frequency of aberrant anaphases and telophases in *Triticum aestivum* L. varied slightly around the percentage of the control, the highest percent of aberrant chromosomes (5.07 %) being observed in V3 2.5 %. **Conclusions:** The investigated coriander fruit extracts did not have phytotoxic effects but had a slight stimulating effect on germination and growth of *Triticum aestivum* L. **Key words:** *CORIANDRUM SATIVUM* L., *TRITICUM AESTIVUM* L., PHYTOTOXICITY, CYTOGENETIC EFFECT.

The study of synthetical or natural drugs toxicity is essential in order to assess their pharmaceutical use and the risks for consumer exposure to these products. The safety of botanicals used as food supplements is of great interest, as the market of such products is expanding worldwide. For this reason, in 2009, the Scientific Committee of the European Food Safety Authority (EFSA) adopted a series of regulations regarding the safety assessment of botanicals and botanical preparations widely used as food supplements (1) even though in the past these products were believed side effect-free.

The fruits of *Coriandrum sativum* L. (Apiaceae) are used as spicing and flavoring agents in food products, liqueurs, and tobacco. As well, they are recommended in the treatment of digestive tract disorders due to their digestive, anti-foaming, and spasmolytic properties (2).

Coriander fruits have a long history of use as a spice and in phytotherapy with no record of notable adverse effects(3); by this token, we used an *in vivo* phytotoxicity test for assessing the risks associated with the use of coriander fruits, Omagiu cultivar. We studied the cyto- and genotoxicity of coriander extracts upon *Triticum aestivum* L.(wheat) as test species.

MATERIAL AND METHODS

Coriander fruits, Omagiu cultivar, were harvested during August 2011 from plants cultivated in the fields of the Secuieni Agricultural Research and Development Center (Neamt County, Romania) under the same soil and climatic conditions, the differences being some agrotechnical factors such as the nutritional space; the three experimental samples were named V1, V2 and V3.

The phytobiological studies were carried out using grains of *Triticum aestivum* L., Dor variety (4). We studied the effects of methanolic fruit extracts upon germination, elongation and accumulation of fresh and dry biomass in the plants; we have also investigated the influence of extracts on mythosis in root tissues.

The methanolic extracts were obtained from delipidated coriander fruits with a drug:extract ratio of 2.5:100 g/mL. Total phenolic compounds in the methanolic coriander fruit extracts were determined as gallic acid equivalents by Folin-Ciocalteu method (5).

The experiment consisted in three treatments per sample by diluting the initial methanolic extract with distilled water (1%, 2.5% and 5%) and one control (distilled water) (tab. I). Both for the control and for each dilution we used groups of 200 wheat grains.

*Study on the effect of coriander seeds extracts on wheat plants germination, root and shoot elongation. S*eeds of test plants were soaked for 24 hours in 50 ml of each treatment variant and in the same volume of distilled water for the control. After that, 150 wheat seeds were washed and placed uniformly upon a system made of sieves, with plastic vessels filled with water placed below (6). Cultivation was done in an air-conditioned chamber, at an average air temperature of 22±2 ºC and constant light of 2500 lux. After 7 days, germination was abstained, seed germination rate was calculated and shoot and root length was measured. Seedlings having less than 50 mm in length (both root and stem) were excluded from the experiment.

Study on the effect of coriander seeds extracts on fresh and dry biomass accumulation in Triticum plants. After 7 days, fresh weight (Wf) of roots and shoots for each treatment variant was determined. Their dry weight (Wd) was measured after oven drying at 60ºC for 24 hours.

Biological effects were evaluated by using the equation: Wd/Wf x100.

Study on the effect of coriander seed extracts upon mythosis in root meristems

The remaining 50 pre-imbibed grains were placed in 10 cm diameter Petri dishes, on filter paper moistened with distilled water. After 72 hours, the primary roots were cut off and a squash preparation stained with Schiff reagent was obtained (7). Both normal ana- and telophases and those with different types of aberrations (bridges, fragments, fragments and bridges, tri- and tetrapolar anaphases, chromosomes in ring) were counted.

RESULTS AND DISCUSSION

The amount of total phenolic com-

pounds in coriander fruit methanolic extracts of were determined as gallic acid equivalents (g GAE %) (tab. I); as shown, the differences between the investigated coriander samples are not significant.

Seed germination of *Triticum aestivum* was used to ascertain the effect of methanolic extracts of coriander fruits, Omagiu cultivar, on physiological process during initial seedling growth. Germination rate and the percentage of seedlings less than 50 mm in length are shown in table II.

TABLE I

Total phenolic compounds in methanolic extracts of Omagiu coriander fruits

Sample	g GAE %
V1	0.13 ± 0.00
V2	0.13 ± 0.00
V3	0.14 ± 0.00

TABLE II

The effect of coriander bioactive compounds on wheat plants germination

Treatment variant	Concentration (%)	Germination rate (%)	Seedlings (%) < 50 mm
Control		**97.97**	**0**
V1	1	97.87	0
	2.5	99.33	2.01
	5	98.01	3.31
V2	1	96.73	0
	2.5	97.24	0
	5	98.01	2.65
V3	1	96.58	0
	2.5	97.97	2.70
	5	95.36	3.97

At the used concentrations the differences in germination rate between the tested treatments were insignificant and similar with those of the control (fig. 1).

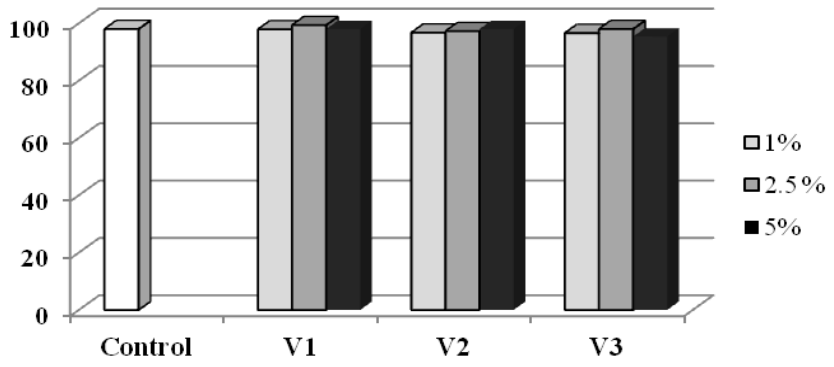

Fig. 1. Germination rate of wheat seeds on coriander fruit extracts treatment

Also, when the percentage of undeveloped seedlings (smaller than 50 mm) was analyzed, we concluded that of a total of nine treatment variants four caused normal growth while the plants treated with the remaining five variants were relative small.

The influence of the treatment with coriander fruit extracts on the growth of *Triticum aestivum* plants (fig. 2) was established by measuring the lengths of roots and shoots and also by determining the fresh and dry weight of seedlings (tab. III).

Fig. 2. *Triticum aestivum* L. seedlings at the end of the experiment

TABLE III

Effect of coriander extracts on root and shoot length and fresh and dry matter accumulation in *Triticum aestivum* L.

Treatment variant	Conc. (%)	Root length (mm)±SD	Shoot length (mm)±SD	Root weight (g)		Water content (%)	Shoot weight (g)		Water content (%)
				fresh	dry		fresh	dry	
Control		**137.37±19.24**	**115.46±17.78**	**6.55**	**0.85**	**87.02**	**9.54**	**1.40**	**85.33**
V1	1	165.39±35.13	117.33±18.06	6.10	0.90	85.25	9.44	1.43	84.85
	2.5	154.50±18.52	121.50±16.91	7.68	0.88	88.54	9.95	1.44	85.53
	5	146.41±20.36	120.54±13.73	6.36	0.86	86.48	9.67	1.43	85.21
V2	1	168.74±28.32	126.69±22.56	7.43	0.92	87.62	10.62	1.58	85.12
	2.5	158.75±19.39	121.63±16.41	6.01	0.81	86.52	10.13	1.50	85.19
	5	146.88±17.87	115.53±20.06	6.07	0.83	86.33	9.20	1.33	85.54
V3	1	148.15±15.61	117.48±23.48	6.80	0.89	86.91	9.92	1.44	85.48
	2.5	154.98±21.56	124.75±14.34	6.76	0.83	87.72	9.80	1.43	85.41
	5	140.57±19.56	118.75±21.38	6.66	0.82	87.69	9.55	1.42	85.13

As shown in the table, coriander extracts had a slight stimulating effect upon root and shoot elongation of wheat plants; at a concentration of 5%, V3 determined the lowest increase (2.33 %) in root length over the control, while V2 1% exhibited the highest increase (20 %). Likewise, shoot elongation was stimulated, the lowest value being calculated for V1 1% and the highest for V2 1% (9.73 %).

The weight of fresh and dry roots and shoots are important parameters in the assessment of coriander extracts phytotoxicity, as the water content of these vegetative organs can be the result of increased/decreased tissue turgescence.

Four treatment variants (V2 2.5%, V2 5%, V3 2.5% and V3 5%) of the nine investigated variants had a detrimental effect on dry weight accumulation in roots (with a 2.35-4.71% decrease over the control) induced by the inhibitory action of the extracts upon the meristems growth and an increase in its water content. Contrary, the remaining 5 treatment variants (V1 1%, V1 5%, V2 2.5%, V2 5% and V3 1%) enhanced root growth, as revealed by the increase in dry weight and low water content, in comparison with the control.

Coriander extracts exhibited a stimulating effect on dry weight accumulation in shoots, their turgescence being close to that of the control.

It remains to be seen if the increase in tissue weight is induced through stimulation of plant growth or by a higher amount of bioactive compounds synthesized by the test plants (tab. IV).

TABLE IV

Frequency of aberrant anaphases and telophases in *Triticum aestivum* L. on coriander extracts treatment

Treatment variant	Concentration (%)	Number of analyzed anaphases and telophases	Aberrant anaphases and telophases	
			Number	%
Control		**894**	**33**	**3.35 ± 0.01**
V1	1	1231	56	4.53 ± 0.02
	2.5	1196	36	3.01 ± 0.01
	5	1056	36	3.41 ± 0.01
V2	1	1470	45	3.06 ± 0.01
	2.5	829	32	3.86 ± 0.02
	5	1121	47	4.19 ± 0.03
V3	1	820	29	3.54 ± 0.01
	2.5	945	48	5.07 ± 0.01
	5	780	36	4.61 ± 0.02

Cytogenetic analysis showed the presence of chromosomal aberrations with a frequency of 3.35 % in the control, considered to be normal with *in vivo* phytotoxicity testing. Consequently, a certain genotoxicity induced by coriander extracts should be considered when the prevalence of chromosomal aberrations is higher than 6 %. The graphic representation of aberrant anaphase and telophase frequency in the analyzed samples (fig. 3) revealed the lack of mutagenic effect of coriander fruit extracts.

The highest percent of aberrant chromosomes (5.07 %) was observed for V3 2.5 % treatment; this value is below the accepted frequency of 6 %.

Using anaphase analysis, we established anaphase and telophase bridges, as well as chromosome fragments (fig. 4).

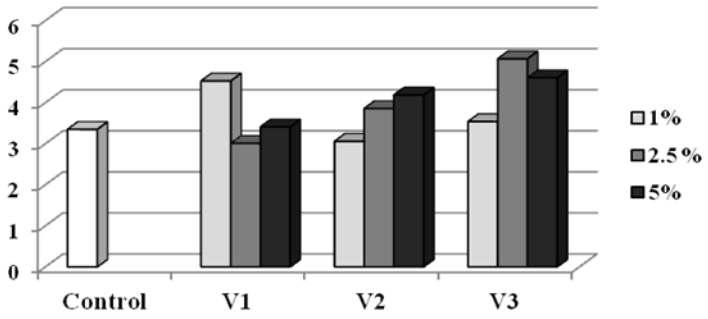

Fig. 3. Frequency of coriander extracts-induced chromosomal aberrations in *Triticum aestivum* root meristem

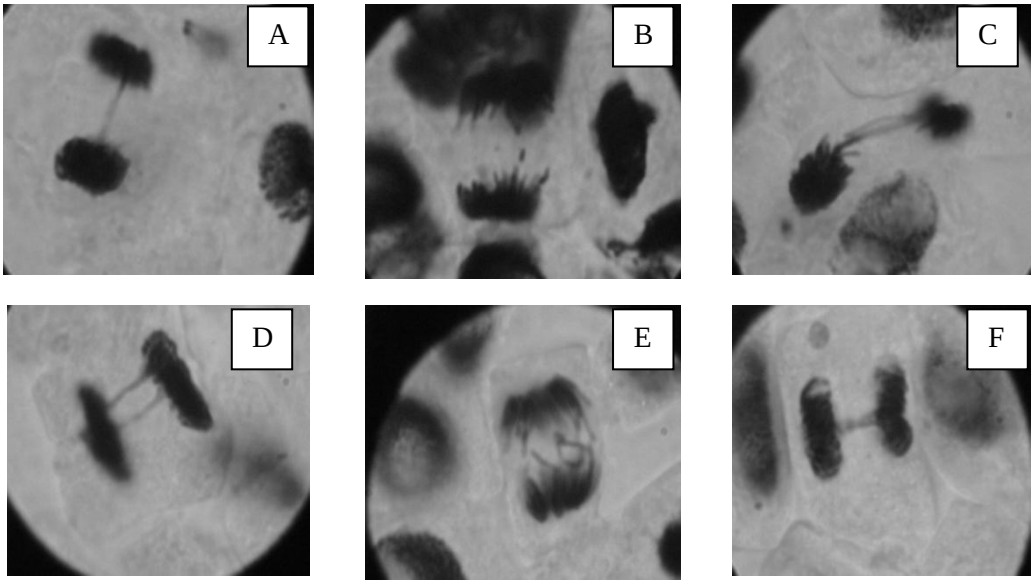

Fig. 4. Photomicrographs of coriander extracts effects on mitotic activity in wheat root meristems cells (A- ana-telophase bridge; B- anaphase with chromosome fragments; C- anaphase bridge; D- telophase with multiple bridges, E- anaphase with a vagrant chromosome; F-telophase bridges and micronuclei).

CONCLUSIONS

In summary, we tested the phytotoxicity of coriander fruit extracts, Omagiu cultivar. The present study showed, as we expected, no significant influence upon the germination and growth of *Triticum aestivum* plants. Likewise, the investigated extracts and their constituents did not inhibit root and shoot elongation of wheat plants (a 20 % increase in root length over the control being recorded for V2 1%).

Frequency of aberrant anaphases and telophases in *Triticum aestivum* L. varied slightly around the percentage of the con-

trol; the highest percentage of aberrant chromosomes (5.07 %) was observed for V3 2.5 % treatment.

The general conclusion is that the investigated coriander fruit extracts do not possess phytotoxic effects and have a slight stimulating effect on *Triticum aestivum* L. germination and growth.

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UTILIZAREA TEHNOLOGIILOR DE TIP CAD/CAM ŞI DE FABRICARE RAPIDĂ ÎN PRODUCEREA PROTEZELOR ŞI ORTEZELOR

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THE USE OF CAD/CAM AND RAPID FABRICATION TECHNOLOGIES IN PROSTHESIS AND ORTHOTICS MANUFACTURING (Abstract): Modern CAD/CAM and rapid fabrication technologies used in the prosthesis and orthotics manufacturing are based on 3D scanning, 3D reconstruction and computer aided fabrication. **Aim:** Paper approaches a study on the manufacture of customised orthosis (foot insole) through CAD/CAM and rapid fabrication technologies, using most economical devices and software available in Romania. **Material and methods**: Paper describes a method used in the data acquisition, reconstruction and fabrication of a foot insole made of acrylonitrile butadiene styrene (ABS). Data acquisition is accomplished using non contact 3D scanner with structured light. Structured-light 3D scanner is a device for scanning the three dimensional shape of an anatomical surface using projected light patterns and a camera system. All working steps from 3D scanning to computer aided fabrication of the foot insole through rapid ptototyping are described. Peculiar attention has been given to the image processing steps from 3D reconstruction to the final 3D CAD modelling of the foot insole. **Results**: The approached method showed a good accuracy in scanning of anatomic surfaces and in speeding up fabrication of leg orthosis. The 3D printer was able to build durable ABS thermoplastic insoles without post treatment. **Conclusions**: Approached fabrication method showed the advantages of manufacturing of orthosis and prosthesis components through CAD/CAM and Rapid Fabrication technologies. New prosthetic laboratories may be equipped with Rapid Fabrication technologies like 3D scanners and 3D printers. **Key words**: PROSTHESIS, FOOT INSOLE, CAD/CAM, RAPID MANUFACTURING

Dezvoltarea continuă a pieţei dispozitivelor de protezare şi de ortezare a determinat înfiinţarea unor societăţi naţionale de profil, care împreună cu Ministerul Sănătăţii, Casa Naţională de Asigurări de Sănătate şi medicii specialişti au dezvoltat programe naţionale de sănătate cu scopul de a susţine, cel puţin parţial, costurile procedurilor de protezare.

Casa Naţională de Asigurări de Sănătate a încheiat anual contracte cu furnizorii de dispozitive medicale, numărul acestora fiind de exemplu, de 2.922 în anul 2009, în creştere faţă de anii anteriori. În anul 2009, Casa Naţională de Asigurări de Sănătate a făcut plăţi în valoare de 95.218 mii lei pentru dispozitive şi echipamente medicale (1).

În acest context apare necesitatea studierii şi implementării unor metode moderne şi eficiente de fabricare bazate pe tehnologiile de tip CAD/CAM şi de fabricare rapidă care să vină în întâmpinarea atât a cere-

rilor de dispozitive de protezare, ortezare sau de tehnologie asistivă cât şi a cererilor de reparaţii sau de fabricare a pieselor de schimb.

Tehnologiile de tip CAD/CAM se referă la sisteme de scanare şi măsurare a suprafeţelor anatomice sau a obiectelor şi la maşini unelte de prelucrat orice tip de material, dotate cu echipamente numerice şi cu comandă prin calculator. Prin tehnologiile de tip CAD/CAM se pot fabrica în serie mică sau în serie mare, componente din orice material, dar cu o pregătire a fabricaţiei mai lentă şi un preţ de cost mai ridicat.

Tehnologiile de fabricare rapidă (sau de prototipare rapidă) se referă la maşini de prelucrat (de regulă pentru materiale plastice) numite în unele cazuri şi imprimante 3D comandate printr-un calculator. Tehnologiile de fabricare rapidă pot fabrica rapid diverse componente personalizate (unicate) mai ales din materiale plastice, la un preţ scăzut.

În această lucrare s-a efectuat un studiu în vederea realizării unei orteze de picior personalizate cu aplicarea tehnologiilor de tip CAD/CAM şi de fabricare rapidă, de preţ moderat, disponibile în România. Prin modelul de orteză (talonetă) personalizată studiată în această lucrare se permite parcurgerea detaliată a tuturor etapelor de lucru, de la scanarea pacientului cu un scaner 3D până la producerea fizică a ortezei. Tehnologiile de tip CAD/CAM şi de fabricare rapidă acoperă fabricarea unei game foarte largi de componente de proteze şi orteze, de dispozitive şi aparate de tehnologie asistivă, de componente de echipamente de fitness, aparate tehnico-medicale, etc.

MATERIAL ŞI METODE

Metodele moderne de fabricare a ortezelor şi a protezelor se bazează pe utilizarea intensivă a tehnologiilor de tip CAD/CAM şi de prototipare rapidă. Dintre tehnologiile moderne de tip CAD/CAM se remarcă: scanarea 3D, reconstrucţia 3D şi fabricarea componentelor protezelor şi ale ortezelor cu mijloace de tip CAD/CAM sau cu imprimante 3D.

Pentru protezele personalizate se întâlnesc în general, următoarele metode principale de fabricare (2,3), considerând cazul protezării unui membru:

- Fabricarea manuală, cu fazele de lucru prezentate în fig.1;
- Fabricarea cu mijloace CAD/CAM, cu fazele de lucru prezentate în fig.2;
- Fabricarea cu mijloace de fabricare rapidă (sau prototipare rapidă), cu fazele de lucru prezentate în fig.3.

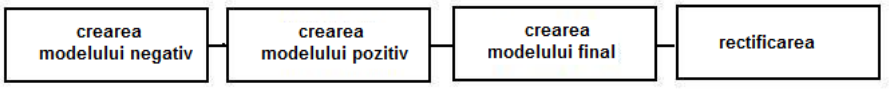

Fig. 1. Fabricarea manuală

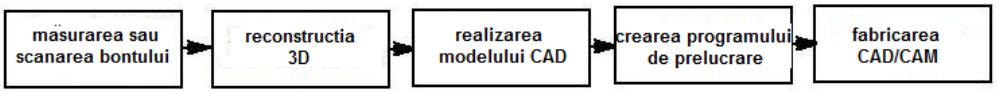

Fig. 2. Fabricarea cu mijloace de tip CAD/CAM

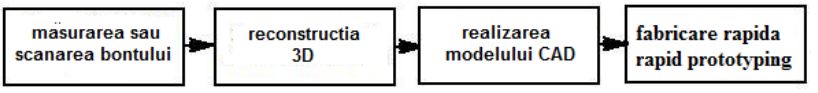

Fig. 3. Fabricarea cu mijloace de fabricare rapidă (sau prototipare rapidă)

Modelul de orteză personalizată studiată în această lucrare permite parcurgerea tuturor etapelor de lucru, de la scanare la prelucrarea imaginilor până la producerea fizică a ortezei. Orteza poate fi fabricată atât cu tehnologii de tip CAD/CAM (fig.2) cât şi cu tehnologii de fabricare rapidă (fig.3). Studiul de faţă a abordat fabricarea cu tehnologii de fabricare rapidă întrucât implică cheltuieli mai reduse.

Principalele etape de lucru parcurse sunt următoarele:

1. Scanarea cu un dispozitiv cu lumină structurată a piciorului unui pacient;
2. Reconstrucţia 3D a piciorului scanat;
3. Prelucrarea imaginii şi obţinerea modelului CAD sau a desenului 3D al unei orteze de picior;
4. Fabricarea ortezei prin introducerea desenului 3D într-o maşină de prelucrare de tip imprimantă 3D.

Orteza abordată în acest studiu face parte din categoria ortezelor corectoare de statică a piciorului, denumite şi susţinători plantari (talonete) recomandate în talalgii, osteofitoza calcaneană, în prevenirea, corectarea şi înlăturarea presiunii în cazul piciorului diabetic ş.a.

Studiul abordează tehnologiile de fabricare rapidă disponibile în România, utilizabile la fabricarea ortezelor şi a componentelor de proteze, din materii prime de tipul maselor plastice.

REZULTATE

1 Scanarea piciorului

Una dintre operaţiunile de obţinere a datelor anatomice personalizate este scanarea. Scanarea permite captarea unor date despre geometria suprafeţelor anatomice, din care se pot obţine, prin prelucrări de imagine, reconstrucţii 3D ale suprafeţelor cercetate.

O posibilă clasificare a metodelor de scanare aplicabile în protezare este următoarea (3, 4):

1. Scanarea cu raze X (computerul tomograf);
2. Scanarea prin rezonanţă magnetică nucleară (RMN);
3. Scanarea cu dispozitive cu laser;
4. Scanarea cu dispozitive cu lumină structurată (distribuită sub formă de linii, puncte).

Fig. 4. Scaner 3D tip Artec

Scopul tomografiei computerizate constă în a produce imagini 2D ale secţiunilor transversale prin corpului unui pacient. Dezavantajele constau în următoarele: pacienţii sunt expuşi unor doze mai mari de radiaţie decât în cazul radiografiilor clasice, echipamentele sunt costisitoare, procedurile sunt mai lente şi mai laborioase decât în cazul radiografiei tradiţionale.

Scanarea prin rezonanţă magnetică (RMN) are unele limite reprezentate de eventuale contraindicaţii (pacemakere, corpuri metalice straine, clipuri anevrismale), claustrofobie (pacientul trebuie sedat), vizualizare slabă a calcificărilor.

Scanarea cu dispozitive cu laser sau cu lumină structurată elimină dezavantajele menţionate în cazurile anterioare. Principalul avantaj constă în eliminarea expunerii la razele X. Totuşi costul unui dispozitiv de scanare cu tot cu programul de reconstruc-

ție 3D este relativ ridicat, fiind cuprins între 10.000 şi 30.000 Euro, în funcție de firma producătoare.

Scanarea piciorului s-a realizat cu un dispozitiv cu lumină structurată care are ca principal avantaj neutilizarea razelor X. S-a folosit în acest scop un scaner 3D portabil tip Artec MH (fig. 4) oferit pentru aplicații de firma Zspotmedia SRL (5). Scanerul are distanța de lucru 0,4 – 1 m şi viteza de achiziție de date 500.000 puncte/sec. Scanarea se execută luând imagini prin deplasarea manuală a scanerului în jurul piciorului (fig. 5).

Fig. 5. Scanarea piciorului

2 Reconstrucția grafică 3D a piciorului

Reconstrucția 3D se referă la obținerea modelului 3D al piciorului pacientului în formatul STL, specific maşinilor de prelucrat prin depunere de material şi imprimantelor de tip 3D. Prelucrarea se poate face de regulă cu un program de tip CAD (AutoCAD, SolidWorks, Catia etc.) sau cu programul de grafică 3D livrat împreună cu scanerul 3D (softul Artec).

Reconstrucția se face mai uşor cu programul ataşat scanerului şi constă în asamblarea imaginilor obținute la scanare, în aşa fel încât să se obțină modelul 3D al piciorului de ortezat (fig.6). Este etapa care consumă cel mai mult timp întrucât operațiunile sunt meticuloase şi cer potrivirea corectă a suprafețelor scanate din unghiuri diferite.

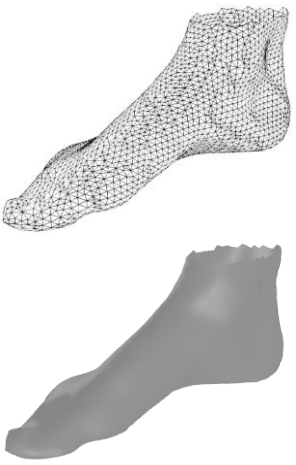

Fig. 6. Reconstrucția grafică 3D a piciorului (3D mesh în stânga şi cu randare în dreapta)

3 Prelucrarea modelelor şi generarea desenului 3D al ortezei personalizate

Prelucrarea modelelor 3D urmăreşte în acest studiu obținerea amprentei piciorului pe suprafața unui semifabricat din care se va confecționa taloneta. Prelucrările se fac cu un program de calculator şi constau în combinarea booleană (în cazul de față operația de scăderea) a modelelor 3D ale piciorului şi semifabricatului ortezei cu scopul obținerii modelului ortezei (fig 7).

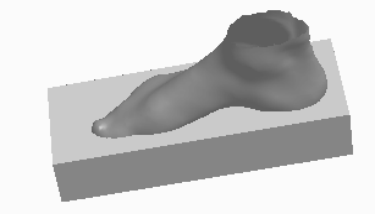

Fig. 7. Modelului piciorului şi modelul semifabricatului

Modelul 3D al semifabricatului are o formă paralelipipedică urmând ca după obținerea amprentei piciorului să fie finisat şi adus la forma finală. Cele două modele

3D se aduc în contact şi apoi din volumul paralelipipedic al semifabricatului ortezei se scade volumul piciorului rezultând orteza cu amprenta piciorului (fig. 8). Prelucrarea modelelor 3D se poate face cu programe de tip CAD care permit combinațiile booleene sau cu programe de grafică 3D (3D Studio Max, Blender, etc.) care permit combinații booleene de suprafețe 3D. În urma prelucrărilor de imagini rezultă modelul sau desenul 3D al ortezei.

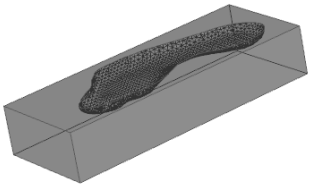

Fig. 8. Amprenta piciorului pe modelul semifabricatului (desenul 3D al ortezei)

După obținerea modelului 3D al semifabricatului ortezei, se ajustează forma ortezei încât să aibă conturul încălțămintei şi se teşesc sau se rotunjesc muchiile ascuțite prin câteva operațiuni de tip CAD. Modelul 3D al ortezei trebuie să fie în format STL.

4 Imprimarea 3D

Înainte de introducerea desenului 3D în imprimantă (5), se face o prelucrare pregătitoare care adaptează formatul desenului (STL) la formatul echipamentul numeric al imprimantei. Desenul 3D se introduce în programul AXON 2, care se vinde împreună cu imprimanta şi care converteşte fişierele STL, feliind modelul ortezei în secțiuni longitudinale (format G-Code), specifice imprimantei.

Desenul 3D al ortezei personalizate se introduce cu ajutorul unui stick de memorie în imprimantă. Imprimanta 3D utilizează ca materie primă, o fibră de material plastic (acrilonitril butadien stirenic ş.a.) cu grosimea de 3 mm, care se topeşte prin încălzire cu ajutorul unui cap termic si apoi se aplică strat cu strat, in funcție de coordonatele X, Y şi Z ale obiectului desenului.

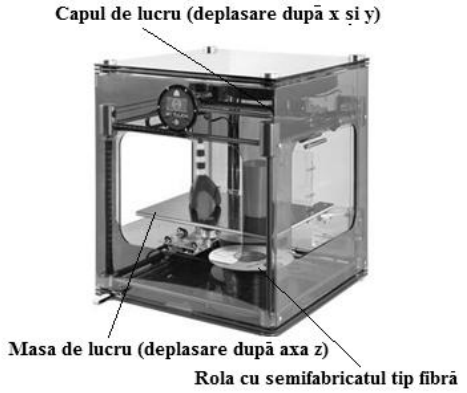

Fig. 9. Imprimantă 3D tip BfB 3D Touch

Imprimanta utilizată, 3DTouch 3DPrinter (fig. 9) este o imprimantă 3D preasamblată low-cost pentru următoarele aplicații: design, proiectare, medicină, arhitectură, inginerie, aplicații tip office sau hobby. Prețul de achiziție este cuprins între 1.500 şi 3.000 Euro. Gabaritul imprimantei este 515x515x590mm, greutatea 37 kg iar consumul de 90 w. Costul imprimării este de 0,06 euro/cm³ imprimat. Gabaritul de prelucrare este de maxim 275x275x210mm. Viteza de imprimare este de 15 mm³/secundă. Figura 10 prezintă forma finală a ortezei fabricate de imprimantă.

Operațiuni la pornirea imprimantei:

1. Conversia desenului 3D al ortezei din format STL, în formatul imprimantei;
2. Transferul modelului în formatul imprimantei, pe un card de memorie;
3. Introducerea cardului în imprimanta 3D şi transferarea datelor;
4. Alegerea unor parametri de lucru şi pornirea imprimantei.

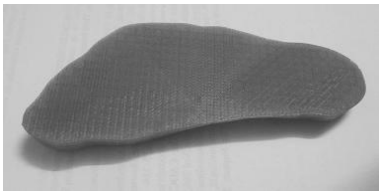

Fig.10 Forma finală a ortezei personalizate fabricată cu imprimanta 3D

DISCUȚII

Din parcurgerea etapelor de lucru se constată că personalul implicat în protezarea cu tehnologii de tip CAD/CAM sau de fabricare rapidă trebuie să aibă cunoştinţe temeinice de proiectare 2D şi 3D, să cunoască programele tip CAD, de reconstrucţie 3D, programele pretabile la operaţii booleene între modelele 3D şi cunoştinţe despre maşinile de prelucrat comandate prin calculator.

Tehnologiile de tip CAD/CAM sunt încă de scumpe la ora actuală şi în consecinţă s-au răspândit destul de puţin în domeniul protezării şi ortezării. Datorită acestui fapt, în Europa şi în SUA se manifestă următoarele tendinţe în dezvoltarea laboratoarelor de protezare cu tehnologii de tip CAD/CAM (6):

1. Dezvoltarea cu precădere a laboratoarelor de protezare bazate pe fabricarea manuală care sunt dotate de regulă cu maşini clasice de prelucrat;

2. Dezvoltarea unor laboratoare de protezare care apelează la calculatoare şi la programe de tip CAD şi uneori la scanere 3D; în aceste laboratoare se obţine forma 3D a suprafeţei anatomice de protezat sau de ortezat dar fabricarea se execută prin subcontractare, la altă organizaţie;

3. Dezvoltarea unor laboratoare de protezare ultramoderne, dotate cu tehnologii complete de tip CAD/CAM sau de fabricare rapidă care produc pe loc toate componentele de protezare;

4. Apariţia unor laboratoare regionale sau centre regionale dotate cu tehnologii complete de tip CAD/CAM sau de fabricare rapidă, care pot fabrica tot felul de componente şi la care apelează prin subcontractare laboratoarele de protezare din regiune, pentru fabricarea protezelor, ortezelor, a dispozitivelor de tehnologie asistivă etc.

În studiul prezentat, costul investiţiilor pentru achiziţionarea unui scaner (11.000 Euro) şi a unei imprimante 3D (3.000 Euro) cu programele aferente, care prelucrează materiale plastice este suportabil pentru un întreprinzător începător. Există şi scanere mai simple, cu performanţe mai slabe dar care se pot achiziţiona la preţuri de circa 300 Euro (7). De fiecare dată la achiziţia tehnicii trebuie analizat tipul componentelor care se pot fabrica şi materialul acestora. Există şi imprimante 3D care pot fabrica unele componente de proteze din oţeluri sau aliaje dar preţul acestora este mult mai ridicat iar materialul de lucru are de multe ori preţuri necompetitive.

CONCLUZII

Studiul arată fezabilitatea aplicării tehnologiilor moderne în producerea unor componente de proteze şi orteze. O mare parte din componentele unor proteze şi orteze se poate fabrica la preţuri competitive cu tehnologii de moderne de fabricare. Prin utilizarea tehnologiilor de tip CAD/CAM sau de fabricare rapidă scade timpul de obţinere a geometriei suprafeţelor anatomice, de proiectare, fabricare şi timpul de modificare a protezelor şi ortezelor. Protezele şi ortezele personalizate au ca principal efect îmbunătăţirea calităţilor ergonomice şi creşterea confortului pacientului. Protezele şi ortezele personalizate au efecte medicale (îndepărtarea handicapului şi

recuperarea funcţionala şi organică), sociale (reintegrare socio-profesională, menţinerea şi prelungirea vieţii active a individului) precum şi psihologice

Volumul investiţiilor necesare în dezvoltarea laboratoarelor de protezare cu tehnologii de fabricare rapidă, pentru fabricarea de componente din materiale plastice este suportabil pentru un întreprinzător începător. Piaţa internaţională a produselor de protezare şi ortezare este în continuă creştere, atât prin creşterea volumului de vânzări cât şi prin diversificarea acestora (8).

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NOUTĂŢI

ACTIVITATEA ANTIMICROBIANĂ A HIDROGELULUI POLICATIONIC

În scopul reducerii riscului infecţiilor asociate dispozitivelor medicale implantabile, Li P. şi colab. au dezvoltat un hidrogel pe bază de chitosan, cu proprietăţi antimicrobiene, eficace împotriva unor bacterii precum *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*. Majoritatea materialelor folosite actualmente nu corespund simultan cerinţelor legate de activitatea antimicrobiană şi biocompatibilitate. Activitatea antimicrobiană a hidrogelului policationic se datorează capacităţii de aspirare a unor porţiuni din membrana microbiană anionică în nanoporii interni ai hidrogelului, determinând disrupţia membranei microbiene şi moartea celulară. Studii animale au demonstrat biocompatibilitatea polimerului şi lipsa toxicităţii pentru celulele epiteliale. (Li P, Poon YF, Li W, Zhu HY, Yeap SH, Cao Y, et al. A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. *Nat Mate*r.2011;10(2):149-56).

Teodora Vremeră

BIOCOMPATIBILITATEA FAŢETELOR VESTIBULARE DIN RĂŞINI DIACRILICE COMPOZITE

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CLINICAL BIOCOMPATIBILITY OF DIACRILIC RESIN VENEERS (Abstract): The great variety of modern composite resins developed recently by manufacturers, offered to clinicians multiple choices of restoring esthetic of frontal teeth. **Material and methods**. The present study was performed over a period of 8 months. The patients were devided in two groups: A consisted of patients that received indirect veneers; and B consisted of patients that received direct veneers. **Results**. The clinical evaluation showed no major differences between group A and B, concerning the gingival recesion data and also concerning the plaque index. **Conclusions**. The data obtained from the study made us assess that direct and indirect composite materials have a clinically acceptable result in terms of biocompatibility when used for frontal veneers. **Key words**: RESIN VENEERS, BIOCOMPATIBILITY, GINGIVAL RECESSION.

Varietatea de materiale compozite moderne şi adezivi au oferit în ultimii ani facilităţi pentru îmbunătăţirea aspectului estetic al dinţilor frontali.

Metoda directă de faţetare are avantaje evidente de costuri şi timpi clinici dar şi dezavantaje legate de stabilitatea coloristică, rezistenţă şi biocompatibilitate.

Compozitele au fost iniţial gândite pentru a reface estetica şi funcţionalitatea dinţilor frontali, dar ulterior ramforsarea şi dezvoltarea unor materiale cu încărcătură mai rezistentă au extins aplicarea lor şi la zonele laterale.

Deşi nu sunt, în general, atât de rezistente ca şi restaurările ceramice sau integral ceramice, unele studii au dovedit că răşinile diacrilice compozite (RDC) sunt performante şi pentru zona laterală, cavităţile de clasă I şi II, după Black (cit.1).

Conform datelor din literatură (2, 3), comparaţia între rezistenţa mecanic a faţetelor directe din RDC, nu a dovedit rezultate mai slabe decât cea a echivalentelor, realizate în laborator.

MATERIAL ŞI METODE

Studiul a fost realizat în cabinetul de medicină dentară pe o perioadă de 8 luni şi a comparat biocompatibilitatea faţetelor vestibulare directe şi indirecte realizate din RDC. Lotul de 36 de pacienţi a fost împărţit în 2 grupuri: primul a inclus pe cei protezaţi după metoda indirectă din material compozit SRADORO, iar al doilea, pacienţii pentru care au fost realizate faţete după metoda directă cu răşină compozită Empress Direct. SRADORO® este compozitul folosit în acest studiu, având o umplutură de 65% şi un dimetacrilat alifatic –

aromatic UDMA (3). Fațetarea directă s-a realizat cu compozitul cu nanoumplutură IPS Empress ® Direct compus din nanomer dimetacrilat 20 – 21% (17% transparent). Umplutura a fost compusă din sticlă de bariu, florură de itterbium, dioxid de siliciu, şi copolimeri în masă de 77,5 – 79% nuanțe opalescente şi 83% culori normale. Masa totală de umplutură anorganică a fost de 79 - 59%, iar dimensiunea particulelor între 40 mm şi 3000 mm, cu o medie de 550 mm. Stratificarea s-a realizat în conformitate cu indicațiile producătorului. Examinarea clinică a fost realizată prin calcularea adâncimii şanțului gingival şi indicele de placă (IP). Toate valorile au fost clasificate şi supuse testului de analiză statistică „t Student".

REZULTATE

Fiecare grup a fost compus din câte 18 pacienți. Grupul A, la care am examinat sănătatea gingivală a 18 fațete vestibulare realizate din SRADORO, a inclus: 10 bărbați şi 8 femei cu vârsta medie 38,7 ani; iar în B au fost incluşi 7 bărbați şi 11 femei, cu vârsta medie de 40,5 ani. S-a examinat ataşamentul gingival vestibular la 18 fațete realizate folosind nanocompozitul Empress Direct.

În cazul tuturor pacienților incluşi în studiu, la marginea preparației s-a realizat un chanfrein uşor, juxtagingival şi supragingival, iar etalarea şanțului gingival s-a obținut preoperator cu ajutorul unui fir 0,01 Ultrapak, astringetul folosit fiind clorura de aluminiu. Toți dinții luați în studiu au fost vitali, iar relațiile de ocluzie diferite nu au constituit un obiect al acestui studiu. Valorile medii ale recesiunii gingivale în grupul A la 1, 3 şi 6 luni, postoperator au fost 1,15 (± 0,95), 0,73 (±0,68) şi 0,6 (±0,48). Comparația în cadrul celor 3 examinări la grupul A postoperator a fost semnificativă statistic: astfel s-a înregistrat o scădere a valorii p 0,001 şi 0,000, respectiv la 1 şi 3 luni. Schimbarea lui p între 3 şi 6 luni nu a prezentat o valoare semnificativă statistic ($p > 0,05$) (tab. I).

TABELUL I

Unele date de comparație între recesiunea gingivală verticală (în mm) la grupul A

Grupul A (Perechea)	Timpul de comparație	Valoarea medie	Deviația standard	t	p
1	VR 00 VR 01	4,3 1,15	1,174 0,947	12,13	0,000
2	VR 00 VR 03	4,3 0,72	1,174 0,6781	14,04	0,000
3	VR 00 VR 06	4,3 0,6	1,174 0,475	16,67	0,000
4	VR 01 VR 03	1,350 0,752	0,947 0,678	3,86	0,001
5	VR 01 VR 06	1,350 0,600	0,947 0,4757	4,56	0,000
6	VR 03 VR 06	0,725 0,600	0,6781 0,4757	1,097	0,287

Pentru pacienții din grup B, recesul gingival la 1, 3 şi 6 luni a prezentat o valoare medie de 0, 85 (±0,56), 1,25 (±0,79) şi 1,15(±0,61), având o semnificație statistică de p 0,001 şi 0,007 la 1 şi 3 luni. Între 3 şi 6 luni rezultatele nu au avut diferențe semnificativ statistice (p > 0,005) (tab. II).

În cadrul aceluiaşi grup de pacienți comparația gradului de retracție gingivală verticală a evidențiat că la început şi după o lună, a rezultat o valoare semnificativă statistic (p ≤ 0,05). La 3 şi 6 luni postoperator, valorile s-au menținut semnificativ statistic (p < 0,05). La compararea între grupurile de pacienți, nu s-a obținut nici o valoare semnificativă statistic între 3 şi 6 luni; dar aceasta a fost prezentă între momentul 0 şi 3 luni (tab. III).

TABELUL II

Unele date de comparație între recesiunea gingivală verticală (în mm) la grupul B

Grupul B (Perechea)	Timpul de comparație	Valoarea medie	Deviația standard	t	p
1	VR 00	4,200	1,0052		
	VR 01	0,850	0,5643	1,6833	0,000
2	VR 00	4,200	1,0052		
	VR 03	1,250	0,7864	13,968	0,000
3	VR 00	4,200	1,0052		
	VR 06	1,150	0,6091	16,212	0,000
4	VR 01	0,850	0,5643		
	VR 03	1,250	0,7864	-4,000	0,001
5	VR 01	0,850	0,5643		
	VR 06	1,150	0,6091	-3,040	0,007
6	VR 03	1,250	0,7864		
	VR 06	1,150	0,6091	1,285	0,214

TABELUL III

Evaluări privind comparația retracției gingivale (în mm) pentru lotul de pacienți incluşi în studiu

Timp (luni)	Media		Deviația standard		t'	p
	Grupul A	Grupul B	Grupul A	Grupul B		
1	1,150	0,850	0,9473	0,5643	2,028	0,050
3	0,725	1,250	0,6781	0,7864	-2,261	0,030
6	0,600	1,150	0,4757	0,6091	-3,182	0,003

Indicele de placă, urmărit în evoluție, a prezentat valori diferite în relație cu grupul şi perioada evaluată.

Creşterile de la momentul 0 la 1, 3 şi 6 luni au fost aceleaşi la ambele grupuri, iar diferențele între cele 3 momente nu au prezentat o valoare semnificativă statistic în ambele grupuri. Aceleaşi rezultate nesemnificative statistic au fost obținute şi la compararea între grupul A şi B (p>0,05) (tab. IV, V).

TABELUL IV

Elementele de comparaţie pentru indicele de placă în grupul A

Grupul A (Perechea)	Timpul de comparaţie	Valoarea medie	Deviaţia standard	t	p
1	PI 00	1,420	0,3636		
	PI 01	0,890	0,3978	3,818	0,001
2	PI 00	1,420	0,3636		
	PI 03	0,790	0,2594	7,471	0,000
3	PI 00	1,420	0,3636		
	PI 06	0,700	0,3340	8,786	0,000

TABELUL V

Elementele de comparaţie pentru indicele de placă în grupul B

Grupul B (Perechea)	Timpul de comparaţie	Valoarea medie	Deviaţia standard	t	p
1	PI 00	1,540	0,3235		
	PI 01	1,050	0,3441	7,074	0,001
2	PI 00	1,540	0,3235		
	PI 03	0,875	0,4089	6,808	0,000
3	PI 00	1,540	0,3235		
	PI 06	0,790	0,4844	7,194	0,000

S-au înregistrat diferente ale scorului indicelui de placă în ambele grupuri, comparativ cu momentul preoperator şi la 1, 3 şi 6 luni postoperator (tab. VI).

TABELUL VI

Elementele de comparaţie ale indicelui de placă între grupurile A şi B

Timp (luni)	Media		Deviaţia standard		t'	p
	Grupul A	Grupul B	Grupul A	Grupul B		
0	1,420	1,540	0,3676	0,3235	-1,103	0,277
1	0,890	1,050	0,3878	0,3441	-1,380	0,176
3	0,790	0,875	0,2594	0,4089	-0,785	0,437
6	0,700	0,790	0,3340	0,4844	-0,684	0,498

DISCUŢII

Sistemele adezive moderne şi răşinile nanocompozite oferă clinicianului mijloace rapide pentru a reda pacientului un zâmbet estetic, iar eventualele fracturi ulterioare datorate mai slabei rezistente mecanice, pot fi reparate uşor, comparativ cu feţetele ceramice.

Principalul dezavantaj al feţetării directe este însă contracţia de polimerizare a materialului folosit la restaurare, care duce în final la carii secundare şi acumulare de placă bacteriană în spaţiile rezultate.

Feţetele realizate după metoda indirectă din RDC sunt mai fiabile decât cele directe (4, 5). Principalele avantaje constau în gradul lor superior de finisare şi structura mai compactă rezultată din polimerizarea baro, termo, ultravioletă sau combinată, care creşte gradul de conversie rezultând o

structură mai rezistentă. Este cunoscut faptul că gradul de conversie monomer – polimer creşte atunci când se folosesc monomeri multifuncţionali, oferind mai multe poziţii reactive care vor creşte lungimea lanţurilor de polimer.

Un mijloc suplimentar de creştere a rezistenţei poate fi obţinut prin ramforsarea cu sticlă sau fibre de polietilenă (6, 7). Determinarea lungimii şanţului gingival a fost realizată cu sonda parodontală gradată, examinare realizată la intervalele date de timp, de către acelaşi examinator, iar faptul că au fost solicitări pentru reexaminări a stimulat pacienţii în a-şi întreţine o igienă corespunzătoare pe toată perioada studiului, fenomen observat şi de către alţi autori (8). Pacienţii erau în proporţie de 100% fumători şi consumatori de cafea, iar condiţia de includere în studiu a fost un maxim 8 la indicele de placă, deci prezentau o igienă satisfăcătoare şi înainte de tratament.

Studiul a demonstrat că ambele metode, directă şi indirectă, de faţetare au prezentat rezultate acceptabile clinic, şi comparabile cu datele din literatură, astfel că biocompatibilitatea a fost demonstrată clinic în cazul materialelor compozite utilizate (9).

Studiul s-a desfăşurat însă pe o perioadă scurtă, "îmbătrânirea" cunoscută a compozitelor, cauza frecventă a problemelor parodontale create de acestea, neavând încă loc, aspect relevat şi de literatura de specialitate a ultimilor ani (10).

CONCLUZII

Fiecare restaurare e unică în termeni specifici materialului din care este realizată, cum ar fi: rezistenţă mecanică, la uzură, toleranţa la umiditate, stabilitate dimensională şi coloristică. La aceştia se adaugă factorii ce ţin de procedeul tehnic de realizare, volumul restaurării, adâncimea preparaţiei, uşurinţa accesului în zonele de restaurat.

Datele clinice acumulate cu ajutorul studiului au permis a se considera că restaurarea directă corect realizată în condiţii asemănătoare, este practic la fel de corespunzătoare clinic şi estetic ca şi cea realizată indirect în laborator din RDC la momentul zero al aplicării ei în cavitatea bucală. Eventualele diferenţe de finisare şi estetică se pot explica după o perioadă de timp print-o placofilie mai accentuată sau o pigmentare marginală.

Diferenţa notabilă între faţetele directe şi cele indirecte, constă în preţul de cost şi probabil în rezistenţa în timp, coloristică sau probleme de mecanică.

Principalul factor de luat în considerare, este gradul de igienă dentară care a fost satisfăcător în cazul tuturor pacienţilor examinaţi, iar plasarea subgingivală a marginilor preparaţiei afectează sănătatea gingivală, dar faţetele, prin grosimea lor redusă, sunt de preferat coroanelor integrale.

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NOUTĂȚI

LIPOZOMII- NANOCARRIERS PENTRU ANTITUBERCULOASE

Tuberculoza (TB) este o boală infecțioasă care rămâne departe de a fi eradicată şi controlată. Tratamentul TB este grefat de o complianță scăzută fiind un tratament pe termen lung, cu o combinație de antituberculoase care generează apariția de multiple reacții adverse. Complianța scăzută favorizează apariția de tulpini rezistente şi creşte costurile terapiei. În condițiile în care nu au fost descoperite noi antibiotice antituberculoase devine prioritară dezvoltarea de modalități terapeutice care să crească complianța pacienților la terapiile curente.Un grup de cercetători din Portugalia au demonstrat avantajele utilizării lipozomilor ca sisteme nanocarriers pentru antituberculoase de prima şi a doua linie. Ei au arătat că lipozomii sunt structuri veziculoase auto-închise compuse din fosfolipide care pot încorpora medicamente hidrofile, hidrofobe sau amfifile şi care sunt recunoscute specific de către macrofage. Suprafața lipozomilor poate fi modificată prin adăugarea de liganzi (anticorpi şi alte molecule), care cresc specific capacitatea de a recunoaşte şi a se leaga de celulele-țintă. Consecința creşterii specificității lipozomilor pentru o celulă țintă determină acumularea lor preferențial în țesuturi bolnave şi realizarea unor concentrații mai mici în țesuturile sănătoase care vor fi astfel salvate de un eventual efect toxic al antibioticului livrat de aceste nanoparticule. Astfel, lipozomii reduc toxicitatea drogurilor încapsulate, cresc biodisponibilitatea antibioticelor determinând creşterea eficienței tratamentului şi reducerea duratei acestuia. În plus, lipozomii au avantajul de a fi biocompatibili, biodegradabili şi non-imunogeni deoarece pentru obținerea lor se pot utiliza compuşi endogeni (cum ar fi elementele constitutive de surfactant pulmonar). Compoziția lipozomilor poate fi, de asemenea, reglată pentru a selecta cea mai favorabilă cale de administrare. De exemplu, în caz de TB pulmonară, administrarea inhalatorie în loc de calea intravenoasă ar creşte complianța pacienților la terapie, diminuând abandonul şi riscul apariția rezistenței. Administrarea inhalatorie de lipozomi încărcați cu medicamente anti-TB are avantajul de a realiza concentrații locale ridicate de antituberculoase în macrofagele alveolare, de a reduce efectele adverse sistemice, precum şi de a reduce frecvența administrărilor. Aceste proprietăți permit utilizarea lipozomilor în tratamantul TB ca o alternativă a tratamentului curent (Pinheiro M., Lúcio M., José LFC Lima. Liposomes as Drug Delivery Systems for the Treatment of TB. *Nanomedicine*. 2011:1413-1428).

Cătălina Luncă

INCLUZIA PALATINALĂ A CANINULUI MAXILAR – PREZENTARE DE CAZ

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2. Disciplina Chirurgie maxilo-facială

PALATALLY IMPACTED CANINES – CASE REPORT (Abstract). Maxillary canines have the longest period of development, the deepest area of formation and the most difficult path of all teeth. The maxillary canines are the most likely to remain unerupted or impacted. The maxillary permanent canine is considered important by virtue of its place in the scheme of functional occlusion and its contribution to facial appearance. We successfully orthodontically treated two cases with palatally impacted canines. Although both cases had palatally impacted canines, they were different due to canine position and angulation. **Key words**: PALATALLY IMPACTED CANINES, ORTHODONTIC TREATMENT, CANINE ANGULATION

Incluzia dentară este reţinerea intraosoasă sau submucoasă a unui dinte complet format, după perioada normală de erupţie, sacul pericoronar nefiind deschis. Incluzia de canin superior este întâlnită în populaţie cu o frecvenţă de 0,9-2,2% (1). Dachi şi Howell (2) au arătat că această condiţie este de 2,3 ori mai frecventă la sexul feminin comparativ cu sexul masculin. În 85% din cazuri caninul maxilar este inclus palatinal, iar la 8% din subiecţi incluzia este bilaterală. În ceea ce priveşte etiologia incluziei palatinale a caninului maxilar au fost emise două teorii: teoria „absenţei ghidajului dentar" şi teoria „factorilor genetici". Teoria „ absenţei ghidajului dentar" se referă la existenţa unui exces de spaţiu în zona apicală maxilară, caninul pierzând astfel ghidajul reprezentat de rădăcinile dinţilor vecini. Teoria „genetică" susţine existenţa unor tulburări de dezvoltare ale lamei dentare, tulburări care vor determina poziţionarea anormală a mugurelui caninului superior (3,4). Unii autori citează înghesuirea drept cauză locală a incluziei caninului maxilar, dar această condiţie este mai frecventă în cazul caninilor incluşi vestibular (5).

PREZENTAREA CAZURILOR

CAZUL 1

Pacient I.S., în vârstă de 25 de ani, sex feminin, se prezintă cu persistenţa caninului temporar dreapta. La examenul intraoral se constată prezenţa caninului temporar drept şi absenţa caninului maxilar permanent în cadranul 1. La inspecţie şi palpare se remarcă o uşoară bombare a mucoasei, de consistenţă dură, retroincisiv lateral superior drept. Pacienta prezintă arcade den-

tare relativ corect aliniate şi ocluzie echilibrată clasa I Angle (fig. 1).

Fig.1. I.S., model maxilar pretratament ortodontic

Ortopantomografia arată prezența caninului maxilar inclus cu suprapunerea completă a coroanei peste imaginea rădăcinii incisivului lateral. Rădăcina incisivului lateral nu prezintă rizaliză. Angulația caninului este de 45º, ceea ce conferă cazului un prognostic rezervat (fig. 2).

Fig. 2. I.S., ortopantomografie pretratament

Obiectivele tratamentului chirurgical ortodontic au fost: 1) obținerea spațiului pe arcadă necesar alinierii caninului; 2) descoperirea chirurgicală a caninului inclus; 3) tracțiunea lentă a caninului spre ocluzal, apoi spre distal pentru a-l degaja de sub incisivul lateral; 4) tracțiune vestibulară şi aliniere pe arcadă. Tratamentul ortodontic a constat în aplicarea unui aparat fix *straight-wire* 0.025x0.028-inch şi a unui arc palatal modificat pentru tracționarea caninului (fig. 3).

Fig. 3. I.S., tracționarea caninului inclus

Durata totală a tratamentului a fost de 23 luni, modelele şi ortopantomografia arătând o încadrare corectă pe arcadă a caninului maxilar drept (fig. 4,5). Deşi prognosticul a fost rezervat datorită profunzimii incluziei şi angulației nefavorabile, tratamentul s-a desfăşurat cu prejudicii parodontale minore, înregistrându-se o recesiune gingivală de numai 0,75 mm pe fața palatinală a caninului.

Fig. 4. I.S., model de studiu post-tratament ortodontic

Fig. 5. I.S., ortopantomografia post-tratament ortodontic

CAZUL 2

Pacient M.C., 13 ani, sex feminin, se prezintă cu absența caninului temporar și permanent drept, linia interincisivă superioară deviată spre dreapta și micșorarea spațiului necesar dintelui 1.3. Ocluzia echilibrată, clasa I Angle. La examenul intraoral se constată o ușoară bombare la nivelul bolții palatine înapoia incisivului lateral drept (fig. 6).

Fig. 6. M.C., aspect pretratament ortodontic

Ortopantomografia arată prezența caninului superior drept inclus, înclinat la 45º, imaginea sa suprapunându-se cu cea a incisivului lateral (fig. 7).

Etapele tratamentului au fost: 1) aplicarea unui aparat fix *straight-wire* 0.025x 0.028-inch și a unui arc quad-helix modificat; 2) recâștigarea spațiului pe arcadă pentru caninul superior; 3) descoperirea chirurgicală a caninului și tracționarea lui la arcul quad-helix;, 4) tracțiunea spre vestibular a caninului și alinierea lui pe arcadă. Durata totală a tratamentului a fost de 25 de luni (fig. 8, 9).

Fig. 7. M.C., ortopantomografia pretratament

Fig. 8. M.C., aspect postortodontic

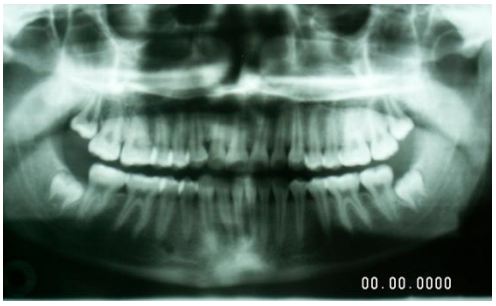

Fig. 9. M.C., ortopantomografie post-tratament ortodontic

DISCUȚII

Incluzia palatinală a caninului maxilar reprezintă o patologie comună în practica

ortodontică, dar care este descoperită târziu de către pacienți din cauza persistenței caninului temporar. În cazurile în care s-a pierdut totuşi caninul temporar şi în absența tratamentului ortodontic, se poate produce devierea liniei interincisive şi rotația premolarului 1 limitrof edentației, ceea ce duce la pierderea spațiului necesar caninului permanent inclus. Un canin inclus palatinal poate, în unele cazuri, să producă rizaliza rădăcinii incisivului lateral maxilar, şi chiar a incisivului central. În tratamentul incluziei de canin maxilar hotărâtoare este poziția, profunzimea, angulația şi raportul caninului cu dinții adiacenți. Un rol deosebit de important în stabilirea diagnosticului şi conduitei terapeutice îl are examenul radiologic care trebuie să includă obligatoriu ortopantomografia, iar în cazurile mai dificile tomografia computerizată cu reconstrucție 3D. În primul caz prezentat caninul inclus palatinal era din grupa a 3-a, conform clasificării lui Becker (6), ceea ce înseamnă un canin situat înalt în osul maxilar, paralel cu linia arcadei. În acest caz prognosticul a fost destul de rezervat, dar, la final, rezultatul obținut a fost mulțumitor din punct de vedere ortodontic şi parodontal. În cel de-al doilea caz a fost nevoie de recâştigarea spațiului pierdut prin extracția caninului temporar şi corectarea devierii liniei mediane, dar caninul inclus a fost situat mai aproape de linia arcadei în plan vertical şi transversal.

Tratamentul incluziei palatinale de canin este un tratament complex, interdisciplinar şi care trebuie să ia în calcul toate aspectele anatomo-clinice legate de dintele inclus şi de dinții limitrofi, durata probabilă a tratamentului şi raportul risc/beneficiu.

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EVALUĂRI ASUPRA PROGRAMELOR DE PREVENŢIE A SIFILISULUI CONGENITAL

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4. Disciplina de Dermatologie

ASSESSMENT OF THE CONGENITAL SYPHILIS PREVENTION PROGRAMS (Abstract): The Romanian program for the management and screening of syphilis includes the recording, follow-up, and antenatal care of pregnant women. It aims at testing all pregnant women for syphilis with the help of VDLR (Venereal Disease Research Laboratory) or RPR (Rapid Plasma Reagin) tests, and in the women with positive tests to confirm the results by treponemal tests (treponemal antibodies): THPA (*Treponema Pallidum* Hemagglutination), FTA-Abs (Fluorescent Treponemal Antibody with Absorption), ELISA-Captia-IgM, and Western Blotting-IgM. In the pregnant women with positive tests two doses of 2.4 million units of penicillin G benzathine were administered at 5 days interval. These pregnant women are in the evidence of a specialist (obstetrician, dermatologist), and District Department of Public Health, and required to come for another serology test in 3 months. In case they still test positive, the same treatment is applied at the beginning of the third trimester of pregnancy. **Aim:** To assess the outcome of congenital syphilis prevention programs in Iasi, Romania. **Material and methods:** In the interval 2005-2011, in the Iasi town, 84 RPR positive pregnant women were recorded. There was no significant difference in the number of pregnant women residing in urban as compared to rural areas. Most of these women were from poor social environments and had a low level of education. The diagnosis of acquired syphilis was made by serological tests as most pregnant women presented in the period of syphilis latency, being asymptomatic. All pregnant women followed the treatment, and were tested periodically. Ultrasound examination was normal in all women (no changes suggestive of fetal malformations). **Results:** Free clinical, laboratory, and ultrasound investigations, history taking, psychological assessment, sex education, rapid identification of contacts of known patients, follow-up of the interaction between health care providers and syphilitic pregnant women, booklets, and leaflets altogether made that in the last 3 years (2008-2010) no new case of congenital syphilis to be reported in the study area. **Conclusions:** Encouraging women to attend antenatal care early in their pregnancy is essential, this way all pregnancy-related problems (syphilis included) could be managed.
Key words: PREGNANCY, PRENATAL DIAGNOSIS, CONGENITAL SYPHILISY

Prevenirea sifilisului congenital nu este dificilă, însă există unele dificultăţi operaţionale de nerespectare a programelor de investigaţie şi a tratamentului prenatal.

Gestionarea cazurilor pozitive este dificil de pus în aplicare atunci când gravidele

nu se prezintă deloc, când prima lor vizită la clinica prenatală se face prea târziu, uneori chiar înainte de naştere. Unele dintre ele consideră că o singură vizită la medicul de familie în timpul sarcinii este suficientă şi nu acordă importanţă examenului ecografic, analizelor curente din timpul sarcinii sau aplicării tratamentului corespunzător în cazul celor care au avut rezultatul pozitiv la testul VDRL (Venereal Disease Research Laboratory) sau RPR (Rapid Plasma Reagin).

În acelaşi timp, clinicianul nu a avut un sistem de urmărire şi dispensarizare a gravidelor VDR/ RPR pozitive, care nu s-au întors după rezultatele lor şi nici posibilitatea de a le obliga să urmeze tratamentul atât pe femeia depistată cât şi pe partenerul ei sexual. O altă metodă de prevenire ar putea fi aplicarea sistemului de poliţie sanitară care exista în România înainte de 1989.

Acesta presupune încurajarea femeilor de a participa la îngrijire prenatală şi dispensarizare la medicul de specialitate încă de la începutul sarcinii, oferind testare serologică gratuită, examene clinice şi paraclinice periodice, astfel încât rezultatele să fie disponibile imediat şi gravidele sero-pozitive să poată fi tratate împreună cu partenerul sexual, dacă şi acesta este seropozitiv.

A doua testare, mai târziu, în timpul sarcinii la începutul trimestrului III, ajuta la gestionarea şi îmbunătăţirea calităţii asistenţei medicale faţă de sifilis în timpul sarcinii, naşterii precum şi în perioada neonatală, şi în acelaşi timp la prevenirea sifilisului congenital.

Diverse metode au fost urmărite pentru acest studiu dintre care: examinări clinice şi para clinice, examenul ecografic, anamneză, evaluare psihologică, interviuri informatoare şi observaţii ale interacţiunii dintre furnizorii de servicii medicale şi gravide cu boala sifilitică, colectarea de date din materiale publicate, rapoarte, ghiduri ale furnizorilor de servicii, manuale de instruire şi alte documente .

Elementele de bază au fost testarea VDRL/RPR, confirmarea prin TPHA gratuită la toate femeile gravide, furnizarea de informaţii despre îngrijirea necesară şi despre servicii medicale corespunzătoare, tratamentul femeilor gravide sero-reactive realizat de asistente medicale la clinica de specialitate obstetricală, prevenirea sifilisului congenital şi notificarea partenerului sexual şi convingerea lui de a fi testat serologic pentru diagnosticarea şi tratarea la clinica de dermatologie.

CAZ CLINIC

În anul 2006 gravida S.A. în vârstă de 15 ani, diagnosticată cu sarcină 7 săptămâni, sifilis latent, urmează tratament cu benzathin penicilină G. La câteva zile după tratament se internează la maternitate cu diagnosticul de Sarcină luna a II-a - avort incomplet. Se aplică tratamentul chirurgical (chiuretaj uterin evacuator şi hemostatic), tratament medicamentos (antibiotice) se practică examenul anatomo-patologic al produsului de chiuretaj în microscopie optică nu se identifică modificări celulare care să incrimineze agentul sifilisului *Treponema pallidum*.

Pacienta urmează tratament, efectuează invistigaţii periodice pentru sifilis, persistând pozitivitatea TPHA, iar soţul ei prezintă serologie negativă.

În anul 2010 pacienta este gravidă din nou, fiind dispensarizată de medici specialişti, făcând analize periodice şi examene ecografice (fig.1) din care rezultă vârsta sarcinii, evoluţie normală, În luna ianuarie

2011 aceasta dă naştere unui nou născut viu,de sex feminin, cu o greutate de 2950 de grame, apgar = 9, fiind identificat cu serologie sifilitică negativă.

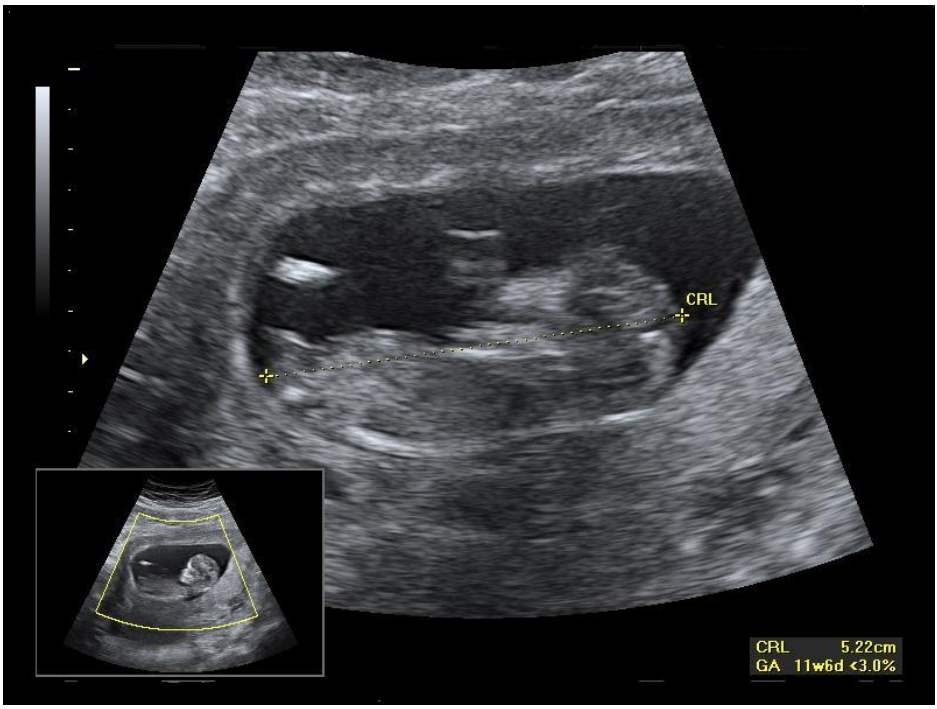

Fig. 1 .Examen ecografic din care rezultă vârsta sarcini cu evoluţie normală

DISCUŢII

Programul de management şi screening al sifilisului prenatal din România cuprinde înregistrarea, luarea în evidenţă, supravegherea gravidelor şi îngrijirea lor prenatală.

Programul îşi propune să testeze toate gravidele pentru sifilis folosind testele VDRL (Venereal Disease Research Laboratory) sau RPR (Rapid Plasma Reagin), iar pentru cele cu rezultate pozitive să realizeze confirmare prin teste treponemice (anticorpi antitreponemici): testele TPHA (**Treponema Pallidum Hemagglutination**), FTA–Abs (Fluorescent Treponemal Antibody with Absorption), ELISA-Captia-IgM, Western Blotting- IgM.

La prima lor vizită, pentru femeile care au prezentat rezultate pozitive s-a aplicat un tratament cu două doze a 2,4 milioane unităţi de benzathin penicilină G la interval de 5 zile. Gravidele au fost luate în evidenţă la medicul de specialitate (Obstetrician, Dermatolog) şi la DSP, urmând să revină la controlul serologic la 3 luni, unora dintre ele reaplicându-li-se aceeaşi schemă de tratament la începutul trimestrului III al sarcinii.

CONCLUZII

În perioada 2005-2011 în care au fost luate în evidentă în Iaşi 84 de gravide cu RPR pozitiv, nu a existat o diferenţă mare de procentaj între gravidele provenite din mediul rural şi cele provenite din mediul

urban. Majoritatea au fost din medii sociale precare şi cu un nivel scăzut al educaţiei.

În practica de rutină, diagnosticul sifilisului dobândit, inclusiv al gravidelor, se face cu ajutorul testelor serologice, deoarece majoritatea pacientelor au venit în perioada de latenţă a bolii şi sunt asimptomatice.

Toate gravide dispensarizate au urmat tratament şi au efectuat analize periodice. Examenul ecografic a fost normal la toate, fără modificări care să arate malformaţii fetale, iar în ultimi 3 ani (2008, 2009, 2010) nu s-a declarat nici un caz nou de sifilis congenital în judeţul Iaşi.

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NOUTĂȚI

DABIGATRAN ETEXILATE – UN NOU INHIBITOR ORAL DE TROMBINĂ

Anticoagularea orală de lungă durată este indicată pentru o serie de boli cardiovasculare: prevenţia tromboembolismului la pacienţii cu fibrilaţie atrială, purtătorii de proteze valvulare mecanice, infarctul miocardic acut, tratamentul şi profilaxia trombozei venoase profunde. În ultimii 60 de ani, antagoniştii de vitamină K (warfarină, acenocumarol, phenprocoumon, fluindione) au fost prescrişi pe scară largă. În tot acest timp, utilizarea anticoagulantelor orale a fost grevată de o serie de inconveniente: lungimea perioadei până la intrarea în acţiune, care duce la creşterea perioadei de spitalizare, şi, deci, a costurilor, fereastra terapeutică îngustă, interacţiunile frecvente cu alte clase de medicamente şi riscurile de sângerare. Dabigratan etexilate şi derivatul său activ dabigratane vor reprezenta o alternativă terapeutică eficientă la antivitaminele K. Dabigratan este un inhibitor direct de trombină şi are un timp de înjumătăţire de 14 – 17 ore, ceea ce permite administrarea sa în două prize pe zi (150 mg de 2 ori pe zi). Dabigatran s-a dovedit eficient şi sigur în profilaxia trombozei venoase profunde (sudiul RE-NOVATE, efectuat la pacienţi spitalizaţi pentru fractură totală de şold) şi în scăderea riscului de sroke la pacienţii cu fibrilaţie atrială (studiul RE-LY) (Hankey GJ, et al. Dabigatran etexilate – a new oral thrombin inhibitor. *Circulation* 2011; 123: 1436-50).

Corina Dima-Cozma

EPITELIOM CALCIFICAT MALHERBE – OBSERVAȚII PE MARGINEA A DOUĂ CAZURI CLINICE

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MALHERBE'S CALCIFYING EPITHELIOMA – COMMENT ON TWO CLINICAL CASES (Abstract): Malherbe's calcifying epithelioma (also called trichomatricoma, pilomatrixoma or pilomatricoma) is an uncommon benign cutaneous tumor, which takes the form of a solitary, firm, asymptomatic, slowly growing nodule, developed subcutaneously on the face, neck, or proximal upper extremity. With an incidence of 1/800-1000 cutaneous tumors, pilomatricoma is more common in the first two decades of life, predominantly affecting the women. Multiple locations and associations between this entity and other diseases such as Steinert's myotonic dystrophy or Gardner syndrome have been described in the literature. The clinical diagnosis is often difficult, most cases being mistaken as sebaceous cysts. The treatment of choice is surgical excision. Incomplete surgical excision can be followed by local recurrence because most lesions are poorly defined; therefore, patients should be monitored after surgical excision to prevent this from happening. We present two cases admitted in 2010 and 2011 to the Iasi Department of Oral and Maxillo-Facial Surgery: a 55-year-old female patient with a Malherbe's calcifying epithelioma located in the left temporal region, and a 51-year-old female patient with the same disease but located in the left masseter region. **Key words:** MALHERBE'S CALCIFYING EPITHELIOMA, PILOMATRICOMA, PILOMATRIXOMA

Pilomatrixomul (pilomatricomul sau epiteliomul calcificat Malherbe) este o tumoră benignă a matricii foliculului pilos. Incidența maximă a fost semnalată în primele două decade de viață dar şi între 50-65 ani, predominant la femei. Localizările cele mai frecvente ale pilomatrixomului sunt: capul, gâtul, fața şi membrele superioare. De obicei, se prezintă ca un nodul nedureros fiind adesea confundat preoperator cu un chist epidermoid (sebaceu). Prima descriere a acestei entități clinice a fost publicată în 1880 de către Malherbe şi Chenantais sub denumirea de epiteliom calcificat. Din 1961 se foloseşte termenul de pilomatrixom (propus de Forbis şi Helwig), acesta fiind considerat mai corect din punct de vedere etimologic. Prezentăm în continuare două cazuri clinice de pilomatrixom şi discutăm principalele aspecte ale acestor tumori cutanate.

PREZENTAREA CAZURILOR

CAZUL 1

În aprilie 2010 s-a prezentat în Clinica de Chirurgie Orală şi Maxilo-Facială Iaşi o pacientă în vârstă de 55 ani, pentru o forma-

țiune tumorală localizată în regiunea temporală stângă, cu evoluție clinică de 2 ani (fig. 1). Examenul clinic a consemnat în regiunea temporală stângă (adiacent regiunii sprâncenare stângi) o tumoră rotundă, de circa 2 cm diametru, situată în planul subcutanat, cu tegumentul acoperitor congestionat, aderent la formațiune şi prezentând central o zonă ulcerată acoperită de o crustă hematică, formațiunea fiind uşor sensibilă la palpare. Formațiunea a fost extirpată în totalitate odată cu insula cutanată suprajacentă (fig. 2), defectul rezultat fiind refăcut printr-o plastie cu lambou cutanat bilobat din vecinătate (fig. 3,4). Examenul histopatologic definitiv a stabilit diagnosticul de certitudine de pilomatrixom (fig. 5,6), confirmând excizia în țesut sănătos. Controlul clinic efectuat după 10 luni de la intervenția chirurgicală nu a indicat semne de recidivă.

Fig. 1. Aspect clinic preoperator

Fig. 2. Defectul rezultat după extirparea leziunii în țesut sănătos

Fig. 3. Lamboul cutanat bilobat din vecinătate folosit pentru plastia defectului

Fig. 4. Aspect clinic după o săptămână de la intervenția chirurgicală

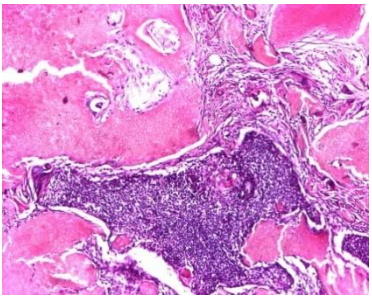

Fig. 5. Aspect microscopic de pilomatrixom (HE, obX4; proliferare bazaloidă, arii întinse de keratinizare cu umbre celulare – „celule fantomă")

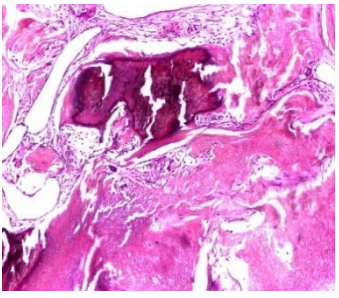

Fig. 6. Aspect microscopic de pilomatrixom (HE, obX4; arie de calcificare)

CAZUL 2

În februarie 2011 s-a internat în acelaşi serviciu o pacientă în vârstă de 51 ani prezentând o tumoră subcutanată la nivelul regiunii maseterine stângi, cu evoluție clinică de un an. Examenul clinic a relevat în jumătatea inferioară a regiunii maseterine stângi o tumoră ovoidală, de 4 cm/2 cm, dezvoltată subcutanat, de consistență fermă, nedureroasă, cu tegumentul suprajacent uşor congestionat şi aderent la formațiune (fig. 7). Diagnosticul clinic de probabilitate preoperator a fost de chist sebaceu. S-a efectuat extirparea formațiunii tumorale cu

sutură (fig. 8). Examenul histopatologic a stabilit diagnosticul de certitudine de pilomatrixom, cu evidențierea plajelor cu umbre celulare – „celule fantomă” (fig. 9).

Fig. 7. Aspect clinic preoperator

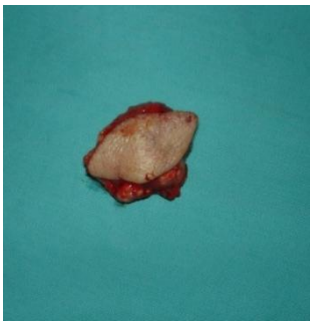

Fig. 8. Piesa operatorie (tumora şi insula cutanată suprajacentă, aderentă la tumoră)

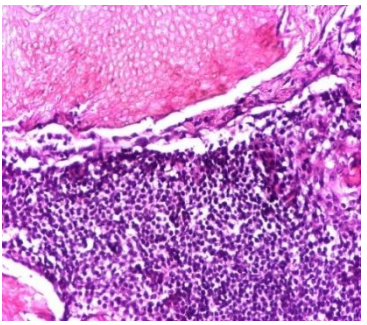

Fig. 9. Aspect microscopic de pilomatrixom (HE, obX10; proliferare bazaloidă – inferior şi plajă cu umbre celulare – superior)

DISCUȚII

Epiteliomul calcificat Malherbe (tricomicomul, pilomatricomul sau pilomatrixomul) este o tumoră benignă care se dezvoltă din celulele matriceale ale foliculului pilos, având o incidență de 1/800-1000 tumori cutanate, afectând predominant femeile (1, 2). A fost descris pentru prima dată de către Malherbe şi Chenantais în 1880 ca fiind o tumoră calcificată, cu originea în glandele sebacee. Ulterior, Forbis şi Helwig (citați de Chuang) au efectuat studii histochimice şi electronomicroscopice ale pilomatrixomului descoperind că originea celulară se află în teaca externă a rădăcinii foliculului pilos (3). Deşi pilomatrixomul poate apare la orice vârstă, s-a observat că este mai frecvent întâlnit în primele două decade de viață şi respectiv în decada a şasea (1). După naştere nu se mai formează noi foliculi piloşi iar în timpul pubertății doar o parte dintre foliculii piloşi existenți sunt activați. Dacă aceştia sunt localizați foarte profund, agenții de inducție a diferențierii nu vor acționa corespunzător asupra lor. Se crede că aceşti foliculi parțial diferențiați vor forma pilomatrixoamele (4).

Cele mai afectate regiuni de prezența pilomatrixoamelor sunt ariile capului şi gâtului, urmate de extremitățile superioare, trunchi şi extremitățile inferioare (5). Tabloul clinic obişnuit este cel al unei tumori nedureroase subcutanate sau intradermice, de consistență fermă şi creştere lentă în dimensiuni (luni sau ani), localizate la nivelul gâtului, regiunilor frontale, periorbitare şi preauriculare. În general, pilomatrixoamele sunt asimptomatice, dar uneori se asociază cu inflamarea şi ulcerarea tegumentului suprajacent (ca şi în cazurile prezentate aici); sunt însă mobile pe planurile subjacente.

Deşi au fost descrise în literatură şi localizări multiple asociate cu alte boli generale (distrofia miotonică Steinert, sindromul Gardner), pilomatrixomul apare adesea ca leziune unică (6,7,8).

Diagnosticul clinic preoperator este dificil de stabilit, pilomatrixomul fiind confundat de cele mai multe ori cu un chist sebaceu. Diagnosticul de probabilitate poate fi formulat atunci când o tumoră

subcutanată cu localizare în segmentele capului şi gâtului are consistenţa fermă spre dură, cu aspectul de „piatră faţetată”. Diagnosticul diferenţial se va face cu: chistul dermoid şi epidermoid, lipomul calcificat, chistul branhial, adenopatia calcificată, condromul, reacţiile de corp străin, hematomul calcificat, osteomul cutanat, hemangiomul sau tumora de glandă parotidă (9).

Investigaţiile imagistice (radiografiile simple, ultrasonografia, imagistica prin rezonanţa magnetică, tomografia computerizată) au valoare redusă în diagnosticarea pilomatrixoamelor. Tomografia computerizată, de exemplu, relevă doar situarea subcutanată a leziunii, delimitarea ei netă şi prezenţa calcificărilor în interior (1).

Caracteristicile histologice ale pilomatrixomului includ: leziune bine-delimitată alcătuită din celule bazale cu diferenţiere scuamoasă localizate la periferie şi celule „fantomă” dispuse spre centrul leziunii (10). Pot fi prezente şi depozite de calciu, osificări, reacţii de corp străin. Activitatea mitotică ridicată este o caracteristică a celulelor bazale dar nu indică malignitatea. Atipiile celulare, comportamentul agresiv local, invazia vasculară şi nodulii bazali infiltrativi sunt indicatorii pilomatrixomului malign („pilomatrix carcinoma”).

Puncţia-aspiraţie cu ac fin este utilizată ca o metodă de diagnostic preoperator, principala eroare diagnostică fiind rezultatul fals pozitiv pentru malignitate (11, 12). Dar, istoricul medical al afecţiunii coroborat cu prezenţa celulelor fantomă caracteristice, absenţa atipiilor celulare şi a mitozelor, prezenţa depozitelor de calciu şi a celulelor gigante multinucleate pot fi utile în stabilirea corectă a diagnosticului de pilomatrixom.

Întrucât regresia spontană a pilomatrixoamelor nu a fost niciodată observată, tratamentul standard constă din extirparea completă a leziunii. Se recomandă ca marginile extirpate din ţesut sănătos să fie de 1-2 cm, pentru a minimaliza riscul de recidivă locală. Uneori, datorită aderenţelor dermice ale tumorii, aceasta se extirpă împreună cu insula cutanată suprajacentă (similar cazurilor prezentate aici). Chirurgia Mohs poate asigura un control mai bun al marginilor exciziei. Suspiciunea de „pilomatrix carcinoma” apare în cazurile recidivelor locale multiple (13).

CONCLUZII

În cazul unei mase localizate superficial (subcutanat sau intradermic) la nivelul ariilor capului şi gâtului, de consistenţă fermă, cu textură de „piatră faţetată” trebuie avută în vedere şi entitatea denumită pilomatrixom.

Tratamentul de elecţie al pilomatrixomului este extirparea chirurgicală completă, cu margini largi de ţesut sănătos.

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DIN ISTORIA ŞCOLII MEDICALE IEŞENE

LA CENTENARUL CATEDREI DE MICROBIOLOGIE DIN IAŞI, OMAGIU ÎNAINTAŞILOR

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Mai curând sau mai târziu cele mai însemnate descoperiri pierd pecetatea personalităţii care le-a dat scânteia vieţii şi devin patrimoniu public : urmaşii le folosesc ca moştenire acumulată de nişte înaintaşi anonimi faţă de care uneori numai se mai simt îndatoraţi.

Constantin Ionescu-Mihăieşti

THE CENTENIAL OF THE DEPARTMENT OF MICROBIOLOGY IAŞI - TRIBUTE TO ANCESTORS • 1892-1913 Bacteriological investigations conducted in Iaşi's hospitals. • 1896 Up to date lectures featuring chapters on microbiology. • 1899-1911 The first interim of the Department. • 1 februarie 1912 Bacteriology Department *de iure et facto* : **Alexandru Slătineanu**. • 1938-39 Second intermediate period. • 1939-1942 Romanian inframicrobiology first started in Iaşi : **Ştefan S. Nicolau**. • 1942-1946 Third intermediate period. • 1946 The Bacteriology Department *redivivus* : Professor **Petru Condrea** • 1953 The Discipline of Virology : Professor **Mihai Duca**

În 1887 Victor Babeş era numit profesor la Facultatea de Medicină din Bucureşti unde a creat Institutul de Patologie şi Bacteriologie. *Regulamentul unificat al Facultăţilor de Medicină din Bucureşti şi Iaşi*, promulgat la 13 ianuarie 1897, deschidea perspectiva unei catedre de Bacteriologie şi la Iaşi, dar marginalizarea fostei capitale a Moldovei a descurajat atragerea personalităţii care să fondeze aici şcoală de bacteriologie. Ioan Cantacuzino, asistent al lui Ilia Mecinikov la Institutul Pasteur din Paris, venea la Iaşi în 1894 ca profesor de Morfologie al Facultăţii de Ştiinţe, dar după 2 ani îşi relua cercetările de bacteriologie şi imunologie în Franţa.

Investigaţii bacteriologice şi seroimunologice în spitale ieşene începeau din 1892 în laboratoare improvizate de profesori familiarizaţi cu aceste metode la Bucureşti, Viena, Budapesta ori Paris. Erau activi profesorii Emil Puşcariu în al său Institut Antirabic (1-5) ; Vasile Negel (6), Mihai Manicatide (7,8,9,10), Vasile Sion (11), Gheorghe Demetriade (12,13), Constantin Bacaloglu (14,15) la *Spitalul Sf. Spiridon* ; profesorul agregat Victor Imerwol la *Spitalul de copii Caritatea* (3,4,16,17) sau doctorul G. Sava-Goiu la *Spitalul pentru boli de ochi Frumoasa* (18). Studenţi, interni, asistenţi aveau acces pentru studii în aceste laboratoare. La Institutul Antirabic utilizau tehnici microbiologice Mircea Vesescu (2), absolvent din 1894, şi asistentul I. Lebell (4,5). În raportul de activitate din 1903 al profesorului Mihai Manicatide citim : *„…în laborator se lucrează cu microscopul nostru personal"* (19), iar în cel din 1097 : *„…am reuşit să facem în clinica infantilă un mic focar unde d-nii studenţi se pot instrui în observarea completă a bolnavilor cu cercetarea ştiinţifică,*

clinică şi bacteriologică" (20). Leon Şeptelici, absolvent din 1903, care lucrase în laboratorul clinicii infantile, conducea din 1911 pavilionul pentru izolarea bolnavilor tifici (21), în 1912 diagnostica bacteriologic şi serologic o complicaţie post-tifoidică la o bolnavă cu chist hidatic al ficatului (14), iar în 1913 publica analiza pneumocociilor diagnosticate în Spitalul Sf. Spiridon (22). Ioan Nimereanu, absolvent din 1905, preparator de Anatomie patologică al lui V. Negel (1901), asistent la clinica Dermato--Venerologică a lui G. Demetriade (1905--1908), apoi asistent, şef de lucrări şi conferenţiar la clinica Medicală a lui C. Bacaloglu, făcea examene bacteriologice (23) şi serodiagnosticul sifilisului (15).

Actualizarea cu capitole de microbiologie o găsim în 1896 în cursul de Clinică Medicală al lui Ludovic Russ jr. : *„...examine microscopice, bacteriologice..."* şi în cel de Patologie generală al lui Constantin Thiron : *„Vegetale parazite : ciuperci inferioare"* şi *„Bacterii infecţioase şi boalele pe care le produc"* (24).

Primul interimat al catedrei de Bacteriologie. La Iaşi Bacteriologia apare în orarul Facultăţii de Medicină abia în 1899. Cursul a fost asigurat 12 ani de profesori suplinitori, fără bază materială şi rar secondaţi de un asistent. S-au succedat V. Negel (1899-1900), V. Roşculeţ (1901-1908) şi E. Puşcariu (1908--1911). *Vasile Negel,* doctor în medicină la Paris în 1882, era profesor titular de Anatomie patologică şi şef de serviciu medical la Spitalul Sf. Spiridon. *Valeriu Roşculeţ* obţinuse doctoratul în medicină la Berlin (1890) şi la Iaşi (1894) cu aceeaşi teză de embriologie. În 1892 era asistent al lui Victor Babeş. Până în 1899 practicase medicina în diferite localităţi. La Iaşi a suplinit catedra de Histologie cât profesorul E. Puşcariu a fost secretar general în Ministerul Cultelor (1899-1901). A suplinit apoi 7 ani cursul de Bacteriologie, dar a lăsat o singură cercetare ştiinţifică în acest domeniu. I-a concurat, fără succes, pe Vasile Sion în 1901 şi pe G. Proca în 1903 la ocuparea catedrei de Igienă din Iaşi (25,26). *Emil Puşcariu,* cu studii medicale la Budapesta, Viena şi Paris, a fost primul şef de lucrări al lui Victor Babeş la Bucureşti. Era legat de mediul academic ieşean în calitate de membru corespondent naţional al Societăţii de Medici şi Naturalişti (27). În 1893 a fost numit profesor de histologie la Facultatea de Medicină şi a organizat Institutul Antirabic din Iaşi, cronologic al 4-lea din lume. Ca suplinitor al catedrei de Bacteriologie (1908-1911) a completat cursul teoretic cu lucrări practice.

Catedra de Bacteriologie *de iure et facto*. Alexandru Slătineanu şi şcolile sale. Deşi la 27 ianuarie 1904 Senatul universitar şi Colegiul Facultăţii de Medicină propuneau Ministerului Instrucţiunii Publice înfiinţarea catedrei de Bacteriologie (28), facţiuni rivale din Consiliul Profesoral au obstrucţionat mulţi ani titularizarea unui profesor (29). Abia la 1 februarie 1912 venea la Iaşi Alexandru Slătineanu, primul profesor titular de Bacteriologie din România. Fără mijloace pentru organizarea unui laborator, pe strada Săulescu 6, într-o încăpere din catedra de Chimie, doar cu o cretă şi o tablă de împrumut, dar cu vaste cunoştinţe şi charismă, Profesorul Slătineanu îşi începea cursul teoretic urmărit cu interes. În acelaşi an a fost mobilizat pentru combaterea holerei pe frontul din Bulgaria. Îşi relua activitatea la catedră în ianuarie 1914 confruntat cu reacţia ostilă a Decanului Nicolae Leon (30,31).

Dar cine era Alexandru Slătineanu şi ce a făcut el pentru Iaşi ? Descendent al unei vechi şi luminate familii de boieri din Ţara Românească, s-a născut la 5 ianuarie 1873 în Bucureşti. În 1892 a plecat să studieze medicina la Paris unde devenea în 1894 extern al spitalelor civile. Convins de importanţa chimiei şi biologiei pentru înţelegerea patologiei, a obţinut şi licenţa în ştiinţe naturale. Şi-a lărgit viziunea interferenţelor patologiei cu condiţia socială audiind, la

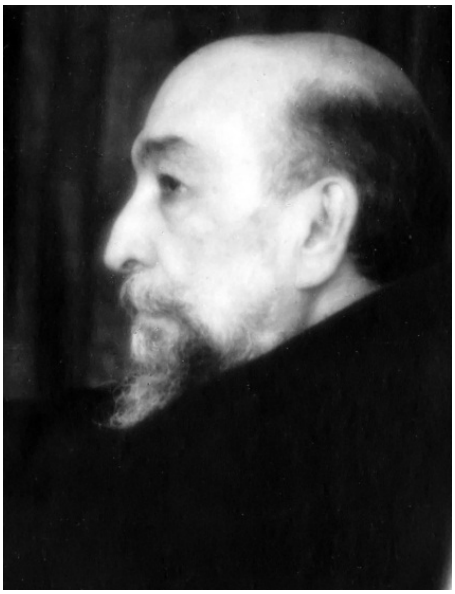
„Amatorul purta barbă şi semăna întrucâtva cu Anatole France. El se întorcea cu bucurie acasă unde regăsea obiectele de artă…”(66)

Combatantul (67,68)

Collège de France, cursul lui Théodule Ribot, care corela psihologia cu stările patologice, şi, la Facultatea de Drept, cursul lui Algave despre doctrinele sociale şi socialiste contemporane. Ioan Cantacuzino l-a introdus în Institutul Pasteur. Aici audia cursul de biochimie al lui Émille Duclaux şi de bacteriologie al lui Émille Roux, iar în laboratorul lui Mecinikov îşi elabora teza de doctorat *Septicémie expérimentale par le coccobacille de Pfeiffer. Essais d'immunisation*, cu care a obţinut în 1901 medalia de bronz a Facultăţii de medicină.

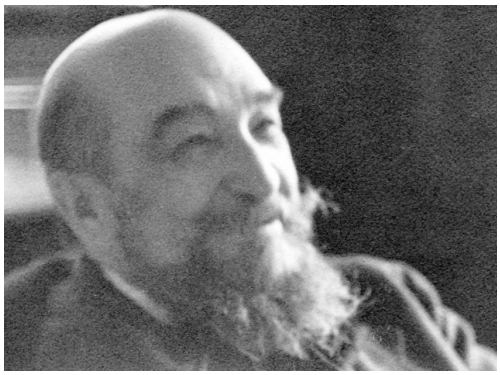
În momente de destindere frecventa, cu alţi tineri români, cafenenele Cluny, Voltaire sau Müller unde au atras simpatia socialiştilor francezi ca Jean Jaurès ori Georges Clemenceau. Vizita muzee, anticariate, cumpăra, cu competenţă de cunoscător, cărţi rare, gravuri, tablouri.

În 1902 a revenit în ţară cu mentorul şi prietenul său Ioan Cantacuzino, care fusese numit profesor la nou înfiinţata catedră de Medicină experimentală de la Facultatea de Medicină din Bucureşti. A acceptat propunerea lui Cantacuzino de a-i fi şef de lucrări şi a organiza laboratorul de cercetări. Bogat înzestrat, energic, organizator de excepţie, cu largă viziune medicală Alexandru Slătineanu nu s-a închis niciodată între pereţii laboratorului. A fost medic secundar în Serviciul pentru boli venerice (1902-1904), apoi în cel de Medicină internă (1904-1906) ale Spitalului Colentina, membru fondator şi colaborator al prestigioasei Revista Ştiinţelor Medicale (1905) şi al Reuniunii Biologice din Bucureşti (1906), prima filială străină a Societăţii Biologice din Paris. Din 1906 a condus spitalul şi sanatoriul pentru tuberculoşi Filaret, la a cărui înfiinţare contribuise. În 1907 a colaborat cu Profesorul Ioan Cantacuzino la reorganizarea serviciului sanitar şi a devenit inspector sanitar pentru epidemii (32). Cu această experienţă şi cu 47 studii de bacteriologie şi medicină experimentală publicate a ocupat catedra de Bacteriologie din Iaşi (33).



Savantul (67,68)

Revenit la Iaşi din Războiul Balcanic, a început, în aprilie 1914, să-şi organizeze laboratorul într-o casă închiriată pe strada Muzelor 18. De la Ministerul Instrucţiunii primise pentru instalare 10.000 lei – sumă cu care putea *„…organiza un laborator de campanie, dar nicidecum un laborator de învăţământ"* – şi un buget anual de 3.000 lei - faţă de 3.500 lei chiria localului (34). Cu bani personali şi donaţii, a realizat ce nu realizase Facultatea de Medicină în 25 de ani : la 22 noiembrie 1915 îşi începea cursul practic pentru studenţi (35), iar în Primul Război Mondial găzduia Laboratorul de Medicină experimentală al Profesorului Cantacuzino evacuat din Bucureşti (36, *vide infra*). După război şi-a dezvoltat laboratorul, care avea acum secţii de bacteriologie, imunologie, chimie fizică, biochimie, hematologie şi histopatologie. Îi solicitau servicii autorităţile medicale şi veterinare, militare şi civile, expertiza calitatea sticlăriei de laborator fabricată pentru prima dată în România. Contracte pentru prestări de servicii îi suplimentau bugetul. Profesorul Slătineanu făcea *avant la lettre* universitate antreprenorială



Spiritualul *„Conversaţia Amatorului era pasionantă. Totul, într-însul, te făcea să uiţi banalul vieţii cotidiene. Problemele de artă, filosofie, literatură şi economie politică alternau în mod natural cu dezbateri de ordine soc*

(37). De la creta şi tabla împrumutate în 1912, a lăsat în 1938, la pensionare, un laborator european modern. O sublinia urmaşul său la catedră, profesorul Stefan S. Nicolau : *„Acest laborator bogat şi admirabil dotat poate fi un subiect de mândrie al ştiinţei româneşti… e mai bogat ca cel al cărui titular am rămas încă în Institutul Pasteur din Paris. Aparate moderne şi extrem de costisitoare ; microscoapele* ***cele mai bune din lume*** *; instalaţie cu peste 25 mese cu faţă din lavă… ; material de sticlărie şi coloranţi în cantităţi mari… Preţuiesc între 10 şi 20.000.000 de lei averea laboratorului de Bacteriologie…Domnia–sa a făcut numeroase donaţii personale laboratorului : aparate scumpe, o bogată bibliotecă ştiinţifică, mobile, tablouri etc. Când se ştie că bugetul anual acordat de Ministerul Educaţiei Naţionale acestui laborator e sub 100.000 lei, nu rămâne decât de admirat opera creată de predecesorul meu"* (38).

În anii 1918-1920 conducea Direcţia Sănătăţii Publice din Ministerul de Interne. În 1919 îl suplinea pe Profesorul Ioan Cantacuzino aflat la Paris pentru tratativele de pace. În 1920 încheia, ca Director al Sănătăţii Publice, cu Epitropia Sf. Spiridon convenţia prin care organiza Spitalul Izolarea

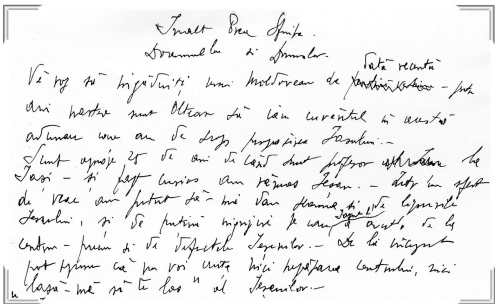
unde a pus bazele învăţământului de Boli Infecţioase la Iaşi (39).

Ca Rector al Universităţii Mihăilene (1923-26) şi membru al Senatului universitar (1928-29, 1933) a continuat lupta contra marginalizării învăţământului ieşean (40-42). Când Moldova rurală era măcinată de pelagră, propunea Ministrului Sănătăţii ridicarea etapizată, pe terenul de la Socola al Facultăţii de Medicină, a unei clinici medicale pentru „*studiul boalelor de nutriţie, dietetică etc.*" Specialistul capabil să organizeze asemenea clinică, Iuliu Niţulescu, tocmai revenise la Iaşi format la şcoala profesorului Mc Collum la Universitatea John Hopkins (40). Nu s-a făcut nimic. Prostie ? Indolenţă ? Rea-voinţă ? Problema a rezolvat-o cu improvizaţii făcute prin Institutul de Igienă şi Spitalul Izolarea. Dar facultatea a pierdut oportunitatea priorităţii şi ...terenul.

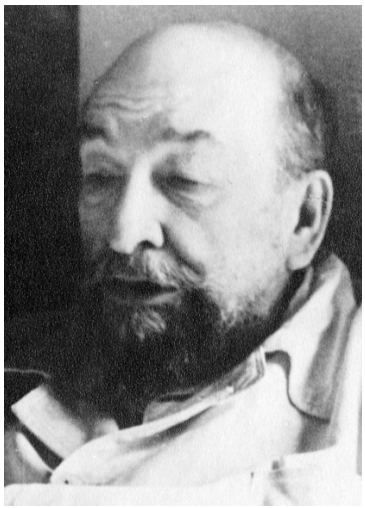
A fost primul Director al Institutului de Igienă din Iaşi (1930-1938), a condus Plasa sanitară de demonstraţie Tomeşti şi a organizat Centrul de tuberculoză şi Sanatoriul Erbiceni. Când Ioan Cantacuzino era Ministrul Sănătăţii l-a secondat în calitate de Secretar general al ministerului (1931-33) (32).

În anii 1926-27, 1930 şi 1933 era Preşedinte al Societăţii de Medici şi Naturalişti din Iaşi şi conducea Comitetul de redacţie al Revistei Medico-Chirurgicale. De-a lungul a 31 de ani a publicat în prestigioasele reviste Viaţa Românească şi Însemnări Ieşene cronică universitară (de la situaţia Facultăţii de Medicină la legenda autonomiei universitare), politică şi socială (de la analiza sindicalismului revoluţionar al lui George Sorel ori nedumeririle unui fost socialist la situaţia reală a ţărănimii române) sau ştiinţifică.

A polarizat în jurul său colaboratori cu care a făcut echipe de lucru competente şi unite. A promovat profesori la Iaşi colaboratori din Laboratorul de Medicină experimentală de la Bucureşti : pe Mihai Ciucă la catedra de Igienă şi Spitalul Izolarea (1922--35), pe C. Ionescu-Mihăieşti la Patologie generală (1925-30), Petru Gălăşescu la Anatomie patologică (1924-38), Ioan Bălteanu la Boli Infecţioase (1927-31), Patologie generală (1930-35), Igienă (1935-42), Ioan Nicolau la Patologie generală şi Medicină experimentală (1936-39), Clinica infantilă (1939-52). Prin ei a creat şcoli care au dus prestigiul Facultăţii de Medicină din Iaşi în ţară şi în lume. A fascinat tineri din facultăţile ieşene de medicină şi ştiinţe. Studenţii medicinişti Ioan Gheorghiu, cu care în 1914 începuse organizarea laboratorului, Ioan Alexa şi Constantin Bart, veniţi în 1920,



Din manuscrisul alocuţiunii Profesorului Slătineanu la adunarea din 1936 a notabilităţilor ieşene pentru propăşirea oraşului (prin bunăvoinţa D-nei Sandra Slătineanu – Sturdza)



Bolnavul (67,68)

i-au rămas discipoli devotaţi. Totdeauna, după 1920, a avut între preparatori şi un chimist. La catedra de Bacteriologie era vie tradiţia Medicinii experimentale: Ioan Gheorghiu studia heterografele de tumori maligne (prioritate bazată pe toleranţa imunologică, citată de J. Bordet, C. Arnouldi, O. Teutschländer şi R. Werner), iar profesorul Alexandru Moruzi făcea chirurgie experimentală. Dintre colaboratorii de la Institutul de Igienă şi de la Spitalul Izolarea remarcabili au fost viitorii profesori ai facultăţilor de medicină din Iaşi – Academicianul Iuliu Niţulescu la Fiziopatologie, Maria Sibi la Biochimie, Maria Franche la Boli Infecţioase, Anton Toma la Ftiziologie, Alexandrina Gaiginschi la Bacteriologie – ori Timişoara – Maria Zalman la Bacteriologie. Coralia Baldovin-Agapi a devenit şefa Secţiei de Streptococi din Institutul Cantacuzino unde şi-a continuat studiile începute la Iaşi.

Intransigenţa şi rectitudinea morală îi erau caracteristice. În 1918, când voci din Consiliul Facultăţii de Medicină îi acuzau de trădare pe profesorii Emanoil Riegler şi Francisc Rainer, apărătorii le-au fost Alexandru Slătineanu, Amza Jianu şi Constantin Parhon. Dar în marele colegiu consecvent, respingând orice compromis, a rămas doar Alexandru Slătineanu (43,44). În 1922 a semnat alături de Victor Babeş, Ioan Cantacuzino ş.a. protestul Asociaţiei generale a Medicilor contra murdarei lucrături politice prin care se urmărea îndepărtarea profesorului Iuliu Moldovan din postul de inspector general sanitar (45).

La catedra de Bacteriologie lucrau voluntari pentru specializarea în medicina de laborator. La Institutul de Igienă organiza cursuri de malariologie, igienă şi medicină preventivă, medicină şcolară, formarea surorilor de ocrotire, cursuri de igienă pentru învăţători.

Alexandru Slătineanu a venit şi a luptat pentru Iaşi până s-a stins din viaţă la 27 noiembrie 1939 cu crezul său *ubi patria ibi bene*. Prea mulţi ieşeni îşi părăseau atunci vatra din convingerea meschină că *ubi bene ibi patria*.

Al doilea interimat. Profesorul *Ioan Bălteanu* a suplinit catedra de Bacteriologie de la pensionarea Profesorului Slătineanu

Requiem aeternam dona eis Domine, et lux perpetua luceat eis (novembris Anno Domini 1939) (67,68)

(1938) până la numirea profesorului Ştefan S. Nicolau (1939). Era titular la Direcţia Institutului de Igienă şi a Spitalului Izolarea. A respectat spiritul şi unitatea acestor şcoli.

Inframicrobiologia* **românească la Iaşi a început, 1939-1942. Ştefan S. Nicolau** (Bucureşti 1896 – Bucureşti 1967) prelua Catedra de Bacteriologie prin Înaltul Decret Regal Nr. 2402 din 7 iunie 1939 *„pe baza legii pentru autorizarea Ministerului Educaţiei Naţionale de a ocupa prin numire directă această catedră".* În 1920 devenise doctor al Facultăţii de Medicină din Cluj, unde a fost remarcat de profesorii Iuliu Moldovan şi Constantin Levaditi.

Inframicrobiologie era sinonimul conceput şi preferat de Ştefan S. Nicolau pentru virologie

Între 1921 şi 1939 a lucrat la Institutul Pasteur din Paris şi la Institutul Naţional pentru Cercetări Medicale din Londra în laboratoarele profesorilor C. Levaditi şi I.A. Golloway. L-au atras mai ales cercetările privind variate viroze şi patogenia acestora. Încă din acea perioadă are priorităţi recunoscute : conceptele de septinevrită (propagarea virusurilor neurotrope prin nervi periferici) şi sincronofilaxie (interferenţa virală), efectul fotodinamic al unor coloranţi asupra virusurilor. Şi-a publicat studiile în prestigioase reviste franceze, engleze şi româneşti. A redactat capitole în *Traité de Microbiologie* al lui Nattan Laurier (1927). A editat monografii ca *Borna Disease and Enzootic Encephalo-Myielitis of Sheep and Cattle* – S. Nicolau and I.A. Golloway (1928). A devenit Doctor în ştiinţe naturale la Sorbona, a fost şef de laborator la Institutul Pasteur şi laureat al Academiei de Ştiinţe din Paris cu premiile *Bellion* (1925), *Bréant* (1930) şi *Montyon* (1935) (43).

La Iaşi, impresionat de realizările predecesorului său, a numit catedra preluată *Institutul de Bacteriologie Dr. Alexandru Slătineanu* şi în faţa localului a pus, cu ceremonial deosebit, bustul în bronz al Profesorului Slătineanu (44).

Cu excelenta dotare la dispoziţie, şi-a continuat cercetările de la Paris. A publicat 11 studii şi a comunicat 8 privitoare la : septinevrita rabică, ultravirusuri, morfogeneza incluziilor în febra galbenă, absenţa neurotropismului la unele tulpini de virus herpetic izolate la Iaşi, herpesul recidivant. Între colaboratori reţinem studentul Radu Portocală şi preparatoarea Artemiza Moţoc. Radu Portocală, student medicinist în Bucureşti, şi-a lucrat la Iaşi, îndrumat de Ştefan S. Nicolau, teza de doctorat *Studiul experimental, histopatologic şi morfologic asupra inframicrobului herpetic,* laureată în 1942 cu premiul *Dr. Gălăşescu* al Facultăţii de Medicină din Iaşi (45,46).

Preda studenţilor cursurile despre virusuri şi conducea lucrările practice privind diagnosticul turbării. Bacteriologia o lăsase pe seama şefului de lucrări, doctorul Constantin Bart, care avea o experinţă de peste 20 de ani în acest domeniu (47). Organizase în 1940 un curs postuniversitar de Microbiologie şi Patologie experimentală. Maria Golăescu, asistentă la Clinica Chirurgicală a profesorului Alexandru Cosăcescu, l-a absolvit în 1942 cu lucrarea *Morfologia corpusculilor elementari vaccinali în ţesuturile animalelor infectate* (46). Aşa a păşit Maria Golăescu spre cariera de profesor de Boli Infecţioase la Spitalul Colentina din Bucureşti.

În septembrie 1942 profesorul Stefan S. Nicolau a plecat la Facultatea de Medicină din Bucureşti unde a pus bazele catedrei de Inframicrobiologie. Relua totul de la capăt. Primii alături i-au fost Radu Portocală (*vide supra*) şi preparatoarea sa de la Iaşi Artemiza Moţoc, care obţinuse de la Ministerul Educaţiei Naţionale şi Cultelor transferul „*în post vacant la catedra de Bacteriologie (Inframicrobiologie) de la Facultatea de Medicină din Bucureşti*" (48). Acelaşi Minister aprobase profesorului Nicolau să împrumute de la Institutul de

Bacteriologie Alexandru Slătineanu din Iaşi un microscop cu fluorescenţă Reichert, un microscop binocular Leitz cu toate anexele, un microtom Spencer, cutii pentru preparate microscopice, cărţi – adresa Nr. 244.888 din 16 noiembrie 1942 cu menţiunea : *„se obligă a restitui aceste aparate, cărţi şi materiale când Ministerul o va cere”*, încuviinţată de Consiliul Profesoral al Facultăţii de Medicină din Iaşi în şedinţa din 7 decembrie 1942 (49,50).

Al treilea interimat a fost asigurat de patru profesori. Episodică a fost trecerea profesorilor, de Anatomie patologică, *Constantin Vasilescu* (octombrie 1942) şi de Parazitologie, *Virgil Niţulescu* (noiembrie 1942). Gheorghe Năstase, profesor de Dermato--Venerologie, a suplinit catedra între ianuarie 1943 şi noiembrie 1945, perioada grea a refugiului facultăţii la Alba Iulia şi revenirii în Iaşii devastaţi de trecerea frontului. Ioan Nicolau, cu experienţa unui fost şef de secţie în Institutul Cantacuzino la Bucureşti, a urmat între decembrie 1945 şi august 1946. Preocupat de viitorul şcolii ieşene de Bacteriologie căuta studenţi talentaţi şi cu pasiune pentru munca de laborator.

Catedra de Bacteriologie *redivivus* : Petru Condrea şi elevii săi. Petru Condrea (Bârlad 1888 - Bucureşti 1967) a venit titular al catedrei de Bacteriologie la 1 noiembrie 1946. Era doctor în medicină de la Bucureşti din 1915. Dobândise ample cunoştinţe de patologie generală, anatomo- şi histopatologie, medicină experimentală, microbiologie şi epidemiologie în şcolile profesorilor Victor Babeş (preparator onorific 1911-13), I. Bălăcescu (şef de laborator la Clinica de Chirurgie infantilă 1919), I. Nanu – Muscel (prozector şi şef al laboratorului de histopatologie şi bacteriologie la Clinica I-a Medicală 1920), Ioan Cantacuzino (preparator 1914--1918, asistent din 1921, şef de lucrări 1925--28, conferenţiar de Patologie comparată din 1929). Se adăuga experienţa anilor medicinei de campanie din Războiul Balcanic şi primul Război Mondial. Au urmat studiile aprofundate la Berlin (1922 : Institutul de Anatomie Patologică) şi Paris (1925-26 : Institutul Pasteur, Institutul Medico-Legal). De la înfiinţarea Institutului de seruri şi vaccinuri *Dr. I. Cantacuzino*, în 1921, a participat la cercetarea ştiinţifică şi producţie ca şef de laborator, apoi Director de secţie. A reprezentat institutul cu diferite ocazii la Zagreb (1927), Praga (1938), Paris (1939) (51). Cu Iaşii avea contacte vechi. În 1918-19 căpitanul medic Condrea Petru era mobilizat, alături de medicii colonel Slătineanu Al., locotent-colonel Ciucă Mihai şi căpitanii veterinar Ciucă Al., medic Nicolau Ioan şi medic Gheorghiu Ioan, în laboratorul de Medicină experimentală : *„… la ordinile M.C.G. pentru a prepara serurile şi vaccinurile necesare în situaţia actuală”* (36,*vide supra*).

Ca profesor titular de Bacteriologie şi Inframicrobiologie, la Iaşi, Petru Condrea a continuat studiile pentru ameliorarea randamentului producţiei de anatoxine şi antitoxine, a contribuit esenţial la studiul şi combaterea epidemiei de tularemie din Moldova anilor 1948-49, a organizat o filială a Institutului Cantacuzino, a refăcut şcoala de microbiologie. Vechii colaboratori ai Profesorului Slătineanu nu mai erau : profesorul agregat Ioan Gheorghiu fusese epurat din universitate chiar când era mobilizat în război (1945) şi arestat în 1950 (52,53,54), conferenţiarul Ioan Alexa îndepărtat în 1945 prin „avansare” în centrala Ministerului Sănătăţii, şeful de lucrări Constantin Bart, cu postul comprimat la 1 octombrie 1947, se retrăsese la Alba Iulia urmat de „dosarul de cadre”. Rămăsese doar asistenta Alexandrina Gaiginschi. Profesorul Condrea a polarizat studenţi şi absolvenţi. În primii şapte ani îi trecuseră prin laboratoare 21 preparatori. Aparent izolat, dar cunoscător de talente şi caractere, impunea

disciplină şi scrupulozitate. Când în 1953 o normare rigidă i-a redus numărul colaboratorilor la şase, profesorul Condrea formase deja mulţi microbiologi de perspectivă. Pe unii i-a reţinut la catedră (viitorii profesori Alexandrina Gaiginschi, Grigore Teodorovici, Eugenia Duca, Natalia Stavri, Sofia Timoşca) sau i-a orientat spre Institutul Cantacuzino la Bucureşti (Alexandru Petrovici, Aurica Cheptea) ori Iaşi (Ioan Pencea, Nicolae Laşcu, Marcus Lazăr), alţii au ajuns şefi ai unor laboratoare de microbiologie (Constanţa Cniş-Dragomir, Maria Olănescu-Antonescu, Carol Feldi). Pentru studenţii medicinişti şi pentru formarea specialiştilor bacteriologi a editat apreciate manuale la redactarea cărora selectase cei mai competenţi colaboratori. La solicitarea Ministerului Chimiei, între 1 februarie 1954 şi 31 ianuarie 1955, catedra a organizat cursul de calificare microbiologică personalului Fabricii de Antibiotice Iaşi, care în decembrie 1955 producea prima şarjă de penicilină românească. Cursuri de perfecţionare au fost apoi solicitate periodic.

Alexandrina Gaiginschi (Iaşi 1904 – Iaşi 2001) era formată în şcoala Profesorului Slătineanu la Institutul de Igienă din Iaşi. Între 1933-36 aprofundase studiul microbacteriilor în laboratoarele profesorilor Albert Calmette şi Alfred Bouquet la Institutul Pasteur din Paris. Era medic primar de laborator când a fost numită asistent al catedrei de Bacteriologie la 1 ianuarie 1939 (55). Profesorul Condrea i-a preţuit competenţa, calităţile umane şi morale promovând-o şef de lucrări (1949) şi conferenţiar (1952). Devenea profesor titular în 1963. A fost un cercetător pasionat – la catedră şi ca şef de secţie la Institutul de Igienă din Iaşi (1941-1951), şef de lucrări la Institutul de Ftiziologie (1951-56), şef de sector la Institutul de Cercetări Medicale al Academiei Române filiala Iaşi (1957 până la pensionarea din 1969) – al fiziologiei şi variabilităţii bacililor tuberculozei, al bacteriilor cu perete defectiv. Studiile sale sunt actuale şi în prezent (56). A format generaţii de bacteriologi dintre care mulţi au ajuns cunoscuţi profesori la Universităţile de Medicină din Iaşi şi Târgu Mureş.

Grigore Teodorovici (Faraoanele, Vrancea 1922 – Iaşi 1992). Caracterizarea făcută de exigentul profesor Condrea la 7 ianuarie 1953 spune multe: *„…numit în postul de preparator în mai 1946 după recomandarea Tov.Prof.Dr. Ion Nicolau,* [care] *mi-a făcut aprecieri elogioase asupra activităţii Tov. Gr. Teodorovici la acea dată. …relevându-se ca cel mai valoros dintre asistenţii laboratorului, a fost propus de noi şi numit ca şef de lucrări în 1949 în care calitate a funcţionat până la recenta restructurare a cadrelor didactice când a fost încadrat ca asistent"* (57). Reîncadrat şef de lucrări la 1 octombrie 1957, prelua în 1959 catedra de Epidemiologie unde a ctitorit noua şcoală ieşeană de epidemiologie. În şcoala profesorului Condrea Grigore Teodorovici şi-a însuşit înalte competenţe de bacteriolog, pe care le-a dovedit ca şef al Secţiei de Microbiologie din Institutul de Cercetări Chimico-Farmaceutice, filiala Iaşi (1957-59), de consilier pentru probleme de microbiologie al Fabricii de antibiotice din Iaşi ca şi în viitoarea carieră de epidemiolog. Anchetele sale epidemiologice, în lungul şi în latul Moldovei, erau lecţii de epidemiologie, de microbiologie şi de patologie infecţioasă.

Omagiem în profesorul Grigore Teodorovici nu numai pe dascălul formator de competenţe şi caractere, ci şi pe cel care 32 de ani a fost sufletul Revistei Medico-Chirurgicale din Iaşi.

Eugenia Duca (Iaşi 1923) rămâne în istoria bacteriologiei prin descoperirea capacităţii nefritogene a *Streptococcus zooepidemicus*, care a determinat în 1968, la Piatra Neamţ, o epidemie de angine transmisă

prin lapte de la vaci cu mastită (58). Abia în 1982 *S. zooepidemicus* a fost înscris pe lista agenţilor transmişi prin lapte şi derivate (59), iar capacitatea nefritogenă i-a fost confirmată de Barnham et al. în 1983 (60) şi de Francis et al. în 1993 (61). Din 1984 prioritatea ieşeană a descoperirii nefritogenităţii acestui streptococ de grup C este citată în repetate ediţii ale marilor tratate ca *Topley and Wilson's Principles of Microbiology and Microbial Infections, Harrison's Principles of Internal Medicine, Oxford Textbook of Medicine* etc.

Şcoala de virologie : Mihai Duca, 1953. În iunie 1948 profesorul Condrea cerea : *„…înfiinţarea unei conferinţe de* ***Inframicrobiologie şi Morfologie Bacteriană*** *anexată catedrei de Bacteriologie"* (62). **Mihai Duca** (Ripiceni 1922 - Iaşi 1998) ocupa acea conferinţă din 15 septembrie 1953. În anii 1950-53, ca tânăr şef de lucrări cu delegaţie de conferenţiar la catedra de Igienă generală, îşi însuşise virologia experimentală în laboratorul de Virologie al Institutului de Igienă din Iaşi unde doctorul Nicolae Constantinescu cerceta turbarea, chorio-meningita limfocitară şi rubeola. În scurt timp a organizat laboratorul de Virologie al catedrei de Microbiologie, a format o echipă de lucru cu care a început cercetări pentru prepararea de vaccinuri virale inactivate cu ioni de argint şi a elaborat, sub îndrumarea Academicianului Ştefan S. Nicolau, dizertaţia *Virusul Coxsackie şi coxsackiozele. Studiu clinic, epidemiologic şi virusologic* susţinută în 1960 pentru titlul de candidat în ştiinţe medicale.

După stagii, în anii 1959-60, la Institutul Pasteur din Paris, *Central Public Health Laboratory* din Londra şi *Staatens Bacteriologiska Laboratorium* din Stockolm, urmate de schimburi de experienţă la Moscova şi Leningrad, a organizat la Iaşi un modern laborator recunoscut ca for metodologic în *World List of Virus Laboratories* (O.M.S., Geneva 1964). A studiat mixovirusurile, adenovirusurile, arbovirusurile şi standardizarea diagnosticului de laborator al virozelor corespunzătoare. Mai mult de 35 de ani a cercetat ecologia virusurilor gripale, a definit *gradientul stratificat* de imunitate antigripală şi relaţia sa cu variaţia naturală a acestor virusuri. Ca recunoaştere a valorii acestor cercetări, Organizaţia Mondială a Sănătăţii i-a acreditat laboratorul ca Centru National pentru studiul şi supravegherea gripei, iar profesorul Mihai Duca a fost numit expert pentru boli virale începând din 1968 şi a lucrat ca ofiţer medical superior în Serviciul de Boli virale din Centrala O.M.S. de la Geneva (1971-73). A fost consultant cu drept de vot al *International Committee on Nomenclature of Viruses*.

Cu prestigiu şi talent organizatoric, profesorul Duca a fost mulţi ani în conducerea Universităţii de Medicină şi Farmacie din Iaşi : Decan al Facultăţii de Igienă (1952-53), prorector pentru activitatea ştiinţifică (1954-67), Rector (1967-71).

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FORMULAREA PRIN DESIGN EXPERIMENTAL A COMPRIMATELOR CU CEDARE IMEDIATĂ CARE CONŢIN ASOCIEREA METFORMIN CLORHIDRAT/GLIBENCLAMIDĂ

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FORMULATION BY DESIGN OF EXPERIMENTS OF IMMEDIATE RELEASE TABLETS WITH METFORMIN HYDROCHLORIDE AND GLIBENCLAMIDE (Abstract): The present investigation was based on the latest quality by design principles, using the design of experiments technique. The aim was to attain an immediate release formulation of metformin hydrochloride and glibenclamide and to optimize the delivery of these two different antidiabetic agents within a single-tablet combination. **Materials and methods**: Due to the poor flow properties of metformin hydrochloride, in order to attain the dose uniformity, a wet granulation based manufacturing process was used. The prepared tablets were evaluated for the release of metformin hydrochloride and glibenclamide using validated HPLC methods. The similarity factor was calculated, taking into consideration as reference profile the mean *in vitro* dissolution data of Glucovance®. The formulation process was undertaken using a reproducible DoE generated model, attained by the variation of each of the formulation factors on two levels, followed by the filling of the data resulted from the analytical testing of the tablets. **Results**: The model was fitted using Partial Least Squares method. For the assessment of the level of fitting, Q2, R2 and Anova tests were performed. The desired drug release pattern can be achieved by using a proper percent of superdesintegrant, by reducing the filler and by the presence of extragranulary added binder. **Conclusion**: The results have shown that the experimental responses match the statistical generated model and that the investigation is reproducible. **Key words**: METFORMIN HYDROCHLORIDE, GLIBENCLAMIDE, FORMULATION, DESIGN OF EXPERIMENTS, TABLETS

Prin mecanisme de acţiune complementare, glibenclamida şi metforminul (agenţi diferiţi din punct de vedere chimic) s-au dovedit ideali pentru administrarea concomitentă la pacienţii diabetici într-o formă farmaceutică monodoză de comprimate.

În timp ce sulfonilureele acţionează prin stimularea sintezei de insulină de către pancreas, biguanidele acţionează prin amplificarea metabolismului glucozei, reducând rezistenţa la insulină şi gluconeogeneza hepatică (1).

Formulările efectuate în cadrul acestui studiu corespund unor forme solide orale care conţin 500 mg clorhidrat de metformin şi 2,5 mg glibenclamid pe comprimat.

Obiectivul studiului a constat în optimizarea formulării la scară de laborator a comprimatelor cu eliberare imediată care conţin asocierea metformin/glibenclamid, în raport de 500 mg/2,5 mg.

Cele două substanţe sunt foarte diferite în ceea ce priveşte solubilitatea în apă: clorhidratul de metformin este uşor solubil

(clasa BCS III, care cuprinde substanţele cu permeabilitate mică şi solubilitate crescută), iar glibenclamida este greu solubilă în apă şi prezintă şi un uşor caracter acid (clasa BCS II, care cuprinde substanţele cu permeabilitate mare şi solubilitate scăzută). În consecinţă, obiectivul acestui studiu constă în obţinerea unor comprimate prin combinarea acestor două substanţe, care să asigure eliberarea şi dizolvarea glibenclamidei în timp util manifestării acţiunii terapeutice.

MATERIAL ŞI METODE

Substanţele medicamentoase şi excipienţii (tab. I) folosiţi în formularea comprimatelor corespund normelor de calitate pentru substanţele de uz farmaceutic prevăzute de *Farmacopeea Europeană 6.0*. (3).

Formula generală de experimentare (tab. II) prezintă două variante, în funcţie de masa comprimatului (600 mg sau 620 mg), proporţia excipienţilor fiind aceeaşi în cazul liantului, dezagregantului şi lubrifiantului. Reglarea masei comprimatului se realizează prin modificarea procentului de excipient majoritar (diluantul).

Dispozitivele utilizate: omogenizator în V de 60 L, maşină de comprimat Kilian S250 cu un poanson de diametru ? 12 mm biconvex, Logan HDT 300 Hardtester, tambur rotativ Electrolab EF-2 friabilator, tester de dizolvare Electrolab TDT-08L, HPLC Surveyor Plus.

TABELUL I

Substanţele active şi excipienţii utilizaţi în formulare

Substanţe active şi excipienţi	Producător
Clorhidrat de metformin	Microsin Romania
Glibenclamidă micronizată	USV India
Glibenclamidă nemicronizată	Cadila India
Celuloză microcristalină (Vivapur® Type 101)	JRS Pharma Germania
Lactitol monohidrat granulat	Purac Biochem Olanda
Croscarmeloză sodică (Vivasol®)	JRS Pharma Germania
Polivinilpirolidonă (Plasdone® S-630)	ISP, Elveţia
Stearat de magneziu (Kemilub® EM-F-V)	UNDESA, Spania

Etapele procesului de preparare prevăd granularea umedă a clorhidratului de metformin, urmată de dispersarea geometrică a glibenclamidului în granulat. La amestecul obţinut se adaugă diluantul, liantul (extragranular) şi dezagregantul. După omogenizarea pulberilor, urmează lubrifierea cu stearat de magneziu şi comprimarea.

Planurile de experimente sunt utilizate în prezent pentru majoritatea proceselor de optimizare. Ideea de bază constă în

TABELUL II

Compoziţia calitativă şi cantitativă a formulei generale de experimentare

Formula generală de experimentare		Metformin clorhidrat	Glibenclamidă micronizată/ nemicronizată	Copovidonă intragranular	Celuloză microcristalină/ Lactitol monohidrat	Copovidonă extragranular	Croscarmeloză sodică	Stearat de magneziu	Total
Var. I	%	83.333	0.4166	3	5.45-10.45	0-3	2-4	0.8	100
Var. II		80.645	0.4032	3	8.15-13.15	0-3	2-4	0.8	100
Var. I	mg/ comprimat	500	2.5	18	32.7-62.7	0-18	12-54	4.8	600
Var. II		500	2.5	18.6	50.536 - 81.536	0-18.6	4.8	4.96	620

definirea unui plan experimental în care toţi factorii variază sistematic. Aspectul cel mai important al acestei metodologii se referă la furnizarea unui cadru matematic riguros de ecuaţii liniare sau polinomiale.

Analiza rezultatelor permite identificarea condiţiilor optime de desfăşurare a experimentelor, depistarea influenţei factorilor de formulare, precum şi prezenţa interacţiunilor şi a sinergismelor (2).

Planul de experimentare s-a realizat cu ajutorul programului statistic Modde 9.0 (Umetrics, Suedia).

Pentru experimentare s-a ales un plan factorial cu cinci factori şi două nivele de variaţie.

Variabilele independente (tab. III) : tipul de diluant (Celuloză microcristalină 101/ Lactitol monohidrat) – variabilă calitativă, procentul de dezagregant (2%/4%) – variabilă cantitativă multilevel, masa comprimatului (600 mg/620 mg)–variabilă cantitativă multilevel, procentul de liant extragranular (0%/3%)–variabilă cantitativă multilevel, sortul de glibenclamidă utilizat (micronizată/nemicronizată) – variabilă calitativă.

Variabilele dependente, respectiv proprietăţile farmacotehnice ale comprimatelor (tab. IV), sunt : friabilitatea (intervalul de variaţie : 0-1 %), rezistenţa mecanică (intervalul de variaţie : 50-120 N), dezagregarea (intervalul de variaţie : 0-240 sec), testul de dizolvare *in vitro* cu evaluarea factorului de similaritate f2 (intervalul de variaţie : 30-100%, valoarea ţintă 60%) (F2G, F2M).

Determinările analitice pentru parametrii friabilitate, rezistenţă mecanică, dezagregare şi pentru testul de dizolvare au respectat prevederile Farmacopeei Europene 6.0 (3).

Friabilitatea comprimatelor neacoperite reprezintă fenomenul prin care suprafaţa comprimatelor se deteriorează sau prezintă semne de abraziune sau de rupere, sub acţiunea şocurilor mecanice şi a frecărilor.

TABELUL III

Variabilele independente şi nivelurile corespunzătoare de variaţie

Variabilă independentă	Abreviere	Unitate de măsură	Tip	Nivele de variaţie
Diluant	Dil	-	Calitativă	Celuloză microcristalină, Lactitol monohidrat
Procent de dezagregant	Rap	%	Multilevel	2,4
Masa comprimatului	Mas	mg	Multilevel	600,620
Procentul de liant extragranular	Lia	%	Multilevel	0,3
Sortul de glibenclamidă	Gli	-	Calitativă	Micronizată, Nemicronizată

TABELUL IV

Variabilele dependente şi intervalele de admisibilitate

Variabilă independentă	Abreviere	Unitate de măsură	Tip	Valoare		
				Min.	Ţintă	Max.
Friabilitate	Fri	%	Regular	0	-	0.1
Rezistenţă mecanică	Rez	N	Regular	50	-	120
Dezagregare	De2	sec.	Regular	0	-	240
Factor de similaritate profil glibenclamidă	F2G	%	Regular	30	60	100
Factor de similaritate profil metformin clorhidrat	F2M	%	Regular	30	60	100

TABELUL VI
Evaluarea calităţii fitării datelor – Testul Anova

Variabilă dependentă		Friabilitate	Rezistenţă mecanică	Dezagregare	Factor de similaritate profil glibenclamidă	Factor de similaritate profil metformin
Valoare p	Model	0.000	0.000	0.000	0.000	0.000
	Eroare	0.295	0.159	0.107	0.229	0.677

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Exp No	Exp N	Run Order	Incl/Excl	Diluant	Procentul de dezagregant	Masa comprimatului	Procentul de liant extra	Glibenclamida	Friabilitate	Rezistenta mecanica	Dezagregare	F2 profil G	F2 profil M
2	1	N1	18	Incl	MCC 101	2	600	0	Micronizata	0.42	107	116	42.7	52.2
3	2	N2	25	Incl	Lactitol	2	600	0	Micronizata	0.72	75	100	46.3	54.3
4	3	N3	5	Incl	MCC 101	4	600	0	Micronizata	0.48	98	56	47.13	57.2
5	4	N4	22	Incl	Lactitol	4	600	0	Micronizata	0.89	65	43	56.2	63.5
6	5	N5	15	Incl	MCC 101	2	620	0	Micronizata	0.72	99	176	40.5	50.2
7	6	N6	12	Incl	Lactitol	2	620	0	Micronizata	0.92	72	156	44.5	52.3
8	7	N7	32	Incl	MCC 101	4	620	0	Micronizata	0.8	97	122	43	55.7
9	8	N8	6	Incl	Lactitol	4	620	0	Micronizata	0.98	69	102	52.2	61.7
10	9	N9	3	Incl	MCC 101	2	600	3	Micronizata	0.38	110	113	57.2	63.1
11	10	N10	35	Incl	Lactitol	2	600	3	Micronizata	0.67	82	97	58.1	62.2
12	11	N11	4	Incl	MCC 101	4	600	3	Micronizata	0.41	102	54	60.11	60.21
13	12	N12	29	Incl	Lactitol	4	600	3	Micronizata	0.75	70	40	71.2	62.3
14	13	N13	13	Incl	MCC 101	2	620	3	Micronizata	0.62	101	172	52.5	58.2
15	14	N14	19	Incl	Lactitol	2	620	3	Micronizata	0.86	80	150	57.5	61.3
16	15	N15	10	Incl	MCC 101	4	620	3	Micronizata	0.71	100	116	55	63.2
17	16	N16	17	Incl	Lactitol	4	620	3	Micronizata	0.91	73	96	65.3	65.3
18	17	N17	23	Incl	MCC 101	2	600	0	Nemicronizata	0.4	103	117	38.6	50.2
19	18	N18	26	Incl	Lactitol	2	600	0	Nemicronizata	0.73	78	97	41.3	52.7
20	19	N19	14	Incl	MCC 101	4	600	0	Nemicronizata	0.52	97	57	42.5	54.3
21	20	N20	24	Incl	Lactitol	4	600	0	Nemicronizata	0.85	67	45	52.3	60.5
22	21	N21	33	Incl	MCC 101	2	620	0	Nemicronizata	0.78	100	188	48.9	51.2
23	22	N22	20	Incl	Lactitol	2	620	0	Nemicronizata	0.9	76	162	51.6	56.7
24	23	N23	27	Incl	MCC 101	4	620	0	Nemicronizata	0.82	96	127	52.7	63.5
25	24	N24	34	Incl	Lactitol	4	620	0	Nemicronizata	0.97	70	107	63.5	67.8
26	25	N25	9	Incl	MCC 101	2	600	3	Nemicronizata	0.37	109	114	54.2	63.2
27	26	N26	11	Incl	Lactitol	2	600	3	Nemicronizata	0.65	86	93	56.1	65.25
28	27	N27	30	Incl	MCC 101	4	600	3	Nemicronizata	0.45	103	58	58.13	60.25
29	28	N28	8	Incl	Lactitol	4	600	3	Nemicronizata	0.76	71	41	62.3	68.5
30	29	N29	31	Incl	MCC 101	2	620	3	Nemicronizata	0.65	104	179	53.2	5.5
31	30	N30	16	Incl	Lactitol	2	620	3	Nemicronizata	0.83	85	159	65.3	67.3
32	31	N31	1	Incl	MCC 101	4	620	3	Nemicronizata	0.78	101	120	69.3	68.2
33	32	N32	21	Incl	Lactitol	4	620	3	Nemicronizata	0.92	83	99	70.1	69.7
34	33	N33	28	Incl	MCC 101	2	600	0	Micronizata	0.41	106	112	40.5	50.3
35	34	N34	2	Incl	MCC 101	2	600	0	Micronizata	0.42	107	116	42.6	52
36	35	N35	7	Incl	MCC 101	2	600	0	Micronizata	0.44	109	122	45.1	58.3

Fig. 1. Matricea ce cuprinde condiţiile şi rezultatele experimentării

Testarea friabilităţii reprezintă media a 20 determinări consecutive.

Rezistenţa mecanică a comprimatelor trebuie să fie suficientă pentru a permite procesarea, ambalarea şi utilizarea comprimatelor. Aceasta reprezintă media a 10 determinări consecutive.

Dezagregarea comprimatelor reprezintă capacitatea acestora de a se dezagrega în mediu apos, în timpul prevăzut şi în condiţii bine definite. Testul s-a efectuat cu o limită de dezagregare pentru comprimate neacoperite de maxim 15 minute.

Condiţiile pentru testul de dizolvare *in vitro* au fost următoarele: aparatul nr. 2 (palete), mediu de dizolvare (soluţie tampon pH 7.4 + polisorbat 80 0.03 %), temperatura mediului de dizolvare $37 \pm 0{,}5°C$, viteza de agitare 100 rpm, timpii de prelevare a probelor: 15, 30, 45, 60 min, metode HPLC validate pentru dozarea celor două substanţe active.

Evaluarea factorului de similaritate f2 s-a efectuat pentru a compara curbele de dizolvare a formulelor studiate cu cea corespunzătoare medicamentului de referinţă. Factorul de similaritate f2 defineşte distanţa dintre două curbe de dizolvare şi se calculează folosind următoarea formulă:

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	1	2	3	4	5	6	7
1	**Friabilitate~**	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
2	Total	35	1.85461	0.0529888			
3	Constant	1	1.20797	1.20797			
4							
5	Total Corrected	34	0.646637	0.0190187			0.137908
6	Regression	15	0.640583	0.0427055	134.025	**0.000**	0.206653
7	Residual	19	0.00605413	0.000318638			0.0178504
8							
9	Lack of Fit	16	0.00556013	0.000347508	2.11039	**0.295**	0.0186416
10	(Model Error)						
11	Pure Error	3	0.000493996	0.000164665			0.0128322
12	(Replicate Error)						
13							
14		N = 35	Q2 =	0.927	Cond. no. =	1.496	
15		DF = 19	R2 =	0.991	RSD =	0.01785	
16		Comp. = 5	R2 Adj. =	0.983			
17							

Fig. 2. Rezultatele testului Anova pentru parametrul friabilitate

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	1	2	3	4	5	6	7
1	**Rezistenta mecanica**	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
2	Total	35	291223	8320.66			
3	Constant	1	283680	283680			
4							
5	Total Corrected	34	7542.97	221.852			14.8947
6	Regression	15	7446.91	496.461	98.199	**0.000**	22.2814
7	Residual	19	96.0576	5.05566			2.24848
8							
9	Lack of Fit	16	91.3076	5.70672	3.60425	**0.159**	2.38888
10	(Model Error)						
11	Pure Error	3	4.75	1.58333			1.25831
12	(Replicate Error)						
13							
14		N = 35	Q2 =	0.938	Cond. no. =	1.496	
15		DF = 19	R2 =	0.987	RSD =	2.248	
16		Comp. = 5	R2 Adj. =	0.977			
17							

Fig. 3. Rezultatele testului Anova pentru parametrul rezistenţă mecanică

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	1	2	3	4	5	6	7
1	**Dezagregare**	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
2	Total	35	477126	13632.2			
3	Constant	1	417362	417362			
4							
5	Total Corrected	34	59763.6	1757.75			41.9256
6	Regression	15	58374.6	3891.64	53.2342	**0.000**	62.383
7	Residual	19	1388.98	73.1042			8.5501
8							
9	Lack of Fit	16	1337.98	83.6237	4.91904	**0.107**	9.1446
10	(Model Error)						
11	Pure Error	3	51	17			4.12311
12	(Replicate Error)						
13							
14		N = 35	Q2 =	0.874	Cond. no. =	1.496	
15		DF = 19	R2 =	0.977	RSD =	8.55	
16		Comp. = 5	R2 Adj. =	0.958			
17							

Fig. 4. Rezultatele testului Anova pentru parametrul dezagregare

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	1	2	3	4	5	6	7
1	**F2 profil M**	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
2	Total	35	134498	3842.81			
3	Constant	1	132294	132294			
4							
5	Total Corrected	34	2204.55	64.8396			8.05231
6	Regression	15	2011.17	134.078	13.1736	**0.000**	11.5792
7	Residual	19	193.378	10.1778			3.19027
8							
9	Lack of Fit	16	156.518	9.78239	0.79618	**0.677**	3.12768
10	(Model Error)						
11	Pure Error	3	36.86	12.2867			3.50523
12	(Replicate Error)						
13							
14		N = 35	Q2 =	0.740	Cond. no. =	1.496	
15		DF = 19	R2 =	0.912	RSD =	3.19	
16		Comp. = 5	R2 Adj. =	0.843			
17							

Fig. 5. Rezultatele testului Anova pentru parametrul F2M

$$f_2 = 50 \cdot \log\left\{\left[1+\frac{\sum_{i=1}^{P}(\mu_{ri}-\mu_{ti})^2}{P}\right]^{-\frac{1}{2}} \cdot 100\right\}$$

Unde :

- P = numărul de determinări
- m_{ri} şi m_{ti} = media rezultatelor determinărilor la timpul t_i , pentru medicamentul de referinţă şi pentru formulele testate (4).

Valoarea optimă a factorului de similaritate f2 pentru profilul unui comprimat nefilmat este de 60%. Aceasta permite ca, în momentul filmării comprimatelor, curba de dizolvare să fie cât mai apropiată celei corespunzătoare medicamentului de referinţă (Glucovance®), adică Î100%.

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	1	2	3	4	5	6	7
1	**F2 profil G**	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
2	Total	35	100930	2883.72			
3	Constant	1	98174.1	98174.1			
4							
5	Total Corrected	34	2756.2	81.0646			9.00359
6	Regression	15	2595.48	173.032	20.4557	**0.000**	13.1542
7	Residual	19	160.718	8.45887			2.90841
8							
9	Lack of Fit	16	150.111	9.38193	2.65339	**0.229**	3.06299
10	(Model Error)						
11	Pure Error	3	10.6075	3.53583			1.88038
12	(Replicate Error)						
13							
14		N = 35	Q2 =	0.819	Cond. no. =	1.496	
15		DF = 19	R2 =	0.942	RSD =	2.908	
16		Comp. = 5	R2 Adj. =	0.896			
17							

Fig. 6. Rezultatele testului Anova pentru parametrul F2G

Rezultatele obţinute prin analiza parametrilor friabilitate, rezistenţă mecanică, dezagregare, similaritate profil de dizolvare metformin, similaritate profil de dizolvare glibenclamidă au fost interpretate statistic.

Fitarea datelor

Analiza regresională (fitarea datelor) reprezintă o metodă statistică de identificare

a unei relaţii cantitative între variabilele independente (factori) şi variabilele dependente (răspunsuri). Aceasta s-a realizat utillizând metoda Partial Least Squares (PLS), cu o marjă de încredere de 95 % pentru toate răspunsurile obţinute.

Coeficientul regresiei multiple (R^2) şi validitatea ecuaţiei de regresie (Q^2) sunt parametri statistici care evaluează gradul de fitare - corespondenţă cu modelul regresional ales, respectiv al ecuaţiei polinomiale cu datele experimentale. O valoare cât mai apropiată de 1 pentru aceşti parametri indică un model experimental bine ales.

Calitatea fitării a fost evaluată prin determinarea parametrilor statistici R^2 şi Q^2, prin analiza varianţei (testul Anova) şi prin trasarea curbelor de dependenţă.

Analiza varianţei (testul Anova) stabileşte dacă variaţia rezultatelor se datorează modificării factorilor de formulare sau dacă reprezintă o variaţie naturală a fenomenului.

Influenţa factorilor de formulare asupra parametrilor friabilitate, rezistenţă mecanică, dezagregare, factor de similaritate profil de dizolvare (glibenclamidă), factor de similaritate profil de dizolvare (metformin) a fost investigată cu ajutorul diagramelor Coefficient Plot, generate de programul statistic folosit în cadrul cercetării.

REZULTATE

Prin variaţia factorilor de formulare pe cele 2 nivele corspunzătoare au rezultat cele 36 de experimentări cu următoarele rezultate - matricea de experimentare (fig. 1).

Friabilitatea comprimatelor s-a situat în intevalul 0,41-0,97%, sub limita maximă

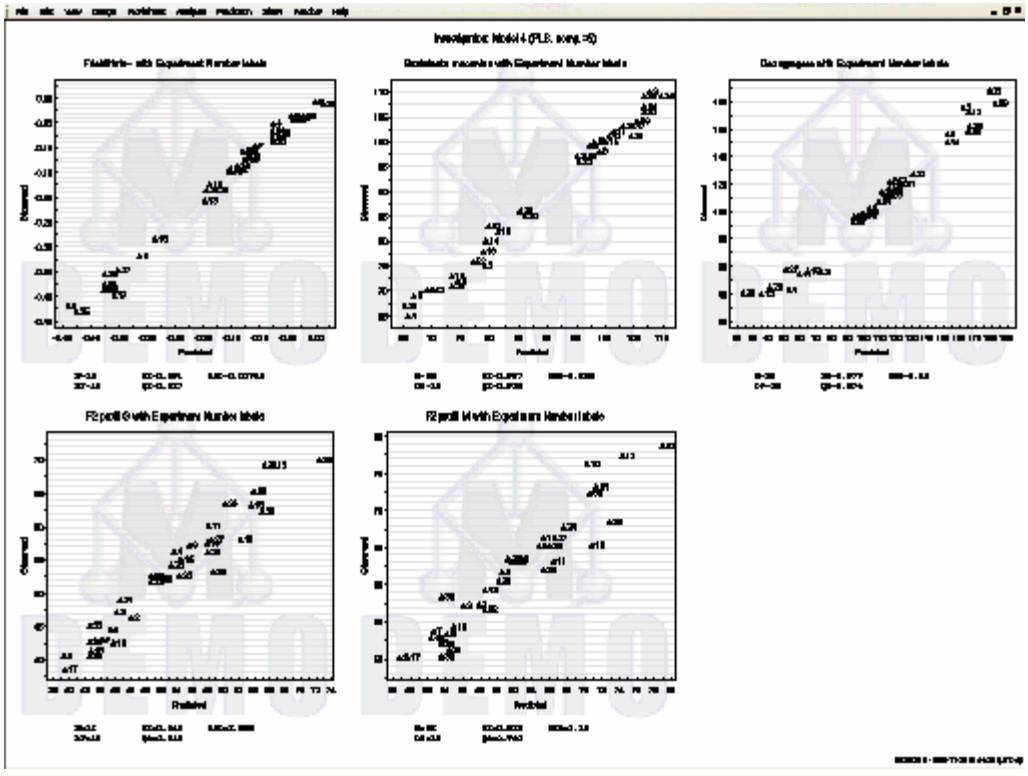

Fig. 7. Curbele reziduale ale răspunsurilor observate în funcţie de răspunsurile estimate pentru parametrii friabilitate, rezistenţă mecanică, dezagregare, factor de similaritate profil de dizolvare (glibenclamidă), factor de similaritate profil de dizolvare (metformin)

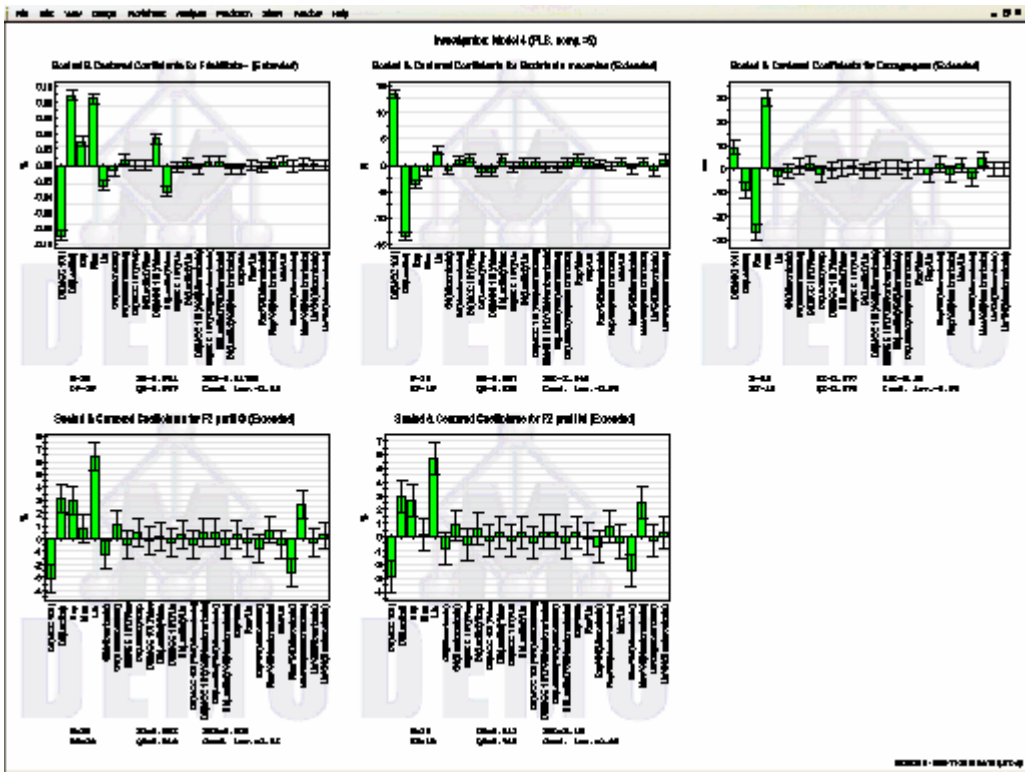

Fig. 8. Influenţa factorilor de formulare asupra parametrilor friabilitate, rezistenţă mecanică, dezagregare, factor de similaritate profil de dizolvare (glibenclamidă), factor de similaritate profil de dizolvare (metformin) (Coefficient Plot).

de admisibilitate de 0,1 %. Rezistenţa mecanică a prezentat valori satisfăcătoare, cuprinse între 67 şi 110 N. Dezagregarea comprimatelor a fost de 40-179 secunde, situându-se sub limita de 15 minute pentru comprimate neacoperite prevăzută de *Farmacopeea Europeană*. Factorul de similaritate F2G a avut valori de 40,5-69,3, iar F2M de 50,2-69,7.

DISCUŢII

Calitatea fitării a fost evaluată prin determinarea parametrilor statistici R_c şi Q_c, prin analiza varianţei (testul Anova) şi prin trasarea curbelor de dependenţă.

Rezultatele obţinute în urma evaluării calităţii fitării (tab. V) au fost satisfăcătoare pentru toţi factorii dependenţi investigaţi, reproductibilitatea modelului prezentând valori de 0,991342 % pentru friabilitate, 0,992863 % pentru rezistenţă mecanică, 0,990329% pentru dezagregare, 0,956383% pentru F2G şi 0,810507% pentru F2M.

Rezultatele testului Anova (tab. VI şi fig. 2-6) arată că, pentru toate răspunsurile "p" este mai mic de 0,05 pentru model şi este mai mare de 0,05 pentru eroare în ceea ce priveşte toţi factorii investigaţi. În consecinţă, variaţia rezultatelor se datorează modificării nivelurilor factorilor de formulare conform planului experimental şi nu unei variaţii aleatoare date de erori experimentale.

Graficele - curbele de dependenţă a valorilor observate (rezultate) în funcţie de valorile estimate de model (fig. 7) - indică o aliniere

satisfăcătoare a punctelor experimentale pe o dreaptă (diagonală la 45°), respectiv o bună fitare a modelului pentru toţi parametrii investigaţi: friabilitate, rezistenţă mecanică, dezagregare, similaritate profil de dizolvare glibenclamidă şi similaritate profil de dizolvare metformin.

În urma analizei diagramelor Coefficient Plot, factorii de formulare care influenţează major friabilitatea comprimatelor sunt: tipul de diluant, masa comprimatului şi procentul de dezagregant utilizat (fig. 8).

Friabilitatea scade o dată cu micşorarea masei comprimatului şi a procentului de dezagregant şi concomitent cu introducerea în formulă a liantului adăugat extragranular.

Factorii de formulare care influenţează rezistenţa mecanică a comprimatelor sunt: tipul de diluant, procentul de dezagregant şi cantitatea de liant introdusă extragranular (fig. 8).

Rezistenţa mecanică a comprimatelor este superioară în cazul utilizării celulozei microcristaline tip 101 ca diluant majoritar, creşte la introducerea liantului extragranular şi scade concomitent cu mărirea procentului de dezagregant utilizat în formulă .

Factorii de formulare care influenţează major dezagregarea comprimatelor sunt: masa comprimatului, procentul de dezagregant, tipul de diluant şi prezenţa liantului extragranular (fig. 8).

Dezagregarea comprimatelor devine mai rapidă o dată cu mărirea procentului de dezagregant şi scade o dată cu introducerea liantului extragranular.

Factorii de formulare care influenţează semnificativ similaritatea profilului de dizolvare a comprimatelor sunt următorii: tipul de diluant, procentul de dezagregant, prezenţa liantului extragranular şi sortul de glibenclamidă (micronizat/nemicronizat) utilizat (fig. 8).

CONCLUZII

Cercetarea unor formulări noi cu substanţe antidiabetice a avut în vedere stabilirea asocierilor cu excipienţi convenabili, prelucrate prin tehnologii care să asigure calitatea produsului final, realizat sub formă de comprimate. În acest sens, au fost studiate asocieri ale substanţelor active cu diferite proporţii de excipienţi, care să asigure cedarea lor corespunzătoare din forma farmaceutică elaborată.

Prepararea comprimatelor cu eliberare imediată ce conţin clorhidrat de metformin/glibenclamid 500 mg/2,5 mg s-a realizat printr-o metodă bazată pe un procedeu de granulare umedă a substanţei active majoritare din formulă.

Conform datelor prezentate (Q_2, R_2, Anova, curbele de dependenţă) se poate concluziona că răspunsurile experimentale se potrivesc modelului experimental ales.

Rata de eliberare a glibenclamidului şi a clorhidratului de metformin s-a îmbunătăţit succesiv cu mărirea proporţiei de dezagregant din formulă, micşorarea proporţiei de diluant din comprimat, introducerea liantului extragranular şi cu folosirea unui sort de glibenclamidă micronizat.

MULŢUMIRI

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STUDIU CLINIC ASUPRA DISTRIBUŢIEI LEZIUNILOR DENTARE NECARIOGENE LA POPULAŢIA ADULTĂ

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CLINICAL STUDY ON THE DISTRIBUTION OF TOOTH WEAR OF THE ADULT POPULATION (Abstract): Tooth wear is becoming increasingly significant in the developed societies, because the etiological factors are frequently present in the daily life. The aim of this study was to assess the distribution of the tooth wear of the adult population in a private practice of dentistry. **Material and method**: The group of study had 614 patients, structured on the following subgroups of age: 18- 30 years, 31-40, 41-50, 51-65 and more than 65 years old. Each patient had a clinical exam and a questionnaire for the diet and the lifestyle, spotlighting the etiology of tooth wear. **Results**: attrition was the most frequent (55.7%), followed by abrasion (32.7%), erosion affected 7.5% of the patients and abfractions are the least frequent (4.1%). Erosions (9.7%) and attritions (59.9%) are more frequent at the feminine gender, and abrasions (40.4%) at the masculine gender. More than half of the abfractions (56%) were found at the youth patients (18-30 years old). Erosions were found in the 31-40 years subgroup at almost 40% of the patients; in the 41-50 years subgroup, abrasion and erosion were found in equal proportions. Abrasion prevails at the 51-65 years subgroup (30.8%). 72% of the consumers of acidic fruits had dental erosions. **Conclusion**: Tooth wear is under the influence of the diet and the age factor. **Key words**: ABRASION, ATTRITON, EROSION, ABFRACTION

Leziunile dentare necariogene sunt descrise ca un fenomen de suprafaţă, spre deosebire de leziunile carioase, în care sunt implicate atât leziuni de suprafaţă cât şi de profunzime. Leziunile dentare necariogene evoluează cu demineralizarea ţesuturilor dentare subiacente, fenomen ce se continuă constant în timp (1).

Leziunile dentare necariogene reprezintă un tip de patologie din ce în ce mai des întâlnită în societăţile dezvoltate, datorită acţiunii factorilor etiologici, care sunt tot mai frecvenţi în viaţa de zi cu zi (dietă, factori de mediu, obiceiuri vicioase, creşterea gradului de igienă orală) (2).

Scopul acestui studiu a fost evaluarea distribuţiei leziunilor dentare necariogene la populaţia adultă dintr-un cabinet privat de medicină dentară.

MATERIAL ŞI METODĂ

Studiul a fost realizat pe un lot de 614 de pacienţi care s-au prezentat la un cabinet de medicină dentară privată din Iaşi pe o perioadă de 3 ani (2006-2009). Lotul a cuprins 285 de pacienţi de sex masculin şi 329 de sex feminin; 155 subiecţi s-au încadrat în intervalul de vârstă 18-30 ani, 170 în intervalul 31-40 de ani, 129 în intervalul 41-50 de ani, 147 în intervalul 51-65 de ani şi 13 de pacienţi aveau peste 65 de ani.

Criteriile de selecţie a subiecţilor au urmărit pacienţi cooperanţi cu vârsta peste 18 de ani şi o valoare a indicelui Smith şi Knight mai mare decât zero.

Pentru fiecare pacient s-a realizat exa-

men clinic şi s-a aplicat un chestionar referitor la obiceiurile alimentare şi modul de viaţă, evidenţiind factorii implicaţi în etiologia diferitelor tipuri de leziuni necariogene, în urma căruia s-au consemnat următoarele date :

- generale (nume, prenume, vârstă, mediu rural sau urban, studii, antecedente generale şi stomatologice) ;
- tipul de băuturi consumate (băuturi acide, băuturi pentru sportivi, ceaiuri, alcool, sucuri naturale din fructe) ;
- eventuale simptome gastrice (vomă, reflux gastro-esofagian, gust acid în cavitatea orală) ;
- tipul de medicaţie ;
- antecedente personale generale, în contextul patologiei asociată altor boli.
- expunerile la mediile acide sau abrazive ;
- gradul de igiena dentară (tehnica, abrazivitatea pastei de dinţi şi orarul de periaj) ;
- obiceiuri vicioase (bruxismul, interpunerea diverselor obiecte între dinţi).
- statusul odonto – parodontal
- leziunile necariogene au fost consemnate diferenţiat în : eroziuni, abrazii, atriţii şi abfracţii.

Datele obţinute au fost centralizate şi analizate utilizând SPSS 16 pentru Windows.

REZULTATE

În urma analizării datelor s-au obţinut următoarele rezultate : 20,19% dintre pacienţi provin din mediul rural şi 79,8% din mediul urban ; 479 de subiecţi au studii medii şi 135 au studii superioare. Din întregul lot de studiu, 4,07% din subiecţi consumă în mod frecvent fructe acide (25), 25,89% băuturi carbogazoase (159), 15,9% sunt consumatori de dulciuri (98), iar 18,89% sunt consumatori de alcool (116). La 44,65% dintre subiecţi am considerat că igiena orală este satisfăcătoare (274), la 24,59% bună (151), la 30,78% igiena fiind nesatisfăcătoare (189). În ceea ce priveşte contactul cu

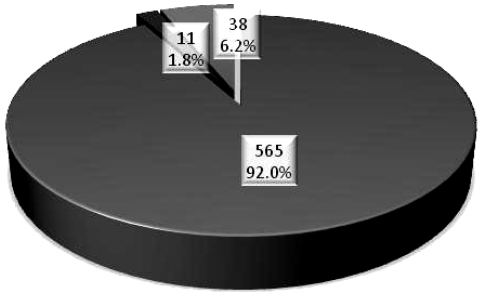

Fig.1. Distribuţia lotului în funcţie de factorii de mediu

factorii de mediu poluanţi 92% (565) dintre subiecţii lotului nu au avut, pe când 6,2% (38) au fost expuşi mediului abraziv şi 1,8% (11) mediului acid (fig.1).

Am evaluat leziunile dentare necariogene în funcţie de sex, constatând existenţa semnificaţiei statistică – datele diferă ca distribuţie semnificativ (tab. I). Din frecvenţa de repartiţie se observă diferenţe majore pentru abrazie unde persoanele de sex masculin se întâlnesc în procent mult mai mare ajungând la 40% (115) faţă de cele de sex feminin, în proporţie de doar 26% (86). Tot diferenţe majore se întâlnesc la eroziune unde persoanele de sex feminin sunt în proporţie aproape dublă faţă de cele de sex masculin (9,7% comparativ cu 4,9%). La leziunile dentare necariogene de tipul atriţiei nu constatăm diferenţe majore în funcţie de sex, pacienţii de sex masculin întâlnindu-se în procent de 50,9%, faţă de pacienţii de sex feminine, care se întâlnesc în proporţie de 59,9%.

În cazul abfracţiei, nu constatăm nici o diferenţă în funcţie de sex (3,9% la sexul masculin, faţă de 4,3% la sexul feminin).

În ceea ce priveşte prevalenţa leziunilor dentare necariogene, 32,7% (201) dintre subiecţi prezintă abrazii, 55,7% (342) prezintă atriţii, 4,1% (25) prezintă abfracţii, iar 46 (7,5%) dintre pacienţi au fost diagnosticaţi cu eroziuni dentare (fig. 2).

TABELUL I

Testul Pearson-Chi pătrat pentru repartiţia leziunilor necariogene în funcţie de sex

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.425[a]	3	.001
N of Valid Cases	614		
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.60.			

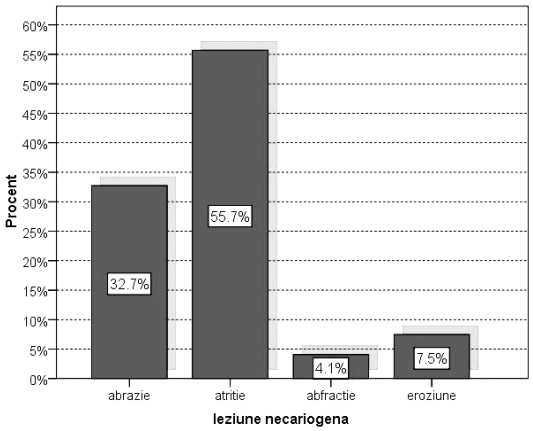

Fig. 2. Distribuţia leziunilor dentare necariogene

Peste jumătate din cazurile cu abfracţie (56%) se întâlnesc la vârstă mică (18-30 ani). Totuşi procente importante din grupele celelalte de vârstă se găsesc şi în celelalte tipuri de leziuni necariogene. Eroziunea se întâlneşte la grupa de vârstă 31-40 de ani în procent de aproape 40% din cazuri. La grupa de vârstă 41-50 de ani, abrazia şi eroziunea se găsesc în procente apropiate (19%), atriţia în procent puţin mai mare (22,8%), iar abfracţia în procent mai mic (12%). La grupul de vârstă 51-65 de ani predomină abrazia cu procent de 30,8% şi atriţia cu un procent de 21,9% (fig. 3). Grupul peste 65 ani este slab reprezentat şi este generator de inconsistenţă statistică.

Am studiat leziunile dentare necariogene în relaţie cu ancheta alimentară, comparând ponderea fiecărui tip de leziune necariogenă (abrazie, atriţie, abfracţie şi eroziune) la pacienţii consumatori de fructe acide cu ponderea tipurilor de leziuni necariogene la lotul martor, acesta constând în grupul de pacienţi la care ancheta alimentară nu a evidenţiat consumul niciunui aliment care poate determina apariţia unei patologii la nivelul cavităţii orale (140 de pacienţi, dintre care 43 prezintă abrazie, 81 atriţie, 3 prezintă abfracţie şi 13 prezintă leziuni erozive).

Studiind leziunile dentare necariogene în funcţie de consumul de fructe acide (fig. 4), am evidenţiat următoarele rezultate:

- dintre cei 25 de pacienţi cu leziuni dentare necariogene consumatori de fructe acide, doar unul prezintă abrazie (4%), comparativ cu 30,7% dintre pacienţii din lotul martor.

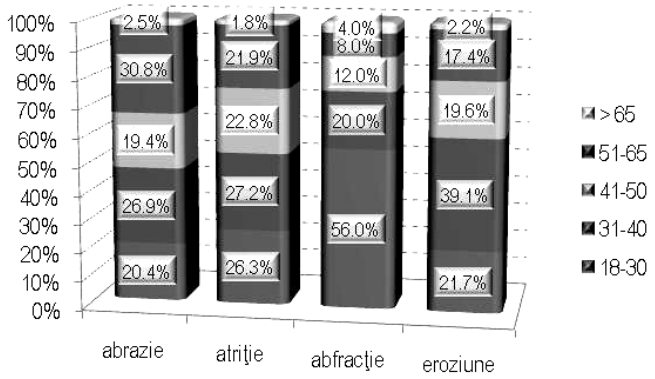

Fig. 3. Prevalenţa leziunilor dentare necariogene comparativ pe intervale de vârstă (%)

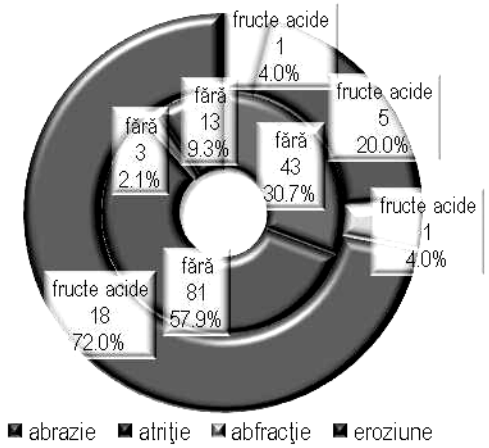

Fig. 4. Prevalenţa leziunilor dentare necariogene la consumatorii de fructe acide

- 20% dintre pacienţii consumatori de fructe acide au fost diagnosticaţi cu atriţie, faţă de 57,9% dintre pacienţii din lotul martor.
- un singur pacient consumator de fructe acide prezintă abfracţie, faţă de 3 pacienţi din lotul martor
- dintre cei 25 de pacienţi consumatori de fructe acide, 18 (72%) prezintă leziuni necariogene de tip eroziv, comparativ cu 13 pacienţi (9,3%) dintre pacienţii din lotul martor.

DISCUŢII

Leziunile dentare necariogene prezintă etiologie plurifactorială, pierderea de substanţă dură dentară având diferite cauze. Eroziunea, atriţia şi abrazia interacţionează şi contribuie la afectarea morfologiei şi funcţionalităţii dentare (1,3). Datele despre prevalenţa uzurilor dentare arată faptul că leziunile dentare necariogene sunt o patologie des întâlnită. Prevalenţa eroziunilor dentare este mai mare la adulţii tineri, ceea ce are ca explicaţie modificările în dietă şi în modul de viaţă la care sunt supuse aceste grupuri de vârstă (4).

Leziunile dentare necariogene tind să devină o patologie relativ frecventă a cavităţii orale, din acest motiv devenind o necesitate identificarea grupurilor cu risc şi aplicarea măsurilor preventive specifice la nivel populaţional (1).

CONCLUZII

Atriţia este diagnosticul cel mai frecvent întâlnit (55,7% din cazuri), fiind urmat de abrazie (32,7%), eroziunile se întâlnesc la 7,5% dintre subiecţi, abfracţiile fiind leziunile cel mai rar întâlnite (4,1%).

Frecvenţa eroziunilor este mai mare la sexul feminin decât la sexul masculin, iar leziunile de tipul abraziei apar mai frecvent la sexul masculin.

Abfracţia se întâlneşte mai frecvent la grupa de vârstă 18-30 de ani, abrazia şi de atriţie apar cu în special la grupele de vârstă 31-40, 41-50 şi 51-65 de ani, pe când leziunile erozive apar la grupa de vârstă 31-40 de ani.

Factorul alimentar influenţează tipul leziunilor dentare necariogene, 72% dintre consumatorii de fructe acide prezentând eroziuni dentare.

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HIPERPLAZIA GINGIVALĂ GENERALIZATĂ – MANIFESTARE CLINICĂ PRECOCE ÎN LEUCEMIA ACUTĂ. PREZENTARE DE CAZ

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GENERALIZED GINGIVAL ENLARGEMENT – EARLY CLINIC MANIFESTATION IN ACUTE LEUKEMIA. CASE REPORT (Abstract): Leukemia is a hematological disorder arises from a hematopoietic stem cell characterized by a disordered differentiation and proliferation of neoplastic cells. Rapidly forming generalized gingival hyperplasia is usually the first sign of this disease (especially in acute forms). This case report describes a 54-year old female who presented rapid gingival enlargement in only three weeks time, heralding the presence of acute monocytic leukemia (AML – FAB M5). The patient was immediately referred for hematologic management, but died five weeks later. Dentists and generalists should always be on guard to observe any oral manifestations that may lead to the early diagnostic of systemic diseases. **Key words**: ACUTE LEUKEMIA, GINGIVAL ENLARGEMENT

HISTO-ANATOMY OF VEGETATIVE ORGANS OF SOME *ARTEMISIA* SPECIES

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HISTO-ANATOMY OF VEGETATIVE ORGANS OF SOME *ARTEMISIA* SPECIES (Abstract): **Aim**: Comparative study of three medicinal species of *Artemisia* widespread in our country, focusing on the anatomical structures that contain bioactive compounds, such as glandular hairs and secretory ducts. **Material and methods**: The roots, stems and leaves of *Artemisia annua, A. vulgaris* and *A. absinthium* were preserved in 70% alcohol. Cross-sections were made using the common methodology and were further analyzed microscopically. **Results**: In the secondary structure of the stem there are differences related to the degree of lignification, more pronounced in *A. annua*, and the special structure of the phloem in *A. vulgaris*. The leaf of *A. vulgaris* presents more vascular bundles in the median rib and *A. annua* has fewer protective trichomes on the leaf lamina. The root has a secondary structure with differences in the number of secretory ducts and cortex size. **Conclusions**: The structure of the stem and root is typical secondary with differences in the number and size of the secretory elements. Depending on light exposure, the leaf presents a heterogeneous symmetric or asymmetric mesophyll. **Keywords**: *ARTEMISIA*, HISTO-ANATOMY, SECRETORY ELEMENTS.

Artemisia is one of the largest genera of *Asteraceae* family, with over 500 species spread mainly in the Northern Hemisphere. The genus includes aromatic plants, used for their antimalarial, antiviral, anticancer, antipyretic, analgesic, anti-inflammatory, antioxidant, hepatoprotective, antispasmodic, anticoagulants, anti-ulcer, antianginal, antiseptics and immunostimulating effects. Researches on *Artemisia* species have intensified along with the discovery of artemisinin, the antimalarial compound from *Artemisia annua* (1).

Artemisia genus is characterized by morphological and phytochemical variability and frequently shows polyploidy. Therefore, the different chemotypes and cytotypes synthesize different compounds with diverse biological activity (2). Romanian flora comprises 20 species of *Artemisia*, and the three species (*A. annua, A. vulgaris, A. absinthium*) selected for this study are widespread and well known in traditional medicine (3).

MATERIAL AND METHODS

The vegetative organs belonging to the three species - roots, stems, leaves - were collected during the flowering period (July-September) in the areas surroundings of Iaşi

town. Plants were identified at the Department of Vegetal Biology, Faculty of Pharmacy, Iaşi "Grigore T. Popa" University of Medicine and Pharmacy, and voucher specimens were stored in the department herbarium. The plant material was preserved in 70% alcohol (v/v). Cross sections were made using a hand microtome and a razor and then stained with carmine red and iodine green (4,5). Microscopic preparations were analyzed using a Nikon Eclipse E400 microscope equipped with a Nikon D90 camera.

RESULTS AND DISCUSSION

The structure of the **root of *A. absinthium*** is typically secondary, built on account of the activity of both secondary meristems. It presents a cork cambium with relatively thick cork on the outside and a slightly collenchymatous phelloderm inward (fig. 1). In the internal part of the cortex, there are large secretory canals, tangentially elongated, and arranged in a circle around the central cylinder (fig. 2).

The vascular cambium produces to the exterior an annular area of secondary phloem and a compact central area of secondary xylem with a high degree of lignification, both crossed by numerous medullary rays, partially lignified in the xylem (fig. 2). In the phloem there are alternating parenchyma zones (in which secretory ducts are found) and tangential areas of sclerenchyma fibers. The elements surrounding the fibers have often strong collenchymatous walls. The actual phloem, located near the cambium, forms a relatively thin zone consisting of sieve tubes and companion cells. The secondary xylem shows wood vessels of different diameters, solitary or grouped, situated disorderly in the mass of sclerenchyma fibers. The xylem parenchyma is reduced and completely lignified.

According to the studies by Toma and Rugină (6), *A. absinthium* is missing the cork area in the secondary xylem, which occurs in many *Artemisia* species from arid climates, having the role of protecting the roots against dehydration. This cork is formed through the activity of a deeper cork cambium which gives rise every year to 1-2 layers of cork cells, arranged at the periphery of the annual growth rings of the secondary xylem (6).

The cross section through ***A. annua* root** has a circular outline. It presents cork on the outside followed by a thin cortex bordered on the interior by a visible endoderm surrounding the bulky central cylinder (fig. 3). In the secondary cortex, there can be seen rare secretory ducts, slightly elongated tangentially, isolated or in small groups.

In the central cylinder there is a circular area of phloem surrounding the xylem. The phloem ring is thin, showing two opposite areas with bundles of sclerenchyma fibers (fig. 3,5). Most of the stele is occupied by the compact xylem area, highly lignified, composed of large wood vessels with radial arrangement. The pith is missing. The vascular bundles are separated by medullary rays which gradually widen from the center to the periphery. All reported anatomical items define, at the root level, a secondary structure with massive areas of secondary phloem and xylem.

The outline of ***A. vulgaris* root** cross section is circular. In the periphery there is an area of thick cork and inward, there is a phelloderm area with parenchyma cells, both resulting from the activity of the cork cambium.

In the central cylinder (fig. 4) there is a thin phloem area, with particular structure and annular shape, then a large central xylem area, consisting of two annual growth rings corresponding to the second-

ary structure. Both phloem and xylem are crossed by several multi-layered medullary rays, formed mostly of cellulosic parenchyma and visibly wider in the phloem.

In the stele, there are isolated secretory canals, scattered in the phloem. More obvious are the grouped secretory ducts in the internal region of secondary cortex, almost glued to the endoderm, near the bundles of phloem fibers (fig. 6).

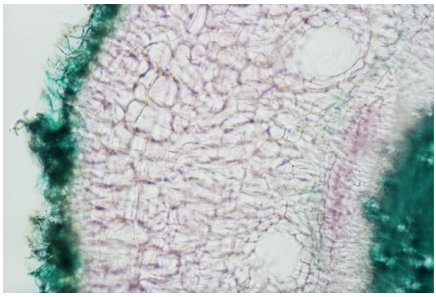

Fig. 1. *A. absinthium* root – cork, phelloderm, cortex with secretory canals (20×)

Fig. 2. *A. absinthium* root – general view (4×)

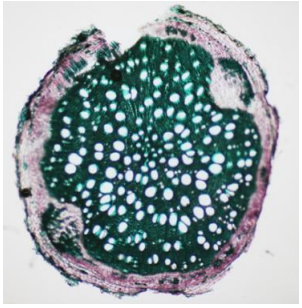

Fig. 3. *A. annua* root – general view (4×)

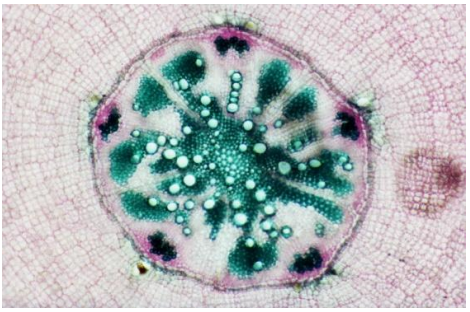

Fig. 4. *A. vulgaris* root – central cylinder (10×)

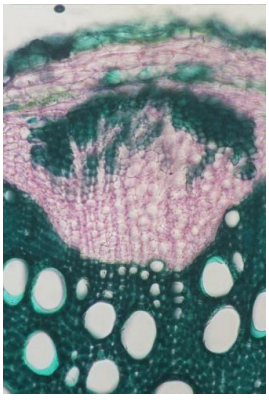

Fig. 5. *A. annua* root – secondary phloem, vascular cambium and secondary xylem (20×)

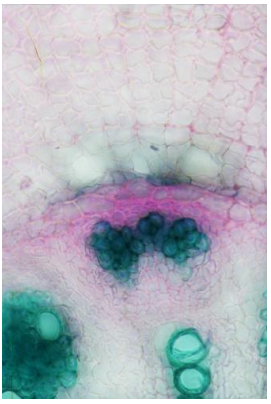

Fig. 6. *A. vulgaris* root – internal cortex with secretory ducts, endoderm, bundles of phloem fibers (20×)

The cross section of ***A. absinthium*** **stem** has a circular ribbed contour and presents an epidermis covered by numerous T-shaped protective trichomes. The trichomes are multicellular, with the terminal part having the shape and position of the compass needle (fig. 8). Near the ribs, under the epidermis there are large collenchyma masses (fig. 7); the rest is represented by a narrow parenchyma cortex. Due to the pronounced lignification of stem, the cross sections were made mostly in the tip area.

In the central cylinder, the secondary structure is formed on the basis of the vascular cambium. Only the intrafascicular cambium works normally, producing less phloem outwards and more xylem inwards. In the primary xylem, vessels are arranged in radial rows, continuously or discontinuously, separated by cellulosic parenchyma (7). Both vascular tissues, although of secondary origin, form a fascicular type structure. Vascular bundles are provided with strong sclerenchyma sheaths on the phloem side (having the aspect of sclerenchyma caps). The vascular bundles seem to touch each other on the sides, being linked by lignified tissue areas (fig. 7). In the center section there is cellulosic parenchyma pith, of lacunose type.

In ***A. annua*** **stem**, depending on the cutting area - top, middle and base - there can be seen a circular shape with more or less pronounced ribs. Compared to the other two species, in this case there is a higher degree of lignification, a reduced bark and greater number of ribs - 19. The epidermis has small cells covered by a cuticle, few T-shaped protective trichomes and characteristic glandular hairs of *Asteraceae* type. Under the epidermis, close to the ribs, there are collenchyma areas followed by a small parenchyma cortex and the endoderm, visible especially next to the ribs.

The central cylinder structure is similar to that of *A. absinthium*. The vascular bundles, of collateral type, are larger near the ribs and smaller between them, connected by a band of sclerenchyma tissue. Outside, above the phloem, there is a sizeable cap of supporting tissue (fig. 9). The multi-layered intrafascicular cambium continues with interfascicular areas, forming a continuous band. The bulk of the section (approx. ¾) is occupied by pith parenchyma.

A. vulgaris **stem** section is similar in shape and contour with *A. absinthium* stem section, except that the number of ribs is smaller and they are more attenuated (fig. 10). On the epidermis, there are no protective hairs or only a small number of broken bases of trichomes can be seen. Next to the ribs, there are large collenchyma areas and a narrow parenchyma cortex.

Both phloem and xylem, although secondary in origin, are organized in bundles (fig. 11). A special type of phloem bundles is present where supporting elements with lignified walls are mixed with others having thin cellulosic walls; to the exterior, there is a consistent sclerenchyma cap (fig. 12). Among these vascular bundles, there are some made only of phloem elements. All of them, together with the cellulosic parenchyma between them, form a ring that shows to its internal face a visible multilayered cambium. Towards the center of the section there is a xylem band consisting of secondary xylem on the outside and primary xylem on the inside. The center of the section is represented by cellulosic parenchyma pith, of lacunose type.

A. absinthium shows an equifacial structure of the **leaf** lamina with symmetrical heterogeneous mesophyll with two rows of palisade parenchyma cells to the upper face and only one layer to the lower face. Between the two areas of palisade tissue there

are two layers of spongy parenchyma cells. The palisade cells are relatively short, with wavy side walls and larger intercellular spaces to the underside of the lamina.

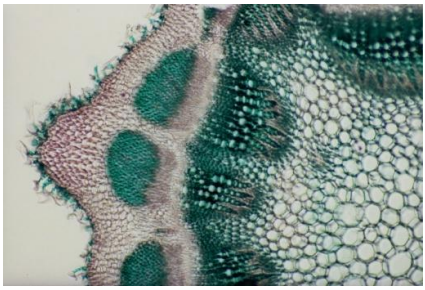

Fig. 7. *A. absinthium* stem – detail (10×)

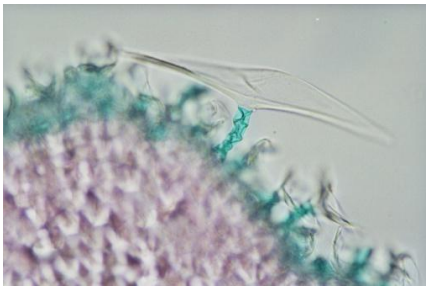

Fig. 8. *A. absinthium* stem – epidermis with T-shaped protective trichomes (40×)

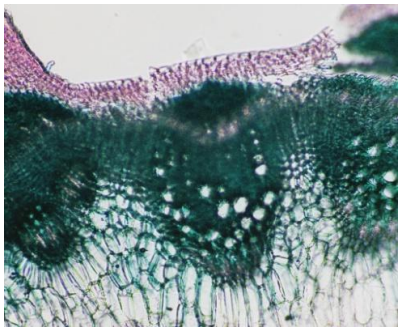

Fig. 9. *A. annua* stem – vascular bundles connected by a band of sclerenchyma tissue (10×)

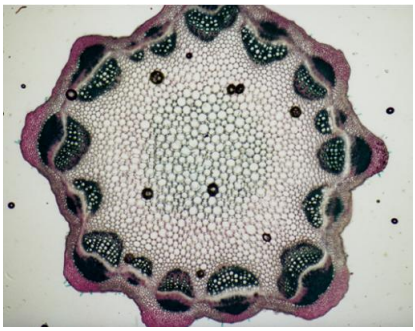

Fig. 10. *A. vulgaris* stem – general view (4×)

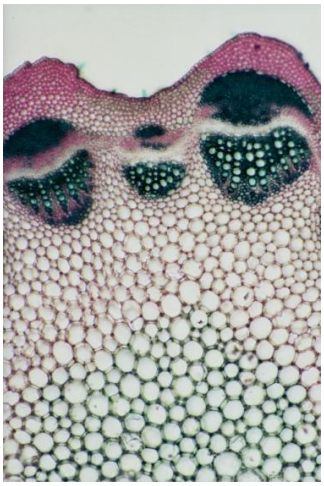

Fig. 11. *A. vulgaris* stem – vascular bundles with sclerenchyma cap (10×)

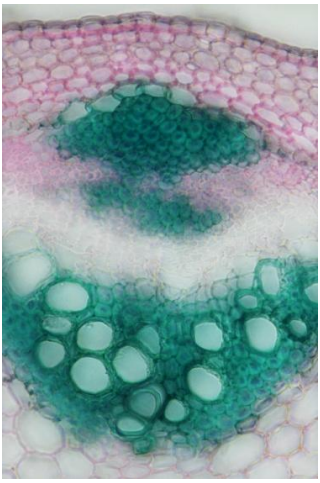

Fig. 12. *A. vulgaris* stem – detail of vascular bundle (20×)

The midrib, prominent on both sides, is more visible on the lower part and presents palisade tissue beneath the upper epidermis (fig. 13). The mid-stem and upper leaves present a single vascular bundle next to the midrib and a reduced collenchyma band above the lower epidermis (fig. 13), whereas the basal leaves show two vascular bundles and collenchyma bands under both epidermises. (fig. 14). In the parenchyma tissue around the vascular bundles of the midrib there are few secretory canals.

The epidermises are wavy, composed of cells with weakly thickened external walls and barely cutinized. On both epidermises - but more frequently on the lower one – characteristic T-shaped protective hairs are present. These trichomes have the terminal cell shaped like a compass needle, arranged perpendicularly to a stalk consisting of 3-5 cells. Biseriate glandular hairs of *Asteraceae* type, with 8 secretory cells, are found in the depressions of lower epidermis.

The lamina is hypostomatic, with anomocytic stomata (8). According to Halmai and Novak (9), in *A. absinthium* only the leaves with both sides exposed to sunlight have equifacial structure. The shaded leaves have bifacial structure due to the asymmetric heterogeneous mesophyll (9).

In the cross section through ***A. annua*** **leaf**, a very evident prominence can be noticed, next to the midrib, towards the upper and lower face (fig. 15). The leaf lamina presents a symmetric heterogeneous mesophyll (fig. 16). Under both epidermises there is a monolayer palisade parenchyma and between palisades there is a spongy parenchyma of 2-3 layers of cells. The median rib (fig. 15) has a collateral vascular bundle centrally located and assimilation tissue.

Next to the midrib, the upper and lower epidermises have collenchymatous cell walls.

In the parenchyma surrounding the vascular bundle of the midrib, there are secretory ducts. On both epidermises can be seen the bases of broken protective hairs, situated mostly on the midrib, and also biseriate glandular hairs of *Asteraceae* type, especially on the lower epidermis. It is important to emphasize the size of *A. annua* leaf, considerably smaller compared to the other two species.

According to Duke et al. (10) there are protective T-shaped trichomes and glandular hairs with biseriate 10-celled glands on the leaves, stems and flowers. There are two biotypes of *A. annua*, with and without secretory trichomes; the biotype lacking glandular hairs does not contain artemisinin. The glandular hairs are more prominent on corolla and floral receptacle than on leaves, stems or bracts (10).

It has been shown that glandular hairs are the site of artemisinin synthesis. Glandular hairs are made of 2 columns of 5 cells which are covered by a common cuticle enveloping the first 3 pairs of apical cells. Since artemisinin is autotoxic for *A. annua*, its location in the subcuticular space prevents the contact of substance with live parts of the plant, while providing protection from insects, herbivores and pathogens (11).

The cross section through ***A. vulgaris*** **leaf** presents a projection next to the median rib, even deeper towards the lower face compared to that of *A. absinthium*. In the midrib there are 3 big vascular bundles of collateral type, centrally located, and 2 smaller ones on the sides, all placed in a general parenchyma devoid of chloroplasts; the assimilation tissue is missing (fig. 17).

The large vascular bundles have small sclerenchyma sheaths at extremities. Above the central bundle there is a secretory canal of volatile oil surrounded by cells arranged

radially (fig. 17). As in *A. absinthium* leaf, next to the midrib, under both epidermises, there is collenchyma bands, the lower one being better developed.

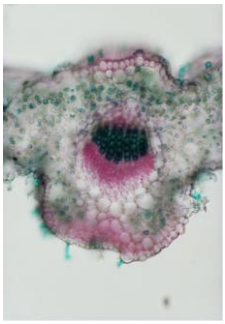

Fig. 13. *A. absinthium* upper leaf – detail of midrib (20×)

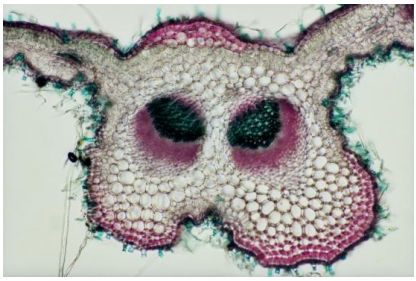

Fig. 14. *A. absinthium* basal leaf – detail of midrib (10×)

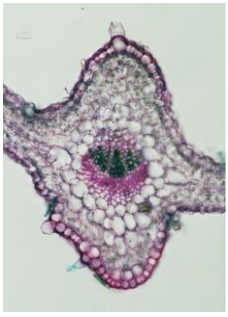

Fig. 15. *A. annua* leaf – detail of midrib (20×)

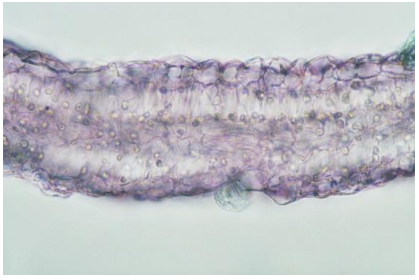

Fig. 16. *A. annua* leaf – detail of mesophyll (40×)

Fig. 17. *A. vulgaris* leaf – midrib detail (10×)

Fig. 18. *A. vulgaris* leaf – detail of mesophyll (40×)

The leaf blade has an equifacial structure with symmetrical heterogeneous mesophyll (fig. 18), showing one-layered palisade tissue under both epidermises, with shorter cells in the lower palisade. The upper epidermis has bigger cells with outer walls thicker than the lower epidermis (fig. 18). Protective trichomes, more frequent on

the lower epidermis, are also T-shaped but slightly different from those of *A. absinthium*: their terminal part has the form of two very long arms.

CONCLUSIONS

Our comparative histo-anatomical study of the three *Artemisia* species revealed a number of resemblances, especially in the stem structure. The stem has many ribs hardened with angular collenchyma, a reduced bark and a central cylinder with an evident secondary structure, and a pith parenchyma in the centre. Differences appear in the degree of lignification, more pronounced in *A. annua* stem and the special structure of phloem in *A. vulgaris* stem.

As to the leaf structure, the similarities are linked to the plant ability to adapt to strong light conditions – mesophyll with symmetrical heterogeneous structure, or to shade – mesophyll with asymmetrical heterogeneous structure. The differences refer to the small size of *A. annua* leaf, a greater number of vascular bundles in *A. vulgaris* midrib and a reduced number of trichomes in *A. annua* leaf. With regard to root anatomy, all the species have a typically secondary structure, with distinctions in the number and dimensions of the secretory ducts, but also in the cortex size – smaller in *A. annua* in comparison with the other two species.

ACKNOWLEDGEMENTS

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GRAPHICAL USER INTERFACE WITH APPLICATIONS IN SUSCEPTIBLE-INFECTIOUS-SUSCEPTIBLE MODELS

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GRAPHICAL USER INTERFACE WITH APPLICATIONS IN SUSCEPTIBLE-INFECTIOUS-SUSCEPTIBLE MODELS (Abstract): Practical significance of understanding the dynamics and evolution of infectious diseases increases continuously in contemporary world. The mathematical study of the dynamics of infectious diseases has a long history. **Aim:** By incorporating statistical methods and computer-based simulations in dynamic epidemiological models, it could be possible for modeling methods and theoretical analyses to be more realistic and reliable, allowing a more detailed understanding of the rules governing epidemic spreading. **Material and methods**: To provide the basis for a disease transmission, the population of a region is often divided into various compartments, and the model governing their relation is called the compartmental model. To present all of the information available, a graphical user interface provides icons and visual indicators. The graphical interface shown in this paper is performed using the MATLAB software *ver.7.6.0*. MATLAB software offers a wide range of techniques by which data can be displayed graphically. The process of data viewing involves a series of operations. To achieve it, I had to make three separate files, one for defining the mathematical model and two for the interface itself. **Results**: Considering a fixed population, it is observed that the number of susceptible individuals diminishes along with an increase in the number of infectious individuals so that in about ten days the number of individuals infected and susceptible, respectively, has the same value. If the epidemic is not controlled, it will continue for an indefinite period of time. By changing the global parameters specific of the SIS model, a more rapid increase of infectious individuals is noted**. Conclusions:** Using the graphical user interface shown in this paper helps achieving a much easier interaction with the computer, simplifying the structure of complex instructions by using icons and menus, and, in particular, programs and files are much easier to organize. Some numerical simulations have been presented to illustrate theoretical analysis.
Keywords: INFECTIOUS DISEASES, SIS EPIDEMIC MODEL, GUI, MATLAB.

Classical research of Kermack and McKendrik (1927) (1) laid the foundations of mathematical modeling in the field of epidemic dynamics. Variables indicate the number of host individuals in various stages - susceptible, infectious and recovered. This formality is the current basis of dynamics modeling and evolution of infectious diseases. To prevent and control their spread, epidemic dynamics has played an essential part in investigating their transmission, predicting trends of development, estimating key parameters from the data published by health departments, under-

standing the characteristics of transmission and implementing prevention and control measures. Both history and reality show that, while people are faced with the threat of several infectious diseases, the importance of the investigation concerning transmission mechanism, spreading rules and strategy for prevention and control have increased rapidly, and such studies are a critical mission to be dealt with as quickly as possible.

In general, the mathematical models applicable in epidemiology divide the population into three compartments (2, 6):

- a susceptible one (marked S), in which all individuals are susceptible to the disease;
- an infected one, marked I, in which all individuals are infected by disease and are infectious;
- an "isolated" one, marked R (removed) in which all individuals are removed from the infected compartment, where S(t), I(t), R(t) denote the number of individuals of the three compartments at the point of time t .

MATERIAL AND METHODS

The Susceptible-Infected- Susceptible model with vital dynamics (births and deaths) is characterized by the following system of differential equations (1):

$$\begin{cases} \frac{dS}{dt} = \mu N - \mu S - \frac{\beta IS}{N} + \gamma I \\ \frac{dI}{dt} = \frac{\beta IS}{N} - \gamma I - \mu I \end{cases} \quad (1)$$

with initial conditions $S(0) = S_0 > 0$, $I(0) = I_0 > 0$ and $S(t) + I(t) = N$, where N is the total number of individuals and β is the total number of contacts. The SIS model shows an increased number of new-born babies in the susceptible compartment at a μN rate and a μS death rate, μI , respectively, where $\frac{1}{\mu}$ is the average life expectancy and $\frac{1}{\gamma}$ is the infectious period (2, 3). Deaths and births are balanced in such a way that total population N remains constant. This model is the simplest epidemiological model and is used for diseases, which do not confer immunity after infection. Susceptible individuals become infected and then become susceptible again after recovery. The model is an endemic one as the disease persists, and it is used in diseases such as gonorrhea, meningitis or streptococcal infections (4).

A graphical interface (Graphical User Interface) is an interface located between the user and electronic devices such as computers, hand-held devices (mp3 players, portable media players), electronic appliances and some office equipment. To present all available information and actions, a GUI provides icons and visual indicators, in contrast to text-based user interfaces, which provide names of commands to be typed in or text navigation (5, 7). This is intended to facilitate the user's work by eliminating the process of writing the software code.

RESULTS

The graphical user interface consists of a panel for displaying different graph views, a panel for controlling graphs and a panel for introducing and controlling parameters (fig. 1).

- **Graph display panel**: This area defines the "axes of coordinates" (abscissa and ordinate) required in graphic view of curves of solutions: S(t), I(t) (fig. 2).
- **Parameter control panel**: In this panel, values of each and every parameter can be changed. To choose the relevant model, one of the models that can be accessed from the top of the graph display panel is selected (fig. 3).

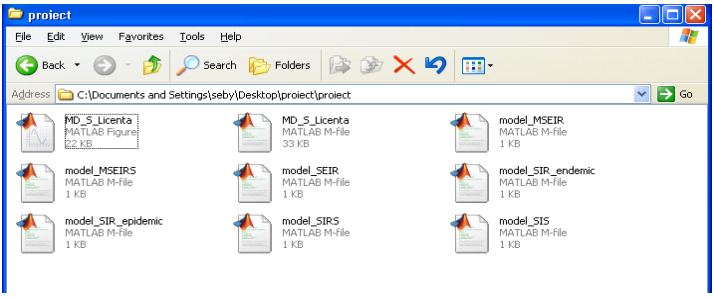

Fig. 1. Files contained by GUI

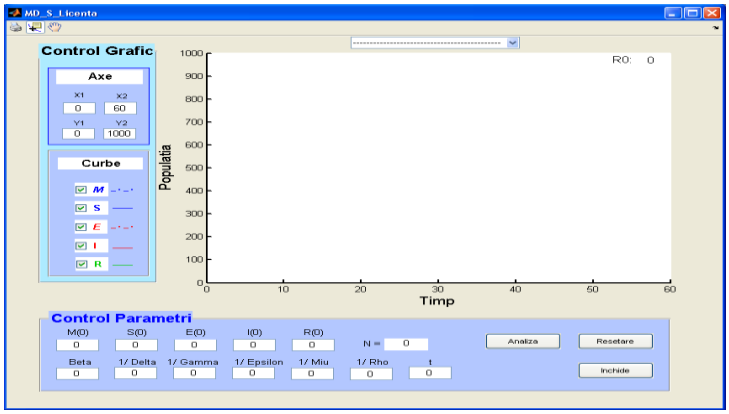

Fig. 2. Graphical user interface with applications in epidemiology

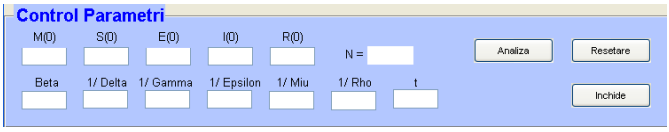

Fig. 3. Parameter control panel

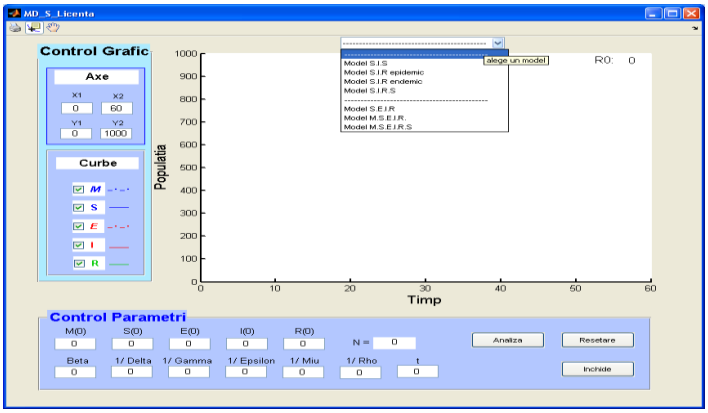

Fig. 4. Example of choosing a model

After having selected a model, the parameter values specific of the models under analysis must be introduced, and parameters that are not specific of the model analyzed will be set to zero. The "Reset" button is pressed to re-enter parameter values and the "Analysis" button is pressed to display the graph. To exit the program, the "Close" button is pressed.

• **Graphic control panel**: At the top, specific values to view the graph obtained will be introduced. Initially, these values are automatically set to the values displayed. Subsequently, according to the user's needs, these values can be modified. The selection buttons to display curves resulting from the analysis are placed at the bottom. Subsequent simulations have been made using epidemiological databases and considering an early time of the epidemic onset.

The SIS model described by the system of equations (1) is characteristic of diseases caused by bacteria. Infected individuals automatically move to susceptible class after recovery. Figure 4 considers a number of contacts of $\beta=5$ during the given time period and an infectious period of $\frac{1}{\gamma}=3$ days. The initial number of susceptible individuals is $S(0)=1000$, while the initial number of infectious individuals is $I(0)=10$. Life expectancy has an average value of $\frac{1}{\mu}=75$, while birth and death rate is $\mu=0.013$.

Should we change the global parameters characteristic of the SIS model, β and $\frac{1}{\gamma}$, we can see a more rapid increase of infectious individuals, corresponding to a lower point of balance reported to the S/I curves (approximately four days). It is also noted that a great variation of the infectious period (from three to ten days) causes a proportional variation of the basic reproductive number, from a value of $R_0=\frac{\beta}{\gamma+\mu}\approx 29$ in the case of a small infectious period, to a higher value, i. e. $R_0\approx 88$ for a incubation period of $\frac{1}{\gamma}=10$. This number R_0 is critical in the beginning of an epidemic (fig. 5, 6).

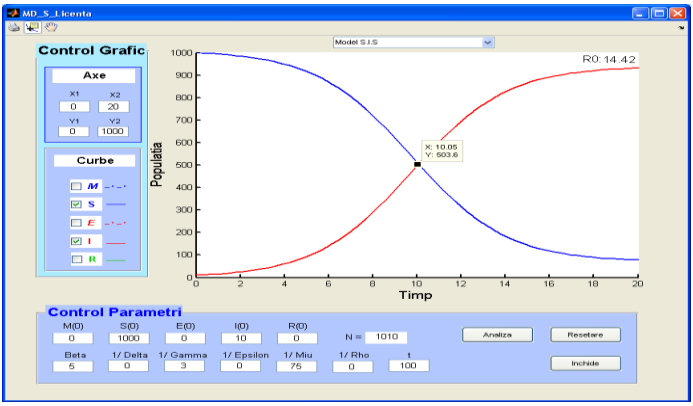

Fig. 5. SIS model analysis for $\beta=5$ and $\frac{1}{\gamma}=3$ values.

I assumed that each contact of an infected individual was with a non-infected, or susceptible. It is more realistic to assume that the number of susceptible contacts is a function of the number of susceptible persons in the population. We also need to specify an initial fraction of the infected population.

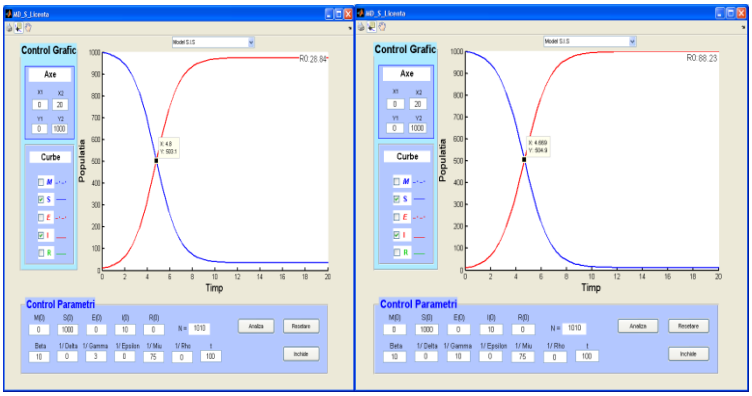

Fig. 6. SIS model analysis a. (up) $\beta = 10$ and $\frac{1}{\gamma} = 3$; b. (down) $\beta = 10$ and $\frac{1}{\gamma} = 10$;

The lack of susceptible individuals slows the epidemic when there are many infective individuals; and many susceptible individuals increase the spread of the epidemic when there are few infective individuals. Because an epidemic usually begins with a small set of infected individuals, let us choose this value to I_0.

CONCLUSIONS

Infectious diseases have always been a fierce opponent. In our study, the use of a graphical user interface provides a much easier interaction with the computer, simplifies the structure of complex instructions, using icons and menus. To prevent and control their spread, numerical and mathematical modeling of epidemic dynamics has played an essential part in investigating their transmission, estimating key parameters from the data published by the departments of health, understanding the characteristics of transmission and implementing prevention and control measures. Using the graphical user interface suggested by this paper, detailed calculation experiments may be carried out, that may help planning the response of authorities in case of a pandemic situation. I have not considered a mathematical model to represent a specific disease in this paper, because my main objective was to demonstrate the idea that the infection transmission can easily be studied using graphical user interfaces.

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PLANT-DERIVED ANTICANCER AGENTS - CURCUMIN IN CANCER PREVENTION AND TREATMENT

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PLANT-DERIVED ANTICANCER AGENTS. CURCUMIN IN CANCER PREVENTION AND TREATMENT (Abstract): Nowadays cancer is still a major public health issue. Despite all the progresses made in cancer prevention, diagnosis and treatment, mortality by cancer is on the second place after the one caused by cardiovascular diseases. The high mortality and the increasing incidence of certain cancers (lung, prostate, colorectal) justify a growing interest for the identification of new pharmacological agents efficient in cancer prevention and treatment. In the last fifty years many plant-derived agents (vinblastine, vincristine, vindesine, paclitaxel, docetaxel, topotecan, irinotecan, elliptinium) played a major role in cancer treatment. Other very promising plant-derived anticancer agents (combrestatins, betulinic acid, roscovitine, purvalanols, indirubins) are in clinical or preclinical trials. Curcumin, a liposoluble polyphenolic pigment isolated from the rhizomes of *Curcuma longa* L. (*Zingiberaceae*), is another potential candidate for new anticancer drug development. Curcumin has been reported to influence many cell-signaling pathways involved in tumor initiation and proliferation. Curcumin inhibits COX-2 activity, cyclin D1 and MMPs overexpresion, NF-kB, STAT and TNF-alpha signaling pathways and regulates the expression of p53 tumor suppressing gene. Curcumin is well-tolerated but has a reduced systemic bioavailability. Polycurcumins (PCurc 8) and curcumin encapsulated in biodegradable polymeric nanoparticles (NanoCurc) showed higher bioavailability than curcumin together with a significant tumor growth inhibition in both *in vitro* and *in vivo* studies. **Keywords:** ANTICANCER AGENTS, PHYTOCHEMICALS**,** CURCUMIN, MOLECULAR TARGETS, BIOAVAILABILITY.

At the beginning of the 21st century, cancer is still a major global public health issue. Recent statistics show that cancer is the second-leading cause of death after cardiovascular diseases. But it is the first most frequent cause of death in 45-65 years old people including a high percentage of working population. The most common cancers in term of incidence are the lung, breast and colorectal cancers. Cancer incidence is supposed to increase dramatically in the next years. In these circumstances, cancer is a research priority in understanding its basic mechanisms, identification of early diagnostic methods and especially in development of efficient treatment methods. In recent years, interest for anticancer drugs with high efficiency and good tolera-

bility has increased and it will probably continue to increase in the near future (1, 2).

PHYTOCHEMICALS IN CANCER TREATMENT

In the last fifty years many plant-derived agents played a significant role in cancer chemotherapy. Numerous phytochemicals with antitumor activity have been isolated from plants. Only few advanced into clinical use; most of them served as prototype chemical structures for semisynthesis and synthesis of more potent analogues (3).

The first plant-derived anticancer agents in clinical use were vinblastine and vincristine, bisindole alkaloids isolated from *Catharanthus roseus* G. Don. sin. *Vinca rosea* L. (*Apocynaceae*). Vinblastine, vincristine, semisynthetic derivatives of vinblastine (vinorelbine, vindesine) block mitosis in metaphase leading to apoptosis. They bind to microtubule ends suppressing their dynamics which is essential for chromosomes movements to the two poles of the mitotic spindle. Vinblastine (Velbe®), vincristine (Oncovin®), vinorelbine (Navelbine®) and vindesine (Eldisine®) are used in the treatment of leukemias, lymphomas, advanced testicular, breast and lung cancers, Kaposi's sarcoma (3, 4, 5).

Podophyllotoxin, an aryltetralin lignan, was first isolated from *Podophyllum peltatum* L. (*Berberidaceae*). Later on, it was isolated from other *Podophyllum* species (*P. emodi* Wall sin. *P. hexandrum* Royle, *P. pleianthum* Hance).

Podophyllotoxin blocks polymerization of tubulin and mitotic spindle assembly arresting mitosis in metaphase. All trials regarding clinical uses of podophyllotoxin failed because its severe side effects (nausea, vomiting, damage of normal tissues). Therefore, extensive studies were carried out in order to obtain more potent and less toxic semisynthetic derivatives. These studies led to etoposide and teniposide, cyclic acetals of epipodophyllotoxin-glucoside.

Unlike podophyllotoxin, these derivatives irreversibly inhibit catalytic activity of DNA topoisomerase II. Etoposide and teniposide form stable complexes with DNA and topoisomerase II. These complexes induce irreversible breaks in single- and double-stranded DNA leading to cell death. Etoposide (Vepesid®, Etopophos®, Eposin®) and teniposide (Vumon®) are used in the treatment of lymphomas, acute leukaemia, small cell lung, testicular, ovarian and bladder cancers (3, 4, 6, 7).

Paclitaxel (taxol) is a polyoxygenated diterpenoid isolated from *Taxus brevifolia* Nutt. (*Taxaceae*, Pacific Yew) and other *Taxus* species (*T. wallichiana* Zucc.). Like other plant-derived antitumor agents, paclitaxel is a spindle poison. But its mechanism of action is completely different and unique. Paclitaxel stabilizes the microtubules supressing their depolymerization to tubulin. In addition, paclitaxel induces apoptosis by inactivating apoptosis stopping protein Bcl-2. Paclitaxel (Taxol®, Abraxane®) is used in refractory ovarian cancer, metastatic breast and lung cancer, Kaposi's sarcoma. Docetaxel, a semisynthetic analogue of paclitaxel, is more active than the latter. Docetaxel (Taxotere®) is used for the treatment of metastatic breast cancer and non-small cell lung cancer (3, 4, 6, 8).

Campthotecin is a quinoline alkaloid isolated from *Camptotheca acuminata* Decne (*Nyssaceae*), *Ophiorrhiza pumila* Champ. ex Benth. (*Rubiaceae*), *Mapia foetida* Miers. (*Acanthaceae*). Campthotecin selectively inhibits DNA topoisomerase I but it was

withdrawn from clinical trials due to its severe toxicity (nephrotoxicity, myelosuppression). Topotecan and irinotecan are the only semisynthetic analogues of camptothecin in clinical use. Both compounds are soluble in water and have the same mechanism of action as camptothecin: they bind covalently to topoisomerase I blocking the enzyme activity. Topotecan (Hycamtin®) is used for the treatment of ovarian cancer and small cell lung cancer while the major therapeutic indication for irinotecan (Camptosar®) is the treatment of colorectal cancer (3, 4, 6, 8).

Other plant-derived anticancer agents in clinical use are homoharingtonine and elliptinium. Homoharingtonine, an indole alkaloid from *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) (*Cephalotaxaceae*), induces apoptosis, tumor growth and metastasis by inhibiting tumor neoangiogenesis. Homoharingtonine (Ceflatonin®) is used in various leukemias, including those resistant to standard treatment. There have been reported cases of complete remission in patients with chronic myelogenous leukemia. Elliptinium is an ellipticine derivative, an indole alkaloid isolated from several genera of the *Apocynaceae* family, especially from *Bleekeria* genera (*Bleekeria vitensis* A. C. Sm.). It acts similar to alkylating agents by forming covalent linkages with DNA. Elliptinium (Celiptium®) is used in the treatment of breast cancer (3).

Numerous plant-derived anticancer agents are in the clinical and preclinical development; some of them proved to be very potent and might become anticancer drugs in the near future.

Combrestatins are compounds with different structures (bibenzyl, stilbene, phenantrene skeleton) isolated from *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (*Combretaceae*).

The most active combrestatin, combrestatin A-4, is a stilbene derivative; it binds to beta-tubulin blocking microtubule and mitotic spindle assembly. Its phosphate, hydrosoluble, is now in phase II clinical trials in USA and Great Britain. Magnetic resonance imaging experiments showed that combrestatin A-4 phosphate reduces blood flow to tumor cells. In bioassays based on animal models a sodium phosphate derivative of combrestatin A-4 blocked blood supply in metastatic tumors leading to tumor necrosis. Numerous structural analogues of combrestatin A-4 have been synthetized; some of them are in clinical and preclinical trials (3, 4, 6).

A promising case is that of betulinic acid, a lupane-type pentacyclic triterpene, very common in many plants. Betulinic acid has selective cytotoxicity against several human melanoma cancer cell lines (MEL-1, MEL-2, MEL-4, B16). Betulinic acid induces apoptosis through multiple mechanisms: rapid increase in intracellular reactive oxygen species production, disruption of mitochondrial membrane potential, DNA fragmentation. Studies on its possible clinical use in topical and systemic formulations are in progress (3, 4, 9).

Many plant-derived anticancer agents, investigated in preclinical and clinical trials, are potent inhibitors of cyclin-dependent kinases. These enzymes play a key role in cell cycle regulation and their overexpression is associated with tumorigenesis. Important examples are roscovitine and purvalanols (synthetic analogues of olomucine, purinic derivative from cotyledons of radish, *Raphanus sativus* L., *Brassicaceae*), indirubins (bis-indoles from *Indigofera tinctoria* L., *Leguminosae* and their substituted derivatives) (3, 10).

CURCUMIN AND MOLECULAR TARGETS IN CANCER PREVENTION AND TREATMENT

In the last years, numerous studies reported that curcumin has a huge potential in cancer prevention and treatment. Curcumin, a polyphenol with a diferuloymethane skeleton, was first isolated from from the rhizome of *Curcuma longa* L. sin. *C. domestica* Valeton (*Zingiberaceae*), an Indian species commonly known as turmeric. The therapeutic potential of curcumin was investigated in numerous inflammatory, cardiovascular, neurodegenerative, immunologic and metabolic disorders. Besides, there are many reports on the capacity of curcumin to induce cell cycle arrest and apoptosis and reduce tumor invasion and angiogenesis. The mechanisms are numerous and complex involving modulation of both cell signaling pathways and enzymes activity (11-16).

The overexpression of COX-2 in different types of tumors has already been reported. In the hormone-dependent breast cancer, COX-2 stimulates aromatase activity increasing estrogen secretion. Curcumin inhibits COX-2 activity and consequently reduces estradiol secretion in patients with hormone-dependent breast cancer (12, 13).

In the malignant cells, the aberrant activity of the transcription factors induces not only the tumor progression but also the resistance of the tumor cells to chemotherapy and radiotherapy. Many of the tumor cells protect themselves against apoptosis *via* nuclear factor-kappa B (NF-kB), one of the main transcription factors that regulate the expression of many genes involved in inflammation, cell proliferation and survival. Curcumin blocks the degradation of the unit alpha of NF-kB inhibitor (IkB); thus NK-kB remains in a latent, inactive form in the cytoplasm being unable to enter the nucleus, bind to DNA and activate transcription (12, 13). In multiple myeloma, lymphomas, leukemias, certain solid tumors there has been reported an increased activity of the signal transducer and activator of transcription (STAT). STAT induces cell growth and proliferation by increasing the expression of Bcl-2 and Bcl-xl thus inhibiting the apoptosis. It is obvious that STAT modulation might be a possible approach in cancer therapy. Curcumin inhibits the phosphorylation of STAT3 thus blocking its translocation to the nucleus. Curcumin also inhibits STAT1 phosphorylation but not STAT5 phosphorylation (12, 14).

The p53 tumor suppressing gene acts as a transcription factor which activates the genes involved in the regulation of the cell cycle. In normal cells, it blocks the cell cycle in the G1/S phase until the DNA lesions are repaired. If the lesions are extensive and cannot be repaired, p53 initiates apoptosis. The mutations in the p53 gene represent the most frequent genetic modification seen in human cancers. In head and neck squamous cell carcinoma curcumin selectively induces apoptosis in the G2 phase of the cell cycle mainly by regulating the expression of p53 (13, 14).

Cyclin D1 is a nuclear protein involved in the cell cycle regulation. Cyclin D1 and the cyclin-dependent kinases Cdk4 and Cdk6 are responsible for the transition from the G1 phase to the S phase of the cell cycle. Cyclin D1 overexpression leads to the abnormal proliferation of the cells by shortening G1 phase. In tumor cells curcumin regulates the aberrant expression of Cyclin D1 and the activity of Cdk4 (13, 14).

The tumor transformation is often correlated with an abnormal activity of the growth factors, their receptors and signal-

ing pathways. The aberrant expression of the epidermal growth factor receptors (EGFR) was reported in numerous solid tumors, including colorectal cancer. By blocking the receptors of the epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), curcumin significantly inhibits the neoangiogenesis which allows tumor growth and metastasis (12).

Curcumin inhibits the tumor invasion and angiogenesis by inhibiting the matrix metalloproteinases (MMPs) overexpression. A significant growth in the synthesis of MMPs is correlated with an aggressive tumor development and a high metastatic potential. MMP-2 and MMP-9 have recorded a significant decrease in the prostate and breast cancer cells treated with curcumin. In the cell line Colo-205 curcumin reduced the activity of MMP-2. There was also reported a reduced degradation of the extracellular matrix that is strongly involved in the tumor dynamics (12, 14, 15).

The tumor necrosis factor (TNF) represents a family of proinflammatory cytokines playing a major role not only in inflammation but also in tumor initiation and proliferation. The proinflammatory effects of TNF are mainly due to its ability to activate NF–kB. TNF-alpha, a vital member of the TNF family, induces the phosphorylation and degradation of IkB; NF-kB is thus released from its cytoplasmic location being able to enter the nucleus and act as a transcription factor. Curcumin suppresses TNF-alpha signaling by inhibiting NF-kB translocation into the nucleus (12, 14).

The mitogen-activated protein kinases (MAPKs) signaling pathway is intensively studied as a possible target in cancer prevention and treatment. In human ovarian cancer cells resistant to cisplatin, curcumin induced apoptosis by modulating the activity of p38 MAPK, kinases involved in NF-kB activation (1).

CURCUMIN IN HUMAN STUDIES

The promising results in the phase I clinical studies regarding the effects of oral administration of curcumin in patients with pancreatic, cervical and cutaneous cancer supported the initiation of phase II studies in which curcumin proved high efficacy in patients with colorectal and pancreatic cancers (12, 15, 16).

In a phase II study, curcumin (8 g per day for 8 weeks) significantly reduced NF-kB, COX-2 and STAT3 expression in patients with pancreatic cancer. At the beginning of the study, most of the pacients had significant higher levels of these markers than those found in healthy volunteers. No toxic effects were recorded. Despite its limited absorption, curcumin was active in patients with pancreatic cancer (17).

In another study, 62 patients with cancerous lesions on some external organs (vulva, breast, skin) were treated topically with curcumin. There were reported a reduction of itching in almost all patients, a reduction of lesions in 10% of the patients and a reduction of exudates in 70% of the patients. An increase of local itching was reported in a single patient with scalp melanoma (12).

A prospective phase I clinical study on 23 patients with high-grade prostatic intraepithelial neoplasia revealed the safety and tolerance of a new plant-based supplement, Zyflamend®, one of its ingredients being curcumin. Zyflamend® was administered orally (780 mg x 3/day for 18 months) both as monotherapy and in combination with nutritional supplements (probiotics, multivitamins, green and white tea extracts, docosahexaenoic acid). The stand-

ard biomarkers and the prostate-specific antigen (PSA) were monitored every three days. At the end of the treatment, reduced values for the C reactive protein and NF-kB were determined in the serum. 40% of the patients showed a 25-50% reduction of PSA. Zyflamend® was well-tolerated by all the patients (17).

CURCUMIN TOLERANCE AND BIOAVAILABILITY

Clinical studies showed that the administration of high doses of curcumin (2-12 g/day) generated few side effects, mild nausea or diarrhea (15). However, curcumin has a reduced systemic bioavailability at oral administration (11). This reduced bioavailability is reflected in its inability to inhibit the pulmonary and mammary tumors in patients treated *per os* and intravenously. Curcumin is practically insoluble in water whereas in neutral and alkaline solutions its degradation occurs very quickly. Several potential transport systems were tested in order to increase the bioavailability of curcumin such as: nanoparticles, liposomes or conjugates with polyethilenglycol.

PCurc 8 is a polyacetal-based polycurcumin, soluble in water and stable under physiological conditions. *In vitro* studies on several cancer cell lines (SKOV-3, MCF-7, OVCAR-3) showed that PCurc 8 blocks the cell cycle in the G0/G1 transition phase or in the G1 phase mainly through a caspase-3 dependent pathway. *In vivo* studies also showed a remarkable tumor-inhibiting capacity of this polymer (18).

NanoCurc, curcumin encapsulated in biodegradable polymeric nanoparticles, has a high systemic bioavailability when compared to pure curcumin. Administration of NanoCurc in association with Gemcitabine determined a full regression of the metastases in orthotopic pancreatic cancer models. The inhibition of the tumor growth was accompanied by a reduction in the NF-kB activity and MMP-9 and cyclin D1 expression (19).

CONCLUSIONS

The capacity of curcumin to modulate numerous cell signaling pathways and to interact with different molecular targets proves the real therapeutic potential of this compound. Both *in vitro* and *in vivo* studies support the therapeutic value of curcumin. Further studies are needed to identify the analogues with high bioavailability and efficiency in cancer prevention and treatment.

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EVALUATION OF CARDIAC PARAMETERS USING ELECTRICAL IMPEDANCE PLETHYSMOGRAPHY

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EVALUATION OF CARDIAC PARAMETERS USING ELECTRICAL IMPEDANCE PLETHYSMOGRAPHY (Abstract): Impedance plethysmography is a method for determining changes in body tissue volumes, based on the measurement of electric impedance at the body surface. Because it is a non-invasive and cost-effective method to measure and analyze hemodynamics, it would bring to physiologists very important diagnostic information for the continuous care of critical patients with hemodynamic disorders. **Methods:** This study aimed at acquiring non-invasively and at analyzing plethysmografic waves by measuring the electrical impedance. For measurements we built a device that was designed as an injection module with a microcontroller, a programmable waveform generator, and a voltage controlled current source which generates a sinusoidal pulse with adjustable frequency (10–200 kHz) and adjustable intensity. In output, the device delivers an analogical signal representing the variation of the impedance of the explored section. The acquisition of the bioimpedance signal on PC was made easier by the use of a National Instrument data acquisition device, the NI USB 6009. **Results:** The bioimpedance signal processing, the user interface and the display were managed by MATLAB. Sample impedance data for a test subject are presented. From the experimental results, it is shown that the proposed bioimpedance measuring system could provide good performance over the frequency range for central or peripheral territory. **Conclusion:** The clinical importance of these findings is that this method can measure vascular parameters noninvasively, continuously and more conveniently in humans. **Key words:** IMPEDANCE, MICROCONTROLLER, BLOOD FLOW, CARDIAC PARAMETERS, VOLTAGE CONTROLLED CURRENT SOURCE.

In biomedical engineering, the term of bioimpedance is used in order to describe the response of a living organism to an external electric current applied on it. This technique is used on humans and animals in order to measure certain parameters, such as the blood flow or organism composition. The accumulation of knowledge in bioimpedance field led to the development of devices for measuring the cardiac flow and the circulating blood volume in different territories. The biologic materials, such as blood and muscular tissue, are poor conductors, as compared to other materials classified as conductors, (for instance, copper), at the frequencies used in order to measure the physiologic activity ($f < 10^6$ Hz). At higher frequencies, these biological materials have resistive properties, and at high frequencies they behave as dielectrics. The interest frequencies in the physiologic activity assessment by impedance are, usu-

ally, comprised between 20 kHz and 200 kHz. In order to discharge of the influence of other physiologic activities, such as ECG, EEG, EMG and the inferior limit frequency is set at 20 kHz. At the same time, the injected currents' intensities should not exceed some milliamperes in order to avoid muscles and nerves' stimulation (1).

The human tissue has both resistive properties and capacitive ones. Nowadays, there are several attempts of equivalent electrical circuits in order to describe the electrical properties of the tissues. The most used attempt comprises three components. It can comprise two resistances and a condenser or two condensers and a resistor. Figure 1 (a) presents the serial equivalent bioimpedance circuit, formed of two resistances and a condenser.

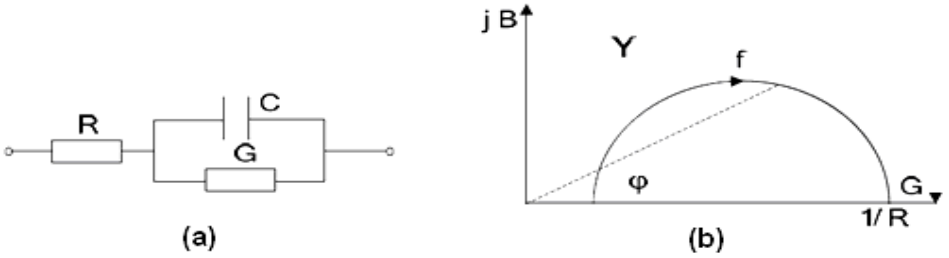

Fig. 1 The serial circuit 2R – 1C (a) and its response in frequency (b)

The circuit allows feeding in alternating current (DC) and current limitation at high frequencies. It is possible to obtain the same values for several frequencies for a single set of values of the components. The series circuit model characterizes the best tissue electrical impedance phenomenon, because the time parameter is uniquely defined (equations 1-7). This circuit was often used as an electrical equivalent of the skin, with resistance (R) representing the more profound series tissues with the skin formed of (G) and (C) in parallel. The response in frequency of this circuit is represented in figure 1 (b). The electrical model of the series circuit 2R-1C can be described by the following equations:

$$Z = R + \frac{G - j\omega C}{G^2 + \omega^2 C^2} \quad (1)$$

$$Z = R + \frac{1}{C(1 + j\omega\tau_z)}$$

$$\tau_z = \frac{C}{G} \quad (2)$$

$$\varphi = \arctan\left(\frac{\omega C}{G(1 + RG) + \omega^2 C^2 R}\right) \quad (3)$$

$$\varphi = \arctan\left(\frac{\omega C}{G(1 + RG + \omega^2 \tau_z \tau_2)}\right) \quad (4)$$

$$Y = \frac{G(1 + RG) + \omega^2 C^2 R + j\omega C}{(1 + RG)^2 + \omega^2 C^2 R^2}$$

$$Y = \frac{G(1+RG+\omega^2\tau_z\tau_2 + jw\tau_z)}{(1+RG)^2+(w\tau_2)^2} \quad (5)$$

$$\tau_2 = CR$$

$$C_{ext} = \varepsilon' = \frac{C}{(1+RG)^2+(\omega\tau_2)^2} \quad (6)$$

$$\varepsilon'' = \frac{G(1+RG+\omega^2\tau_z\tau_2)}{\omega[(1+RG)^2+(\omega\tau_2)^2]} \quad (7)$$

The impedance is the parameter which characterizes the two resistances and a condenser in series, because it is defined by a time parameter (τ_Z) (eq. 2). This time parameter is independent of (R) if the circuit was passed through by current. As a consequence, the impedance parameter has the advantage of measuring the characteristic frequency determined by (τ_Z) and is directly connected to the capacity (C) and conductance (G) connected in parallel (for instance, the membrane effect in tissues), unaffected by an access resistance.

MATERIAL AND METHODS

For the purpose of reducing the action areas derived from the stimulation electrodes and electrodes polarization itself, the technique adopted in the study is the method of the four electrodes. A pair of electrodes injects a constant current and variable frequency in the human tissue, and another pair of electrodes measures the potential difference between them (fig. 2) (3).

Figure 3 presents the block diagram of the proposed system of measuring the bioimpedance. It contains a microcontroller, a programmable wave generator and a source of current controlled in tension. Bioimpedance signal acquisition on a PC was performed by using a data acquisition device, USB NI 6009 from National Instruments. Located between the acquisition system and the PC, it ensures bioimpedance signal digitalization and its transfer on the PC for a subsequent processing.

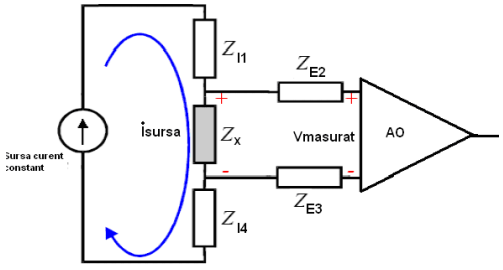

Fig. 2. The method of the four electrodes:
Z_{i1}, Z_{i4} – current injection electrodes;
Z_{E1}, Z_{E3} – signal collection electrodes

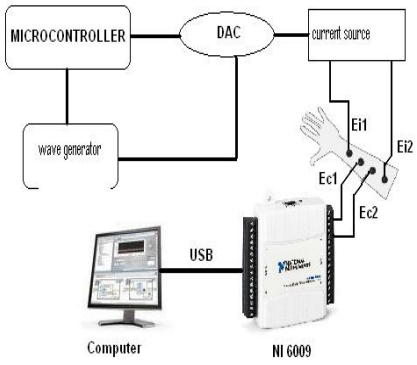

Fig. 3. Block diagram of the measuring system

The microcontroller employed belongs to MSP 430 class from Texas Instruments. They represent a family of microcontrollers with very low energy consumption, consisting of several circuit elements, which, interconnected among them, form different modules specific for medical applications. The microcontroller was modified in order to be fed from a 9 V battery too, for the purpose of being used also in portable monitoring applications.

The current sources are usually designed and produced using the following types of circuit: the mirror current type, feedback multi-operational and modified Howland circuit. The structure of the latter is very simple and has a high performance. The basic diagram of Howland designed circuit is presented in fig. 4.

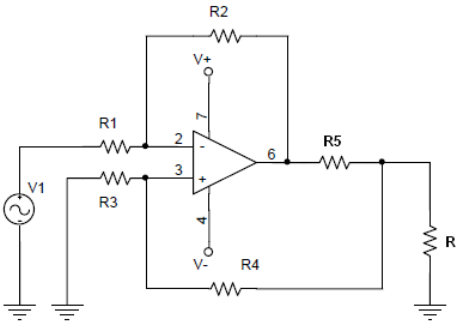

Fig. 4. Current source circuit diagram

After the practical achievement of the current source, a necessary condition is its calibration. In order to do so, resistor networks with tolerance of 1% were built. Networks with different values were tested (10 Ω, 150 Ω, 300 Ω, 510 Ω, 1000 Ω) and their impedance at discharge was measured for different frequencies.

Impedance signal acquisition on the PC was performed by using National Instrument data acquisition device, USB NI 6009. This card, placed between the equipment and the PC, ensures bioimpedance signal digitalization and its transfer to the PC for a subsequent processing. NI USB 6009 features: 8 analogical entrances (14-bit, 48 kS/s), 2 analogical discharges (12-bits, 150 S/s), 12 I/O digital; 32-bit counter, bus-powered for high mobility via the USB; signal connectivity. USB-6009 was configured and tested using MAX (Measurement and Automation Explorer).

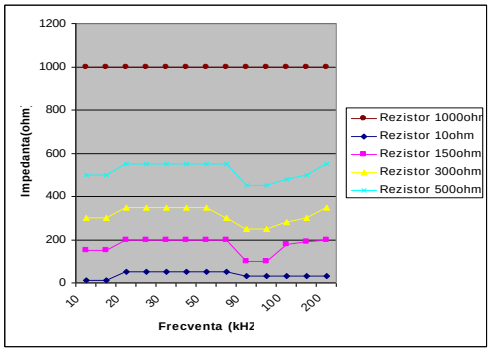

Fig. 5. System calibration – impedance variation for a calibration network value according to frequency

RESULTS AND DISCUSSION

The data analysis program was developed in MATLAB. It offers the opportunity of creating user interfaces by the Graphical User Interface Instrument GUIDE. The purpose of implementing the graphical user interface (GUI) in this project, was of obtaining programs more easy to use by means of certain visual elements associated with control actions and display actions as slides, buttons, menus, diagrams, etc. (5).

A graphical user interface offers to him a manner of interoperating very efficiently with all the elements coordinated by this environment. Its achievement requested the construction of a dendritic structure, based on two directions: one specific to the per-

sonal data associated to each patient, and the other associated to the analysis and determination of certain parameters specific to the plethysmographic waves.

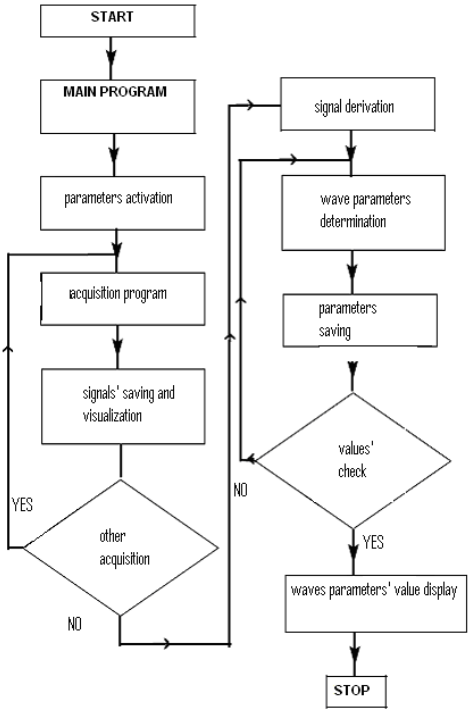

Fig. 6. Logical acquisition and processing diagram of the impedance signal

The total measured impedance Z (t) has three components: Z_0, $Z_R(t)$ and $Z_H(t)$. The component with the higher weight is the thoracic impedance Z_0, which reflects the electrical characteristics of the total thoracic mass (tissues, liquid and air). In a healthy man, Z_0 increases at about 25 Ω, and in a woman the values are, on an average, by 5 Ω higher. The second component, $Z_R(t)$, reflects the respiratory activity, which induces impedance changes of about 1 Ω. The third component, $Z_H(t)$, is synchronized with the cardiac cycle and reaches 0.10-0.15 Ω. As Z_0 does not modify in time, $Z_R(t)$ and $Z_H(t)$ determine together the modifications of the total thoracic impedance. The records were performed on 20 subjects, clinically normal. The values obtained at central level are presented in table I.

TABLE I

The average values of the central cardiac parameters

Parameter measured	Value
Cardiac rhythm (beats/min)	63.8±5.4
Beat volume (ml)	104.2±28
Pre-ejection period (ms)	125.7±9
Cardiac index	8.2±2
LVET (ms)	180±16

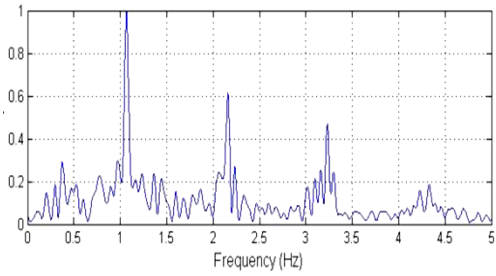

Fig. 7 The power specter of the plethysmografic signal

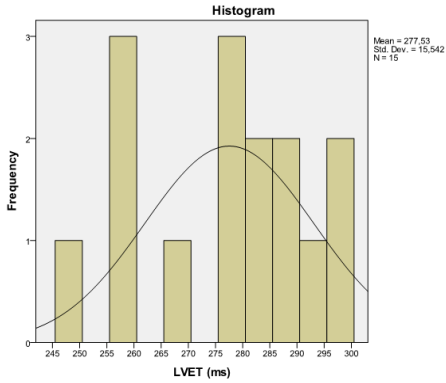

Fig. 8 Variable LVET distribution reported to the normal distribution for the studied group

CONCLUSIONS

The final solution chosen in the design and construction of the acquisition system of the electrical impedance signal proved to be viable and it corresponded from a technical point of view. The software used in calculating the central and peripheral vascular parameters was achieved by means of an accessible working environment.

The electrical impedance plethysmography is a technique which can be used on a wider scale for hemodynamic assessments. It can occupy an important place in assisting the physician in the diagnosis and continuous surveillance at patient's peripheral and central vascular level. Knowing the vascular modifications due to the use of heat and different cold modalities, physical exercises and walking can provide precious information on the circulating system subject to an effort. Determining vascular modifications associated with therapeutical modalities, vasoactive drugs and the disease conditions can offer useful orientations for a future treatment. The electrical impedance plethysmography technique has a high potential for the use in hospital and in the ambulatory, in patient care department, but also in research.

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PATIENTS' SATISFACTION WITH CONVENTIONAL DENTURES AND MINI IMPLANT ANCHORED OVERDENTURES

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PATIENTS' SATISFACTION WITH CONVENTIONAL DENTURES AND MINI IMPLANT ANCHORED OVERDENTURES (**Abstract**): The aim of this study was to assess complete edentulous patient satisfaction with two treatment alternatives - conventional dentures and mini implant anchored overdentures. **Materials and methods**: Study sample comprised 36 patients - 18 treated with newly made conventional prosthesis and 18 with mini implants anchored overdentures, with a ball attachment system (from IMTEC Corporation). **Results:** Patients treated with mini implant anchored overdenture presented a higher satisfaction regarding chewing ability (all of them reported satisfactory mastication, 50% accusing occasionally discontent) compared with the conventional denture group (28% reported impaired masticatory function and 39% occasionally discontent). Denture's retention was better in the mini implant overdenture sample (only 17% reported occasional problems, none of them feel the need to use denture adhesives), compared to the conventional treatment alternative (28% were dissatisfied about this issue). Both treatment alternatives were seen as being highly satisfactory regarding the aesthetic restoration of facial appearance and for performing phonation. Still, in the complete denture group 22% of patients claimed difficulties in articulating certain phonemes. The main reasons why patients refused implant treatment were its higher cost and fear of surgery. **Conclusions:** Patients treated with mini implant anchored overdentures were more satisfied than those treated conventionally, due to the increased retention and stability of dentures, mastication efficiency and improved comfort, these having probably a positive effect on the quality of life. **Key words:** PATIENT SATISFACTION, DENTURE, OVERDENTURE, MINI IMPLANTS, MASTICATION.

According to World Health Organization, complete edentulism - as a result of loss of all teeth, treated with complete conventional denture - is a form of disability for most people, due to the reduced functionality and poor balance of the prosthesis (1, 2). Patient satisfaction with the prosthetic device depends on his/her ability to masticate, speak, and also on the aesthetic appearance after treatment. Berg and colleagues have shown that 66% of patients with conventional dentures were dissatis-

fied due to general discomfort, poor denture retention and chewing ability, and pain associated with wearing dentures, especially in the mandible. Even after making new dentures, 10 to 45% of patients were still dissatisfied with the treatment outcome in general, complaints related to prosthesis instability, mastication or aesthetics being present (3).

Currently, there is an increased interest for treatment alternatives indicated in complete edentulism - especially for implant supported ones. These are seen as modern and successful treatment options, indicated especially in high difficulty clinical cases, where there are lower odds to obtain a successful outcome by restoring the edentulous with conventional dentures (4). Although implant overdentures have become a first choice treatment for mandibular complete edentulism, as McGill Consensus states, still there are many clinical situations where the application of conventional implants is impossible without prior surgical interventions (e.g., bone split, bone addition) (5). Frequently, given the advanced age of patients, an associated systemic pathology is present, that may be related to a poor tolerability of surgical interventions.

Small diameter implants - called mini implants - can be a solution in many high difficulty edentulism cases, in which conventional implants have no applicability. There is a lack of scientific evidence regarding this treatment alternative, being present a limited number of longitudinal studies. The success rate reported by most authors exceeds 92.6%, on an observation period up to six years. Among the advantages of mini implant supported overdentures can be mentioned the minimally invasive characteristic of the technique, the immediate loading of implants, improved prosthetic device functionality and the reduced treatment cost (6-10). These are some of the reasons why, nowadays, this alternative presents an increased popularity.

The aim of this study was to assess complete edentulous patient satisfaction with two treatment alternatives - conventional dentures and mini implant anchored overdentures. Also, it was evaluated the perception of edentulous patient on implant anchored overdentures, as an alternative to the conventional treatment.

MATERIAL AND METHODS

At the Dental Prosthodontic Clinique, Department of Complete Denture, Faculty of Dental Medicine, Bucharest, an observational study was conducted on a group of 36 complete edentulous patients, seen from 2009 -2010. 21 were women and 15 were men, aged between 50 and 85 years. Of the 36 patients selected for study, 18 received conventional complete dentures and 18 mini implants anchored overdentures. The patients considered eligible to be included in the study were complete edentulous, with good oral hygiene, no local inflammatory processes or disorders of the oral mucosa, who had no general conditions to contraindicate surgery.

Conventional complete dentures were performed according to the current guidelines in this field. For implant overdentures, mini implants from the IMTEC Company were used – being applied a minimum of 4 in the lower jaw and 6 in the upper jaw. The mini implants used had different lengths (10, 13, 15, 18 mm) and diameters (1.8, 2.1, 2.4 mm). A ball attachment system was used, the implant being fitted with spherical crown portion. The complete denture pre-

sented a corresponding part - a metallic matrix with O-ring. The type of implant loading was chosen based on the quality of bone and insertion torque measured at the implant site. For values below 35 Ncm delayed loading of implants were used and over this value, the implants were loaded immediately (11-13).

One month after treatment's end, the patients were asked to complete a questionnaire, designed by the authors. The first part of the questionnaire recorded patient's personal data (name, address, age, educational level, and profession, occupation and life conditions), the reason for treatment request, personal and family history – systemic and local. The second part of the questionnaire evaluated patient's satisfaction with the old dentures, as well as with the newly ones - by questioning the level of dentures' retention, mastication ability, and comfort with dentures, phonation, and esthetics. The last part of the questionnaire contained questions used for evaluating patient's perception on dental implants, aspects regarding the financial possibilities and his/her interest in this treatment alternative.

RESULTS

Patients treated with mini implant anchored overdentures and conventional dentures presented similar characteristics (table I).

TABLE I

Characteristics of the patients

Variables		**Conventional dentures**	**Mini implant overdentures**
number of patients		18	18
Age	Mean	68.5	64
	Range	52-85	50-76
Sex	Male	6	9
	Female	12	9
Period of edentulousness	Upper Jaw	3-30 years	3-8 years
	Lower Jaw	3-30 years	6-15 years
Level of education	Low	8	6
	Medium	8	6
	High	2	6

In the conventional denture group, the main reasons of patient dissatisfaction with the previous prosthesis were the following: 33% of women and 50% of men reported problems in mastication and regarding the aesthetics; 17% mentioned denture's poor retention; 17% claimed the fracture of the prosthetic device; 16% accused phonation problems. Other complaints were related to teeth wear and deterioration of the material. The patients received a total of 32 dentures, of which 54% were maxillary and 46% mandibular dentures. After the application of newly made conventional dentures, an improvement in overall satisfaction with the prosthetic device related to aesthetics and mastication efficiency has been documented. Still, 28% reported masticatory deficiencies and retention problems, 17% frequently using denture adhesives. All

patients declared themselves satisfied with the aesthetics and with the restoration of the facial contours. 22% claimed difficulties in articulating certain phonemes (fig. 1). Perceived discontents were different according to sex, men being usually more satisfied. 67% of women and 83% of men reported no retention deficiencies. 25% of women and 50% of men reported a satisfactory mastication.

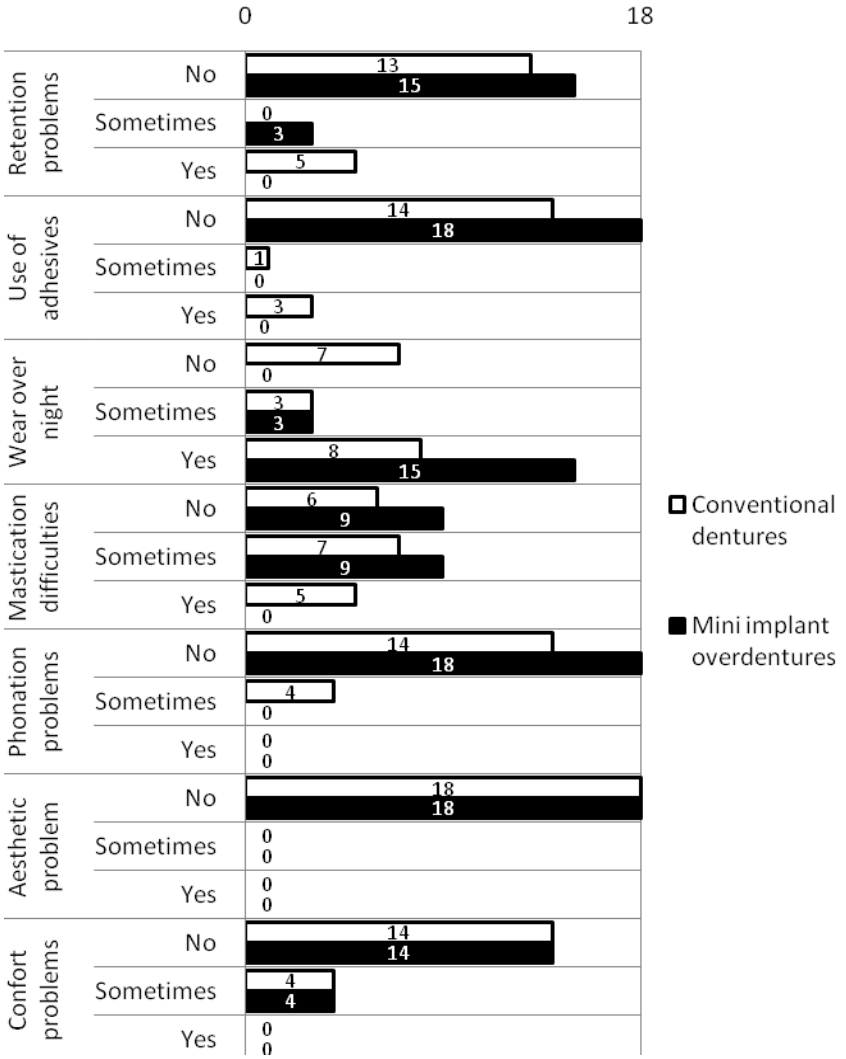

Fig. 1. Comparative assessment of newly made complete dentures and mini implant overdentures

In the mini implants anchored overdenture group, a total of 18 dentures (2 maxillary and 16 mandibulary) were fabricated. Within this group of patients, the main

dissatisfaction reasons with the old conventional dentures were related to poor retention and its stability. 100% of women and 75% of men reported difficulty during chewing. Other reasons were related to aesthetics problems, phonation, and dentures deterioration in time. With mini implant overdentures, an improvement of patient's perception regarding prosthesis's stability and chewing ability was noticed (fig. 1). Denture's retention was not considered a problem anymore by a rate of 89% of women and 78% of men, the rest showing only some occasional problems. Mastication was satisfactory for all patients, occasionally discontents being reported by 33% of women and 67% of men. The aesthetics, phonation and comfort have not been problematic for patients who were generally satisfied by these issues.

Regarding patients' perception on the use of dental implants, with the purpose of retaining conventional dentures, from the conventional denture group, women had a greater interest than men for the use of dental implants and mini implants (75% women compared to 67% men). From them 1 patient declared that he doesn't know about this treatment alternative (fig. 2). Among the main reasons why patients refused implant treatment were their high cost and fear of surgery.

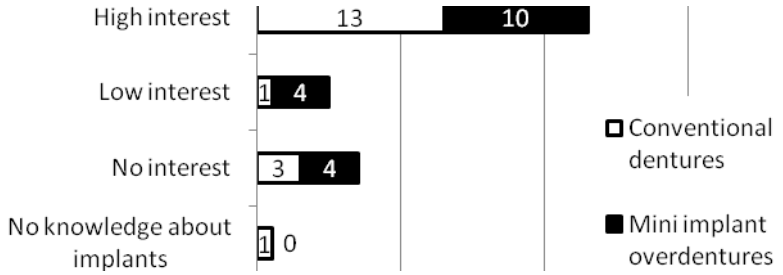

Fig. 2. Patients' perception and knowledge on implant supported overdentures

DISCUSSION

Implants supported overdenture treatment is considered beneficial especially for the patients who had persistent problems related to the retention and stability of conventional dentures, especially in the lower jaw. This treatment alternative favors an improved patient satisfaction with the prosthetic device, however, being quite restrictive in terms of cost, but also in terms of local and general clinical features cannot be considered a routine treatment for all complete edentulous patients.

In literature, studies have reported an overall patient's satisfaction with conventional dentures of 34%, comparing with 60% in patients with overdenture treatment. In the current study, the results are similar, both groups of patients reporting improved functionality with dentures, considerably higher in mini implants overdenture group. Patients with lower levels of education and lower income were more satisfied with chewing ability, aesthetics of denture, and phonation in both groups studied. This may be the consequence of the fact that these patients had lower expectations regarding treatment outcome. Patients who experienced many problems in previous treatments showed more dissatisfaction with the

current prosthesis, unlike those who considered previous prosthesis as being satisfactory. Patients' age influenced the ability to adjust to the dentures - older patients on their first prosthesis reporting more difficulties in adapting compared to the youngest ones.

The improvement in masticatory function is the main benefit brought by the use of implant therapy for edentulous patients. According to literature data, only 15% of patients treated with conventional denture are able to chew harder foods in comparison with a percentage of 88% to 96% patients treated with implant overdenture (10). In our study, among patients with mini implant overdentures, mastication was generally seen as being satisfactory, only occasionally chewing discontent being reported. Patients receiving conventional treatment presented lower chewing ability, being higher in male than in women. In terms of improving social life, patients among overdenture group reported an improvement - retention not being seen as a problem, adhesives not being used at all.

In general, edentulous patients from our sample showed knowledge and increased interest regarding implant treatment alternative. Few patients showed little interest in implant treatment, the main reason being the fear of surgical intervention. Other reasons for refusal of implant treatment were the cost and the insufficient information about this alternative.

CONCLUSIONS

Conventional dentures performed correctly can provide a satisfactory treatment outcome for a large number of complete edentulous patients, treatment's success depending on a number of factors related especially to the doctor's and technician's experience, and to the local conditions and patient's behavior. Patients treated with mini implant anchored overdentures were more satisfied than those treated conventionally, due to the increased retention and stability of dentures, mastication efficiency and better physical comfort. Although the cost of the implant prosthesis is considered high by most patients, they begin to be aware and to understand the benefits of this treatment alternative. Mini implants anchored overdenture offers, beside a minimally invasive intervention, the possibility of immediate functionality, reducing time and cost, being indicated in older people with complete edentulism, especially in the lower jaw.

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CONTRIBUȚII LA STUDIUL COMPOZIȚIEI CHIMICE A ULEIULUI VOLATIL EXTRAS DIN FRUCTE DE CORIANDRU SOIUL SANDRA

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2. Disciplina de Chimie fizică

CONTRIBUTIONS TO THE CHEMICAL STUDY OF THE ESSENTIAL OIL ISOLATED FROM CORIANDER FRUITS (SANDRA CULTIVAR) **(**Abstract): *Coriandrum sativum* L. (*Apiaceae*) is a well known herb, native to the Mediterranean region, also intensively cultivated in Romania. The essential oil obtained from *Coriandri fructus* posseses antimicrobial, antioxidant and anxiolytic effects. Many parameters such as genetic and climatic factors or agronomical practices can influence the yield and composition of the volatile fraction. Plant density is an important factor for the microenvironment in coriander field. **Aim**: In order to study the effect of planting density on the yield of the essential oil and its composition, a bifactorial experiment was carried out on coriander plants (Sandra cultivar). **Material and method**: The experiment was performed with three plant densities on the row (0, 15 and 20 cm); the distance between plant rows was 12.5, 25 and 50 cm, respectively. So, it resulted nine experimental variants. The essential oils obtained by hydrodistillation from fruits have been characterized using gas chromatography and mass spectroscopy analysis (GC-MS). **Results**: The highest yield (7.9866 kg/ha) was obtained for the plants spaced at 20 cm in between and 25 cm row spacing. The highest content of monoterpene alcohols (50.96%) was obtained with 25 cm row spacing and plant spaced at 0 cm on the row. The main components in all oils were monoterpene alcohols (40.75% - 50.96%) and monoterpenes (32.43-38.44%). **Conclusion**: The essential oil of coriander fruits (Sandra cultivar) does not meet the requirements of the European Pharmacopoeia, especially concerning the content in linalool. Nevertheless, the high content in monoterpene alcohols and monoterpenes recommends the use of the essential oil as immunomodulatory, analgesic and anti-inflammatory agent in rheumatology and also as an antibacterial and antiviral agent. Consequently, the changes in yield and composition of the essential oil of Sandra coriander should be assesed during several periods of vegetation in order to conclude on its pharmaceutical quality. **Key words**: *CORIANDRUM SATIVUM* L., ESSENTIAL OIL, COMPOSITION, VARIABILITY.

Coriandrum sativum L. (*Apiaceae*) se cultivă pentru fructele bogate în ulei volatil, utilizându-se ca atare sau sub forma fracțiunii volatile în industria alimentară, în parfumerie şi pentru obținerea unor fitomedicamente sau suplimente alimentare.

Dependent de zona de proveniență şi cultură, uleiul volatil, constituit majoritar

din monoterpene, se regăseşte în fructe în proporţii ce variază de la 0.1% (India) la 1.7% (Norvegia) (1). Componentele majore la un ulei volatil de calitate sunt linaloolul (60%-80%), camforul şi α-pinenul (2, 3, 4).

Influenţa unor factori externi precum soiul, condiţiile pedoclimatice şi de cultură, au fost intens studiate în străinătate cu privire la acumularea uleiului volatil în fructe. La noi în ţară se cultivă în special două soiuri indigene omologate, şi anume soiurile Sandra şi Omagiu. Dacă în cercetările efectuate până în prezent pe cele două soiuri s-a urmărit îndeosebi randamentul la hectar al producţiei de fructe, compoziţia chimică a uleiului volatil nu a fost investigată după cunoştinţele noastre, mai ales în corelaţie cu condiţiile de cultură. Studiul de faţă prezintă rezultatele înregistrate la analiza chimică calitativă şi semicantitativă a uleiului volatil separat din coriandru, **soiul Sandra**.

MATERIAL ŞI METODE

A fost supus analizei uleiul volatil separat din fructele de coriandru Sandra, cultivat la SCDA Secuieni (Neamţ, România), în anul 2009, în cadrul unui experiment în care s-a urmărit influenţa spaţiului de nutriţie (fig. 1) asupra producţiei de ulei volatil şi a compoziţiei sale chimice.

Factorii de cultură investigaţi au fost:

- distanţa între rânduri: a=12.5 cm; b=25 cm; c=50 cm;

- distanţa între plante pe rând: a=nerărit (0 cm); b=15 cm; c=20 cm.

A B C

Fig. 1. *Coriandrum sativum* L., soiul Sandra: A- în faza de răsărire; B- rozetă de frunze; C- în faza de înflorire

S-au obţinut 9 variante experimentale codificate S1-S9, perioada de vegetaţie până la maturarea fructelor fiind de 122 zile.

Fructele recoltate la începutul lunii august au fost uscate şi supuse distilării cu vapori de apă, izolând în acest mod fracţiunea volatilă pentru care s-a determinat randamentul la extracţie. Compoziţia chimică a uleiului volatil a fost determinată prin gazcromatografie cuplată cu spectrometrie de masă (GC/MS), utilizând un **cromatograf de gaze** Agilent Technologies tip 7890A şi un **spectrometru de masă** Agilent tip 5975C. Pentru separarea componentelor s-a folosit o **coloană** DB-5MS (30 m x 0.25 mm x 0.25 µm). **Temperatura coloanei** a fost programată să crească cu rata de 10ºC/min de la 35°C până la 280°C, iar temperatura finală a fost menţinută pentru încă 7,5 minute. **Temperatura injectorului** a fost de 250°C, **rata de splitare** fiind de 50:1. **Faza mobilă** folosită a fost heliul (1mL/min). **Volumul injectat** a fost de 0.2 µL ulei volatil. În ceea ce priveşte spectrometrul de masă, **temperatura sursei** a fost de 230°C, iar **temperatura cva-**

drupolului de 150°C. Componentele separate au fost identificate prin compararea spectrelor obținute cu cele existente în Wiley Library.

REZULTATE ŞI DISCUȚII

La distilarea cu vapori de apă a materialului vegetal s-a stabilit randamentul de extracție, calculându-se producția în ulei volatil exprimată în kg/ha (tab. I).

TABELUL I

Producția de fructe de coriandru şi ulei volatil la hectar

Codificare	Producție fructe (kg/ha)	Ulei volatil, randament (ml/100 g fructe uscate)	Producție în ulei volatil (kg/ha)
S1	591.2	1.4	7.2008
S2	603.9	1.4	7.3555
S3	602.5	1.5	7.8626
S4	654.3	1.4	7.9694
S5	633.2	1.4	7.7124
S6	612.0	1.5	7.9866
S7	403.5	1.4	4.9146
S8	423.6	1.4	5.1594
S9	456.2	1.3	5.1596

Din tabel reiese faptul că soiul face parte din categoria coriandrului cu înaltă productivitate pentru ulei volatil, dar dacă luăm în considerare producția la hectar pentru fiecare variantă în parte, constatăm că S7-S9 (la care semănarea s-a făcut la 50 cm distanță între rânduri) furnizează cantități care nu depăşesc 5,16 kg, fapt care trebuie pus pe seama numărului redus de indivizi (deci şi de fructe)/unitatea de cultură (fig. 2).

Pentru a stabili calitatea uleiului volatil, am determinat prin GC/MS compoziția chimică calitativă şi cantitativă a celor 9 probe de coriandru, rezultatele fiind redate în tabelul II.

După cum reiese din tabel, compoziția chimică variază foarte mult, numărul cel mai mare de componente volatile fiind determinat pentru varianta S3 (44 componente), iar cel mai mic pentru S2 (35 componente), ceea ce nu înseamnă că prima ar fi din punct de vedere calitativ şi cea mai bună.

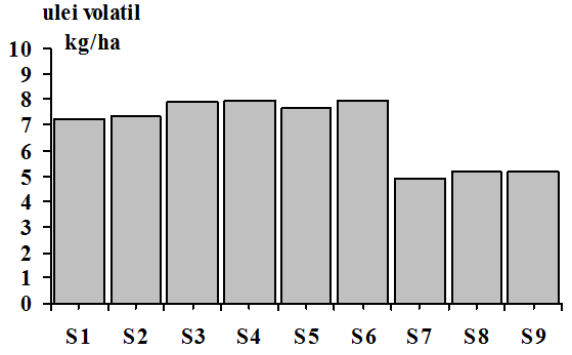

Fig. 2. Cantitatea de ulei volatil produsă/ha

TABELUL II

Compoziția chimică determinată pentru uleiurile volatile de coriandru, soiul Sandra (2009)

TR (min.)	Compus	Aria (%)								
		S1	S2	S3	S4	S5	S6	S7	S8	S9
5.871	α-tuien	0.35	0.33	0.34	0.26	0.29	0.28	0.31	0.24	0.28
6.032	**α-pinen**	**10.66**	**12.89**	**9.76**	**10.58**	**9.46**	**9.42**	**10.43**	**9.45**	**8.97**
6.291	camfen	2.21	2.18	2.04	1.77	1.91	1.66	2.00	1.70	1.86
6.637	sabinen	1.24	1.17	1.16	0.98	1.12	1.07	1.14	0.99	1.06
6.734	β-pinen	1.86	1.81	1.75	1.53	1.69	1.62	1.75	-	1.61
6.852	mircen	2.27	2.16	2.24	1.95	2.14	2.09	2.22	2.01	2.04
7.014	decan	-	-	0.01	-	-	0.01	-	-	0.01
7.079	octanal	-	-	0.01	0.01	0.01	0.01	-	0.01	0.01
7.165	l-felandren	-	0.08	0.08	0.08	0.09	0.09	0.10	0.08	0.09
7.208	Δ-3-caren	0.09	-	0.04	-	-	-	-	-	-
7.338	α-terpinen	0.22	0.18	0.29	0.18	0.21	0.21	0.22	0.20	0.21
7.467	o-cimen	-	4.77	-	4.52	-	-	-	0.03	-
7.478	**p-cimen**	**4.70**	**-**	**4.43**	**-**	**4.48**	**4.56**	**4.86**	**4.94**	**4.43**
7.554	**limonen**	**4.55**	**4.37**	**4.49**	**4.11**	**4.35**	**4.28**	**4.47**	**4.30**	**4.21**
7.770	t-β-ocimen	0.11	0.10	0.23	0.16	0.11	0.12	0.13	0.10	0.12
8.021	cis-sabinen hidrat	0.25	0.23	0.24	0.24	0.28	0.29	0.27	0.29	0.29
8.028	**γ-terpinen**	**11.81**	**11.85**	**10.90**	**10.98**	**11.40**	**11.24**	**11.97**	**11.77**	**10.95**
8.438	terpinolen	1.27	1.09	1.34	1.09	1.28	1.30	1.31	1.30	1.28
8.795	**L-linalool**	**42.44**	**43.28**	**41.18**	**45.17**	**42.97**	**43.12**	**43.32**	**45.12**	**42.81**
9.377	p-ment-2-en-1-ol	0.12	0.10	-	0.09	0.07	-	0.06	-	0.06
9.507	**camfor**	**5.77**	**5.24**	**5.82**	**5.74**	**5.87**	**6.03**	**5.63**	**5.86**	**6.23**
9.701	pinocarvona	0.08	0.08	-	-	0.12	0.11	0.08	0.08	-
9.841	L-borneol	0.16	0.12	0.17	0.14	0.16	0.17	0.15	0.15	0.18
9.949	4-terpineol	0.59	0.47	0.67	0.55	0.63	0.63	0.57	0.60	0.67
10.154	**α-terpineol**	**0.69**	**0.55**	**0.83**	**0.69**	**0.79**	**0.80**	**0.65**	**0.73**	**0.87**
10.262	decanal	-	-	0.07	-	-	-	0.05	-	-
10.370	verbenona	-	-	0.03	-	-	0.02	0.02	-	-
10.424	γ-izogeraniol	-	-	0.03	0.05	0.06	0.03	-	0.05	0.06
10.521	citronelol	0.26	0.20	0.38	0.26	0.31	0.31	0.26	0.26	0.34
10.672	dihidrocarveol	0.05	-	0.07	0.04	0.05	0.05	0.04	-	-
10.737	Z-citral	-	0.06	0.09	0.07	0.08	0.05	0.07	0.07	0.09
10.899	**t-geraniol**	**3.60**	**3.03**	**4.42**	**3.97**	**4.36**	**4.37**	**3.65**	**3.60**	**4.88**
11.028	piperitona	0.03	-	-	-	-	-	-	-	-
11.104	E-2-decenal	-	-	0.04	-	-	0.04	-	-	-
11.158	E-citral	0.14	0.12	0.19	0.15	0.17	0.17	-	0.15	0.19
11.330	carvacrol	-	-	-	0.02	0.02	0.06	-	0.05	-
11.460	izoestragol	-	-	-	-	0.40	-	-	-	-
11.470	t-anetol	0.47	0.28	1.00	0.29	-	0.60	0.43	0.38	0.35
11.589	timol	0.05	0.04	0.10	0.05	0.06	0.04	0.07	0.03	0.10
11.719	undecanal	0.05	0.04	0.05	0.04	-	0.05	0.04	-	-
11.945	metilgeraniat	-	-	0.03	0.02	0.03	-	0.02	0.02	0.03
11.978	mirtenilacetat	0.05	0.03	0.06	-	0.06	-	0.05	-	-
12.398	propionat de neril	0.04	-	-	-	-	-	-	-	-
12.409	nerol	-	0.03	-	-	-	-	-	-	-

TR (min.)	Compus	Aria (%)								
		S1	S2	S3	S4	S5	S6	S7	S8	S9
12.463	neril acetat	-	-	-	-	0.06	0.05	0.04	0.03	0.06
12.679	lavandulil acetat	3.11	-	4.29	3.57	4.04	4.25	-	3.18	4.54
12.862	mirtanil acetat	-	-	-	-	-	-	-	0.03	0.03
13.132	tetradecanal	0.02	0.01	-	-	-	-	0.02	0.04	0.03
13.240	dodecanal	-	-	0.03	0.02	0.09	0.02	-	-	0.06
13.370	junipen	-	-	0.01	-	-	-	-	-	0.01
13.402	β-cariofilen	0.19	0.13	0.23	0.19	0.24	0.23	0.18	0.17	0.27
13.898	α-humulen		0.01	0.05	0.01	-	0.01	0.01	-	0.02
13.930	2-dodecen-1-al	0.06	0.01	-	-	-	-	0.02	-	0.03
13.963	t-2-tridecenal	-	-	-	0.03	-	-	-	-	-
14.092	3-dodecen-1-al	-	-	0.08	0.02	0.09	-	-	0.03	0.05
14.416	biciclogermacren	0.01	0.01	0.03	0.02	0.02	0.03	0.01	0.01	0.04
15.215	cis-farnesol	-	-	-	-	0.01	-	-	-	-
15.592	cariofilen oxid	0.01	-	0.02	-	0.02	-	-	0.02	-

Dacă ne referim la calitatea farmaceutică a fracțiunilor volatile separate din fructele celor nouă variante de coriandru Sandra, trebuie să urmărim cerințele Farmacopeei Europene ed. a 6-a, care impune o serie de limite (5) în cadrul monografiei *Coriandri aetheroleum*. Comparativ cu aceste cerințe, cele nouă uleiuri volatile au prezentat abateri, uneori importante (tab. III).

Din datele prezentate reiese clar că uleiurile volatile analizate conțin cantități insuficiente de linalool (41%-45%, față de minim 65%), în timp ce acetatul de geranil nu a putut fi cuantificat în nicio probă. Pe de altă parte, toate probele depăşesc valorile admise pentru α-pinen, γ-terpinen, p-cimen şi în măsură mai mică, pentru geraniol.

TABELUL III

Calitatea farmaceutică a uleiurilor volatile de coriandru (soiul Sandra)

Componentă	Cerință Ph. Eur. ed. a 6-a	Determinat (%)								
		S1	S2	S3	S4	S5	S6	S7	S8	S9
α-pinen	**3.0-7.0%**	10.66	12.89	9.76	10.58	9.46	9.42	10.43	9.45	8.97
limonen	**1.5-5.0%**	4.55	4.37	4.49	4.11	4.35	4.28	4.47	4.30	4.21
γ-terpinen	**1.5-8.0%**	11.81	11.85	10.90	10.98	11.40	11.24	11.97	11.77	10.95
p-cimen	**0.5-4.0%**	4.70	u	4.43	u	4.48	4.56	4.86	4.94	4.43
camfor	**3.0-6.0%**	5.77	5.24	5.82	5.74	5.87	6.03	5.63	5.86	6.23
L-linalool	**65.0-78.0%**	42.44	43.28	41.18	45.17	42.97	43.12	43.32	45.12	42.81
α-terpineol	**0.1-1.5%**	0.69	0.55	0.83	0.69	0.79	0.80	0.65	0.73	0.87
acetat de geranil	**0.5-4.0%**	u	u	u	u	u	u	u	u	u
t-geraniol	**0.5-3.0%**	3.60	3.03	4.42	3.97	4.36	4.37	3.65	3.60	4.88

u= sub 0.01 %

Datorită acestor carențe, trebuie să tragem concluzia că uleiul de coriandru Sandra (producție 2009) nu întruneşte calitățile farmaceutice (deci terapeutice) cerute de

Farmacopeea Europeană, ediția a 6-a.

Grupând componentele identificate pe subgrupe de terpene, am obținut rezultatul redat în tabelul IV.

TABELUL IV

Compoziția chimică a uleiurilor volatile de coriandru pe subgrupe principale de compuşi

Grupă compuşi (%)	Aria (%)								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
Hidrocarburi monoterpenice	36.81	38.44	34.90	33.91	34.33	33.67	36.32	32.43	32.97
Monoterpenoli	47.91	47.78	47.75	50.96	49.40	49.48	48.70	50.51	49.87
Monoterpencetone	5.88	5.32	5.85	5.74	5.99	6.16	5.73	5.94	6.23
Monoterpenaldehide	0.14	0.18	0.28	0.22	0.25	0.22	0.07	0.22	0.28
Monoterpenesteri	3.20	0.03	4.38	3.59	4.19	4.30	0.11	3.26	4.66
Total monoterpene	**93.94**	**91.75**	**93.16**	**94.42**	**94.16**	**93.83**	**90.93**	**92.36**	**94.01**
Sescviterpene	0.21	0.15	0.34	0.22	0.38	0.27	0.20	0.20	0.34
Compuşi alifatici	0.13	0.06	0.29	0.12	0.10	0.13	0.13	0.08	0.19
Compuşi aromatici	5.22	5.09	5.53	4.88	4.96	5.26	5.36	5.43	4.88

Urmărind valorile înscrise în tabel constatăm că din punct de vedere cantitativ predomină monoterpenolii (47,75%-50,96%), urmați fiind de hidrocarburile monoterpenice (32,43%-38,44%) şi, în mai mică măsură, de monoterpencetone (5,32%-6,23%), compuşi aromatici (4,88%-5,53%) şi monoterpenesteri (0,11%-4,66%). Dacă însă ne oprim atenția doar asupra celor două subgrupe majore de compuşi din compoziția uleiurilor de coriandru Sandra, constatăm că, adunate, ele reprezintă între 82.65% (S3) şi 86,22% (S2).

Din punct de vedere al acțiunii farmacologice, monoterpenolii (linalool, geraniol, 4-terpineol) reprezintă imunomodulatori eficienți, care protejează organismul mai ales față de situații de stres; acționează astfel armonizant asupra sistemelor hormonal, cardiac şi nervos, dermoregenerator, dar şi antibacterian şi antiviral, fără a distruge flora dermică normală. În plan psihic, sunt adaptogene, reglând producția hormonilor de stres, astfel încât calmează starea de agitație, relaxează psihic, cresc percepția pozitivă a mediului înconjurător şi dau senzația de armonizare cu acesta (6).

Pe de altă parte, hidrocarburile monoterpenice, puternic lipofile, penetrează rapid prin piele şi mucoase (inclusiv respirator), având o acțiune stimulant generală, analgezică, antiinflamatoare, iar la aplicare pe piele, blând contrairitantă. Mai ales α-pinenului i se datorează aceste din urmă efecte, din cauza modulării funcției corticosuprarenale. Limonenul, în schimb, pe lângă acțiunea antibacteriană şi antivirală, vine şi cu un efect imunostimulator. Datorită lor, uleiurile bogate în monoterpene reprezintă un tonic psihic, stimulând capacitatea de concentrare şi gândirea logică şi acționând anxiolitic.

CONCLUZII

Se pare că pentru o producție convenabilă de fructe, respectiv de ulei de coriandru, plantele au nevoie de un spațiu de nutriție adecvat. Cele mai bune rezultate s-au obținut, din acest punct de vedere, la

variantele de cultură S4 şi S6, pentru care spațiul între rânduri a fost de 25 cm, iar distanța între plante de 20 cm (S6), respectiv 0 cm (S4). Rărirea excesivă (S7-S9) nu asigură o bună exploatare a terenului agricol, producția de fructe şi deci, de ulei, fiind redusă.

Pentru coriandrul soiul Sandra cultivat la SCDA Secuieni în anul 2009, în condițiile menționate, chiar dacă producția de fructe şi ulei volatil este convenabilă, trebuie să apreciem că materialul vegetal recoltat nu corespunde cerințelor impuse de Farmacopeea Europeană ediția a 6-a, mai ales prin conținutul redus în linalool determinat în fracțiunea volatilă.

Cu toate acestea, având în vedere conținutul mare în monoterpenoli şi hidrocarburi monoterpenice, uleiul de coriandru soiul Sandra ar putea fi utilizat în preparate cu aplicare topică mai ales în reumatologie ca imunomodulator, antiinflamator şi analgetic, dar şi ca antibacterian şi antiviral.

Se impune repetarea studiului pe mai mulți ani de cultură, pentru a putea trage o concluzie finală şi a atenționa producătorii, dacă soiul se dovedeşte a nu corespunde cerințelor de calitate.

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STRATEGIA DE TRATAMENT STOMATOLOGIC LA PACIENTUL VÂRSTNIC

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DENTAL TREATMENT STRATEGIES FOR ELDERLY PATIENTS (Abstract): The elderly are influenced from a various and complex combination of factors, such us individual genetic predispositions, lifestyle, society and environment. the sum of all the individual life experiences concerning the dental care, the caries, the periodontal and iatrogenic diseases. **Aim** : This paper evaluates the dental treatment in the elderly. **Material and methods** : The study was performed on 112 patients, aged over 65, at the Clinical Department of Dental Semiology und Geriatric Dentistry, Iaşi, Romania. **Results** : The treatment interventions were dental hygiene procedures (in 97 patients), restaurations (in 29 patients), removable partial and total denture preocedures (in 50 patients), fixed prosthetic procedures (in 18 patients), rebasing of dentures (in 25 patients) and teeth extractions (in 26 patients). There were 36 endodontic emergencies and 15 periodontal emergencies and 8 emergencies that resulted from other causes. **Conclusions** : The periodical dental examinations and the proper daily oral hygiene routine are still important in the old age and they should not be neglected, because the prosthetical components request also treatment interventions. **Key words** : ELDERLY PATIENT, DENTAL CARE, ORAL STATUS, DENTAL TREATMENT

Modificările survenite prin îmbătrânire influenţează tabloul clinic al unor îmbolnăviri şi fac dificilă culegerea informaţiilor, stabilirea diagnosticului şi a strategiei de tratament şi influenţează negative prognosticul (1).

În cazul îmbătrânirii fiziologice gradul de afectare a organelor este variabil, îmbătrânirea ca proces în sine poate fi evaluată după tipare diferite, în funcţie de corespondenţa ce se stabileşte între vârsta biologică şi vârsta cronologică (2,3).

Îmbătrânirea patologică se referă la îmbătrânirea fiziologică ce interferează cu fenomene patologice, fiind rezultatul acţiunii asupra organismului a unor factori de mediu extern sau intern care schimbă cursul normal al procesului de îmbătrânire (4).

Vârsta înaintată reprezintă prin ea însăşi un element de prognostic nefavorabil pentru pacient, dar acest lucru nu trebuie folosit ca argument împotriva unei examinări atente care să releve potenţialul şi riscul la care este supus pacientul respectiv (5,6).

În general nu există o patologie specifică vârstnicului dar întreaga patologie capătă o tentă caracteristică (6,7).

Scopul lucrării este de a evalua necesitatea, strategia şi răspunsul la tratament stomatologic la pacientul vârstnic.

MATERIAL ŞI METODĂ

Au fost examinaţi 112 pacienţi vârstnici cu vârste de peste 65 de ani, selectaţi dintre bolnavii care au solicitat asistenţă stomatologică în Serviciul Clinic de Semiologie Stomatologică şi Gerontostomatologie Iaşi.

Pacienţii luaţi în studiu au fost supuşi unui examen clinic atent, general şi local, completat de examene paraclinice: studiul de model, examen radiografic, etc, care ne-au permis stabilirea vârstei biologice care, raportată la cea cronologică, să permită stabilirea geroindexului general şi local care stă la baza succesului în practica geriatrică.

Doleanţele pacienţilor s-au raportat atât la dureri, cât şi la tulburări funcţionale. S-a insistat pe valoarea unei bune anamneze şi a unui examen obiectiv, urmărind ca primă etapă a evalua: starea generală, medicaţia, capacităţile psihice, capacităţile fizice, situaţia socială, starea generală.

Examenul clinic gerontologic a urmat acelaşi algoritm ca la adult, dar cu particularităţi de etapă specifice vârstei înaintate şi cu necesitatea consultului interdisciplinar.

S-a realizat anamneza afecţiunilor stomatologice, necesară pentru evaluare atitudinii pacientului în legătură cu tratamentele stomatologice şi motivaţia sa, precum şi o examinare atentă în vederea detectării unor eventuale afecţiuni ale mucoasei orale, modificări ale ocluziei, deviaţii de deschidere şi închidere.

S-a înregistrat şi schimbarea în starea de sănătate orală (ameliorare sau deteriorare), privind cariile şi condiţia parodontală, precum şi alte aspecte ale dentiţiei (dinti, proteze etc). Statusul cariilor a fost clasificat astfel: absenţa cariilor, carii fără implicare pulpară, carii cu implicare pulpară. Starea parodontală a fost clasificată prin scorul parodontal cel mai sever pe o scară după cum urmează: sănătate parodontală (absenţa modificărilor, sângerare la sondare, pungi parodontale ≥5,5 mm, mobilitate verticală (gr III) şi pungi purulente. O schimbare pozitivă în scorul celei mai severe stări a fost considerată o îmbunătăţire, iar o schimbare negativă a fost considerată o deteriorare a sănătăţii orale. Dacă nu a existat nicio modificare a celui mai căzut scor, s-a comparat numărul de dinţi cu cele mai grave scoruri, pentru a indica schimbările survenite între două evaluări de sănătate orală.

Terapia completă a disfuncţiilor sistemului stomatognat a necesitat un număr mare de tratamente odontale, parodontale şi protetice, grupate în urgenţe în caz de durere, paleative, tardive.

REZULTATE ŞI DISCUŢII

Din punct de vedere al repartiţiei pe grupe de vârstă se constată o preponderenţă a celor din grupa 65-75 ani faţă de cei din grupele 75-84 ani şi peste 85 ani (tab. I).

TABELUL I

Repartiţia cazurilor pe grupe de vârstă.

GRUPA DE VÂRSTĂ	NUMĂR CAZURI	PROCENT %
65-74 ANI	87	77,67%
75-84 ANI	14	12,50%
PESTE 85 ANI	11	9,83%
TOTAL	112	100,00%

În urma anamnezei amănunţite şi a examenului obiectiv s-a permis stabilirea unui bilanţ biologic şi a unui bilanţ nutriţional.

S-a evidenţiat o patologie generală, stabilindu-se un procentaj de patologie pentru fiecare grupă (tab. II).

TABELUL II

Repartiţia procentuală a bolnavilor pe grupe de vârstă şi de boală

AFECŢIUNI	GRUPURI DE VÂRSTĂ		
	65-74 ANI	75-84 ANI	PESTE 85 ANI
Boli cardiovasculare	40,9	41,8	46,9
Boli osteo-articulare	16,2	17,3	17,6
Boli metabolice	6,8	5,9	3,7
Boli ale SNC	9,7	11,3	10,9
Boli digestive	7,9	3,8	4,1
Boli ale sistemului respirator	8,1	6,9	5,9
Alte boli	19,1	11,9	12,01

S-a constatat depistarea multor afecţiuni neglijate de bolnavi şi s-a căutat orientarea conduitei terapeutice stomatologice.

Din considerente medicale, psihice (8) sau dentare, din cauza absenţei unităţilor masticatorii, a leziunilor odontale multiple, a bolii parodontale sau a protezărilor nefuncţionale, alimentaţia este necorespunzătoare, incompletă sau neechilibrată (9).

La examenul extraoral s-au observat modificări la nivelul feţei, traduse printr-o tendinţă de lăţire a feţei, datorită creşterii diametrului transversal (Zy-Zy) şi scăderea dimensiunii verticale a etajului inferior, datorată uzurii dentare necompensate prin erupţii, fie edentaţiei şi resorbţiei osoase (6,9). Reducerea texturii şi a elasticităţii buzelor se datorează modificărilor de vârstă la nivel muscular şi tegumentar.

Examenul oral prezintă edentaţii totale, iar pe arcadele dentare au mai rămas dinţi funcţionali. Scăderea numărului de dinţi restanţi pe bolnav, odată cu înaintarea în vârstă, arată că lipsa măsurilor profilactice la vârsta a III-a sunt definitorii pentru pierderea dinţilor, din cauza abandonului îngrijirii, în speranţa unei protezări mobile (10,11).

S-a constatat un număr mare de edentaţi total, protezaţi şi neprotezaţi, la ambele maxilare. Dintre aceştia, protezarea fusese realizată doar la 75% (tab. III, fig. 1).

TABELUL III

Statusul pacienţilor luaţi în studiu

Status	Nr. pacienţi
Dentiţie	
Edentaţe bimaxilară	54
Edentaţe unimaxilară	26
Proteze fixe (totală şi parţială)	59
Neprotezaţi	28
Protezaţi	84
Carii (pacienţi dentaţi)	
Fără carii	25
Carii simple	29
Carii complicate	31
Parodonţiu (pacienţi dentaţi)	
Sănătos	28
Sângerare gingivală	48
Pungi profunde	14
Pioree alveolară	7
Mobilitate gradul III	15

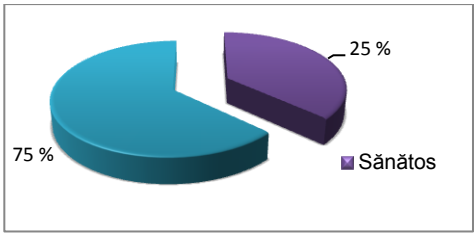

Fig. 1. Statusul pacienţilor luaţi în studiu protezaţi vs neprotezaţi

S-au remarcat diferite tipuri de leziuni carioase, o formă particulară fiind aceea cu localizare la nivelul coletului dinţilor, uneori cu evoluţie rapidă până la fractura dintelui (fig. 2). Pe lângă aceasta, la pacienţii dentaţi, se observă dispariţia treptată a stratului de smalţ, indicând fenomenul de exfoliere şi tendinţa modificării culorii smalţului dentar-cenuşiu. Din cauza periajului intempestiv, se produce în timp o pierdere de smalţ, observată mai adesea pe faţa vestibulară a caninilor şi a premolarilor.

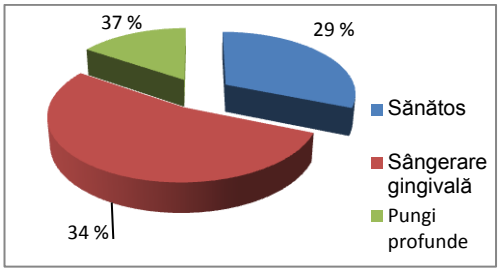

Fig. 2. Leziuni carioase la pacienţii luaţi în studiu

Când se păstrează mai mulţi dinţi restanţi pe arcadă, atriţia la vârstnic este cea mai importantă şi evidentă modificare a smalţului dentar legată de procesul de senescenţă, proces ce determină uzură ocluzală şi proximală a smalţului consecutiv contactului dento-dentar (12). Datorită solicitării funcţionale normale procesul de atriţie apare la toţi dinţii şi determină o micşorare a înălţimii coronare împreună cu aplatizarea conturului proximal, ducând la o reducere progresivă a volumului total al smalţului şi chiar a dentine, cu atingerea camerei pulpare (13,14).

Se observă fenomenul de abraziune sub forma unor faţete de uzură localizate pe suprafaţa obturaţiilor, a coroanelor de înveliş şi pe suprafaţa smalţului. Abrazia de tip patologic este frecventă, ca urmare a dezechilibrelor ocluzale, generate de iatrogenii, prin lipsa unor tratamente corecte, care să restabilească continuitatea arcadelor morfologic şi funcţional.

În cazul patologiei endodontice, s-au avut în vedere calcificările pulpare posibile puse pe seama tulburărilor metabolice prin carenţele vitaminice frecvente la vârstnici.

Boala parodontală la pacientul vârstnic are o evoluţie foarte lentă, cu absenţa fenomenelor dureroase, cu existenţa pungilor parodontale, a recesiunii gingivale şi mobilităţii dentare de diferite grade (3,5) (Fig. 3.

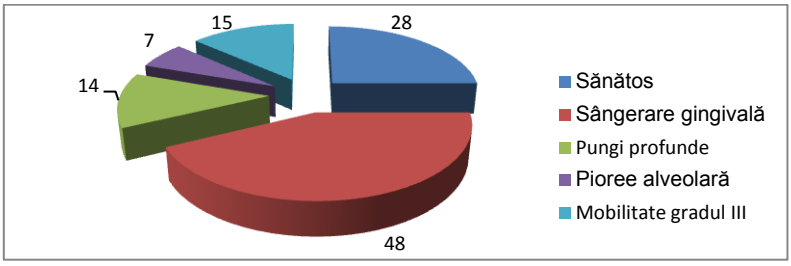

Fig. 3. Statusul parodontal al pacienţilor luaţi în studiu

Analiza clinică a pacienţilor vârstnici protezaţi şi neprotezaţi a pus în evidenţă afecţiuni ale parodonţiului, ale mucoasei orale şi unele disfuncţii ale articulaţiei temporo-mandibulare.

Tratamentul a cuprins manopere odontale, parodontale, protetice (tab. IV).

S-a ţinut cont de starea generală, evitându-se riscul şi temporizarea tratamentului până la echilibrarea constantelor biologice.

În cadrul afecţiunilor odonto-parodontale s-au urmărit eliminarea factorului microbian, creşterea rezistenţei gazdei şi reducerea substratului nutritiv disponibil pentru agenţii microbieni responsabili de apariţia afecţiunilor (15,16). Pentru îndepărtarea plăcii bacteriene s-au indicat metode chimice şi perioada aplicării în funcţie de limitele fizice ale pacientului. S-au realizat aplicaţii topice cu fluoruri, pentru creşterea rezistenţei ţesuturilor dentare la atacul acid, pentru favorizarea remineralizării în cazul leziunilor carioase incipiente şi pentru acţiunea împotriva plăcii bacteriene. Terapia odontală s-a realizat conservator prin tratament convenţional.

TABELUL IV

Tipuri de tratamente efectuate

Tipuri de tratamente efectuate	Nr pacienţi
Igienă dentară: detartraj supra/subgingival, periaj profesional	97
Restaurări	29
Extracţii	26
Tratament protetic conjunct	18
Tratament protetic mobilizabil	50
Proteze totale (uni/bimaxilare)	18
Rebazare/optimizare proteze totale	25
Urgenţe stomatologie	
Endodontice	36
Parodontale	15
Altele	8

Specifice persoanelor în vârstă sunt gingivitele hiperplazice de cauză medicamentoasă, care de multe ori pot da naştere la false pungi parodontale prin hiperplazia marginii libere a gingiei, deşi epiteliul joncţional se găseşte la nivelul joncţiunii smalţ-cement şi nu se observă pierderi osoase (13,17).

Terapia parodontală efectuată a permis preîntâmpinarea apariţiei unei disfuncţii a sistemului stomatognat prin refacerea stării de sănătate parodontală, micşorarea sau dispariţia mobilităţii, limitarea fenomenelor de migrare dentară şi îndepărtarea planurilor înclinate traumatizante.

Tratamentul gnato-protetic a inclus pregătirea preprotetică şi proprotetică, bazate pe principiile punerii în condiţii optime a elementelor sistemului stomatognat, în raport de indicii clinico-biologici generali, loco-regionali şi locali (18,19).

Pentru instituirea unui tratament stomatologic individualizat bolnavilor vârstnici, este necesară cunoaşterea aprofundată a caracteristicilor bio-psiho-sociale şi spirituale ale acestei categorii.

Multe dintre bolile cronice pe care le prezintă vârstnicul cresc riscul aplicării tratamentului stomatologic sau chiar îl contraindică şi de aceea este foarte importantă colaborarea cu medicul generalist (10).

La diabetici există o prevalenţă mai mare a afecţiunilor stomatologice, de tipul candidozelor, uscăciunea neexplicată a cavităţii bucale, boli parodontale şi multiple carii (5,6).

Hemodializaţii şi cei cu transplant renal constituie un grup special care va fi tratat în strânsă colaborare cu specialistul nefrolog,

deoarece riscul intervenţiilor dentare este crescut (5,11).

Datorită dependenţei de medicaţie anticoagulantă, antiplachetară, antiinflamatorii nesteroidiene şi ticlopidine, mulţi dintre vârstnici au un risc crescut de sângerare (10, 11).

Îmbătrânirea oro-facială şi aspectul general constituie un index în aprecierea vârstei persoanei respective (4,5).

Odată cu înaintarea în vârstă apar modificări de culoare, de grosime ale tipului de dentină, care devine săracă în substanţe organice şi anorganice, căpătând un aspect translucid şi devine mai dură.

De asemenea, pulpa scade în volum, datorită formării în continuu de dentină secundară care reduce spaţiul pentru pulpă, care după 75 de ani poate fi complet obliterat.

Distrucţia parodontală se caracterizează prin pierderea ataşamentului periodontal şi reducerea înălţimii osului alveolar.

Bolile structurilor orale şi maxilo-faciale reprezintă circa 13% din totalitatea bolilor întâlnite la pacientul cu vârsta peste 65 de ani, când există o patologie specifică a sistemului stomatognat (11,14,21).

CONCLUZII

Datorită fragilităţii tisulare, a depresiei mecanismelor defensive şi a modificărilor morfo-funcţionale prin procese de involuţie patologia sistemului stomatognat prezintă un indice de gravitate crescut.

Diagnosticul geriatric necesită o cooperare interdisciplinară.

Variantele terapeutice vor fi raportate la prognosticul stării sistemului stomatognat.

Patologia orală întâlnită la pacientul vârstnic impune acordarea unei atenţii deosebite gerontoprevenţiei şi dispensărizării corecte prin controale periodice, în care se înlătură factorii nocivi, se corectează funcţiile şi se depistează leziunile într-un stadiu incipient.

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CERCETĂRI PRIVIND COMPOZIȚIA CHIMICĂ A ULEIURILOR VOLATILE EXTRASE DIN FLORI DE LEVĂNȚICĂ

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STUDIES REGARDING CHEMICAL COMPOSITION OF LAVENDER VOLATILE OILS (Abstract): **Aim**: In this paper, we studied comparatively volatile fractions obtained by hydrodistillation from 5 samples of lavender flowers (*L. angustifolia* ssp. *angustifolia*, L. *hybrida*, *L. angustifolia* spp. p*yrenaica*, *L. angustifolia* spp. *angustifolia* cv. Munstead and cv. Hidcote blue) cultivated during 2009 in the Botanical Garden from Galati. **Material and methods**: The essential oils have been characterized using gas-chromatography and mass spectroscopy analysis (GC-MS). **Results**: The main compounds in all lavender volatile oils were: linalool (20.60%-35.99%), linalyl acetate (12.58%-19.65%), lavandulyl acetate (3.74%-10.48%), t-β-ocimene (1.26%-9.23%), α-terpineol (3.67%-6.73%), nerol (0.81%-3.32%), neryl acetate (0.95%-3.64%) and β-caryophyllene (0.93%-2.43%). **Conclusions**: None of the separated volatile fractions met the minimal quality parameters for the standard volatile oil of fine lavender used in aromatherapy. Low concentration in monoterpene esthers could be explained by the fact that, on one hand, the average daily temperature during the blooming period is below the one from Mediterranean Sea area, and, on the other hand, the city of Galati is only 42 meters above the sea level (the volatile oil from fine lavender used in aromatherapy is obtained from plants cultivated at 800-1200 meters altitude). **Key words**: ESSENTIAL OILS, *LAVANDULA*, CHEMICAL COMPOSITION

Genul *Lavandula* (*Lamiaceae*) cuprinde peste 30 de specii larg distribuite şi cultivate pe întreg globul pentru calităţile medicinale şi parfumistice ale inflorescenţelor lor.

Dintre acestea, oficinală este *Lavandula angustifolia* Miller (sinonim *L. vera* D.C, *L. angustifolia* Chaix ex Vill), care creşte spontan în întreg bazinul mediteraneean, dar se cultivă, mai ales în scopul extracţiei uleiului volatil, extrem de valoros, în Alpii Francezi (Provence), la altitudini de 800-1200 m, Spania, Maroc, Italia, Croaţia, Anglia, Tasmania şi Argentina, precum şi în România.

Şi alte trei specii de *Lavandula* sunt cultivate pe scară largă, în scopul separării, din inflorescenţe a uleiului volatil utilizat în aromaterapie: *L. stoechas* L., cunoscută ca rozmarin sfânt (Romero santo), *L. latifo-*

lia Medikus şi *L.hybrida* Rev. (*L.burnati* Briquet), foarte asemănatoare lavandei adevărate cu toate că este un hibrid între *L. angustifolia* şi *L. spica*, cunoscută sub denumirea de lavandină (1, 2).

Deoarece este cunoscut faptul că atât conținutul în ulei volatil, cât şi compoziția chimică variază mult de la o specie de lavandă la alta, precum şi în funcție de varietate, condiții pedoclimatice, altitudine sau metodă de extracție, ne-am propus în lucrarea de față să realizăm un studiu comparativ al fracțiunilor volatile separate prin hidrodistilare din florile unor specii şi varietăți de *Lavandula* cultivate la noi în țară şi interpretarea, în funcție de componentele identificate a eventualelor calități aromaterapeutice.

MATERIAL ŞI METODĂ

Materialul vegetal a fost constituit din flori de levănțică recoltate în iunie 2009 de la 2 subspecii de *Lavandula angustifolia* (ssp. *angustifolia* şi ssp. *pyrenaica*), 2 varietăți de cultură (*Lavandula angustifolia* ssp. *angustifolia* Munstead şi Hidcote blue), precum şi de la specia *Lavandula hybrida*, cultivate în Grădina Botanică Galați.

Izolarea şi determinarea cantitativă a fracțiunii volatile s-a realizat prin hidrodistilarea timp de 3 ore a materialului vegetal uscat, în aparatul Neo-Clevenger. Conținutul în ulei volatil al produsului vegetal s-a exprimat conform prevederilor Ph Eur. ed a 6-a, şi anume: mL ulei volatil la kg produs vegetal.

Compoziția chimică a uleiului volatil a fost stabilită prin analiza gaz-cromatografică cuplată cu spectrometrie de masă (GC-MS) în următoarele condiții: Gaz-cromatograf Agilent type 7890A; Spectrometru de masă Agilent 5975C; Coloană cromatografică DB-5MS (30 m x 0.25 mm x 0.25 μm); Faza mobilă: Heliu 1 mL/min; Temperatura injector 250ºC; Temperatura detector 280ºC; Regim de temperatură: inițial 35ºC, apoi creştere cu 10ºC/min. de la 35ºC la 280ºC (7.5 minute constant); Volum injectat: 0.2 μL ulei volatil. Rata de splitare: 1:100. Identificarea substanțelor din probele analizate gaz-cromatografic s-a făcut pe baza comparației între spectrul de masă şi spectrele din biblioteca Wiley.

REZULTATE ŞI DISCUȚII

Prin măsurarea volumului de ulei volatil rezultat la hidrodistilare s-a stabilit randamentul de extracție prezentat în tab. I.

TABELUL I

Conținutul în ulei volatil al probelor de *Lavandula*

Proba	Codificare	η (mL) în ulei volatil determinat/kg material vegetal uscat
L.angustifolia ssp. *angustifolia*	**LAA**	90.00
L.angustifolia ssp. *pyrenaica*	**LAP**	40.00
L.*hybrida*	**LAH**	30.00
L.angustifolia ssp. *angustifolia* Munstead	**LAM**	56.20
L.angustifolia ssp. *angustifolia* Hidcote blue	**LHB**	30.00

Concentrația în ulei volatil a probelor de lavandă variază între 30 şi 90 mL/kg produs vegetal uscat. Lavanda oficinală (*Lavandula angustifolia* ssp. *angustifolia*-LAA), precum şi cultivarul Munstead (LAM) sunt cele mai bogate în ulei volatil (90 şi respectiv, 56,20 mL /kg produs vegetal uscat), în timp ce *Lavandula hybrida* (LAH) şi varietatea Hidcote blue (LHB) au cel mai scăzut conținut (30 mL/kg produs vegetal). Toate probele de lavandă corespund cerințelor Ph Eur. ed. a 6-a (min. 13 ml ulei volatil/kg produs vegetal uscat), dar numai trei (LAA, LHB şi LAM) ar putea fi socotite că prelucrează *Lavandula angustifolia* Mill.

Analiza gaz-cromatografică a uleiurilor volatile obținute din florile de lavandă a demonstrat prezența unui număr mare de componente, acesta situându-se între 50 (proba LHB) şi 68 (proba LAP), reprezentând 92,90% şi respectiv 96,12% din totalul fracției volatile. Probele LAA, LAM şi LAH au un număr de 54, 61 şi respectiv 62 de componente, ceea ce constituie 91,99%, 91,66% şi 96,68% din total.

Cei mai importanți componenți identificați în uleiurile volatile analizate sunt redați în tab. II.

Componentele majore în toate probele sunt monoterpenele: linalool (20,60%-35,99%), acetat de linalil (12,58%-19,65%), acetat de lavandulil (3,74%-10,48%), t-β-ocimen (1,26%-9,23%), α-terpineol (3,67%-6,73%), nerol (0,81%-3,32%), acetat de neril (0,95%-3,64%) şi sescviterpena: β-cariofilen (0,93%-2,43%).

Grupând compuşii identificați pe categorii structurale, se obține compoziția chimică indicată în tabelul III.

În baza compoziției chimice uleiul volatil de lavandă se utilizează în fito- şi aromaterapie pentru calitățile sale antiinflamatoare, analgetice, spasmolitice, dezinfectante, febrifuge, imunostimulatoare, dermoregeneratoare, cicatrizante, tensioreglatoare, psihoechilibrante, anxiolitice, antidepresive şi sedativ/stimulante (1,3).

Prevederile standard în aromaterapie pentru uleiul de levănțică cer un conținut de 40-50% monoterpenesteri (componente principale acetat de linalil şi respectiv, lavandulil), 30-40% monoterpenoli (linalool), 7-13% monoterpene (ocimen) şi până la 8% sescviterpene (β-cariofilen) (1,4), dar în uleiurile volatile analizate se înregistrează următoarea situație:

- conținutul în monoterpenoli corespunde strict cerințelor doar în cazul lavandei oficinale (LAA) şi varietății sale de cultură Munstead (LAM);

- nivelul monoterpenesterilor este sub 40% în toate probele, fiind necorespunzător utilizării în aromaterapie;

- exceptând *Lavandula angustifolia* (LAA) şi *L. hybrida* (LAH), hidrocarburile monoterpenice cărora li se atribuie acțiunea tonic generală şi de creştere a capacității de concentrare, prezintă concentrații care se încadrează în standardul de aromaterapie; valorile mai ridicate pentru cele două probe menționate ar putea determina un efect antiinflamator şi analgezic mai pregnant prin reacția contrairitantă pe care o declanşează la aplicarea uleiurilor pe piele;

- varietatea de cultură Hidcote blue (LHB) a lavandei oficinale nu corespunde din punct de vedere al conținutului în sescviterpene, iar

- proba *L.angustifolia* ssp. *pyrenaica* (LAP) prezintă un conținut dublu în monoterpenoxizi față de valoarea standard;

- prezența cumarinei în probele LAP şi LAM impietează de asemenea utilizarea respectivelor uleiuri în aromaterapie.

TABELUL II

Componente principale identificate în uleiurile volatile de *Lavandula*

TR (min.)	Compus	%				
		LAA	**LAP**	**LAH**	**LAM**	**LHB**
5.299	α-pinen	0.43	0.46	0.42	0.27	0.21
5.532	camfen	0.63	0.50	0.26	0.25	1
5.956	β-pinen	-	-	0.12	0.48	-
5.992	1-octen-3-ol	-	0.83	0.11	0.49	-
6.052	3-octanona	0.21	0.54	0.24	0.53	0.09
6.112	mircen	1.39	0.75	1.31	1.09	0.57
6.433	hexilacetat	-	-	0.55	-	-
6.450	delta-3-caren	0.65	0.23	-	0.40	0.29
6.623	o-cimen	0.08	0.13	0.12	0.26	0.14
6.666	p-cimen	0.25	0.34	0.35	0.07	0.38
6.727	limonen	1.32	0.46	0.70	1.42	0.97
6.787	1,8-cineol	0.82	2.47	-	1.17	-
6.831	cis-ocimen	7.13	0.07	-	0.09	4.52
6.987	**t-β-ocimen**	**5.39**	**5.64**	**9.23**	**7.14**	**1.26**
7.411	linalool oxid	-	0.73	0.37	0.23	0.37
7.644	α-terpinolen	0.90	1.06	0.67	0.65	0.59
7.843	**linalool**	**24.35**	**35.99**	**20.60**	**26.90**	**28.06**
7.939	oct-1-en-3-il acetat	2.01	1.91	1.92	1.79	-
8.224	n-allo-ocimen	0.45	0.36	0.30	0.31	-
8.484	n-hexil-izobutirat	-	-	-	-	0.47
8.553	L-camfor	0.47	0.62	0.11	0.24	1.35
8.796	(-)-lavandulol	-	1.70	0.53	1.46	0.80
8.848	L-borneol	1.23	1.76	0.41	1.16	4.32
9.004	**4-terpineol**	**-**	**4.79**	**10.61**	**3.75**	**2.00**
9.125	hexilbutanoat	-	-	0.63	-	-
9.159	criptona	1.54	0.54	-	2.51	-
9.194	**α-terpineol**	**5.20**	**3.67**	**6.73**	**4.42**	**6.71**
9.306	mirtenal	-	0.29	-	0.45	-
9.488	eucarvona	-	0.20	0.18	-	-
9.583	T-carveol	-	-	0.15	0.27	0.43
9.756	acetat de endobornil	-	0.23	-	-	-
9.921	cuminal	0.63	0.29	0.28	1.01	0.62
9.964	L-carvona	0.32	0.18	0.13	0.37	0.52
10.060	**acetat de linalil**	**18.23**	**12.58**	**19.65**	**17.38**	**13.12**
10.535	**acetat de lavandulil**	**3.74**	**8.33**	**10.48**	**8.42**	**9.46**
10.536	γ-terpinen	0.94	0.37	0.60	0.24	-
10.596	cuminol	0.45	0.24	0.26	0.57	-
11.531	**acetat de neril**	**1.59**	**0.99**	**1.68**	**3.64**	**0.95**
11.773	**nerol**	**3.32**	**0.81**	**1.22**	**0.85**	**2.14**
12.362	santalen	0.48	0.38	0.31	0.30	1.25
12.423	**β-cariofilen**	**2.43**	**1.58**	**1.28**	**0.93**	**1.91**
12.544	t-α-bergamoten	0.32	0.15	0.31	0.23	0.66
12.622	cumarina	-	0.05	-	0.05	-
12.743	t-β-farnesen	1.02	0.24	0.66	1.16	0.93
12.847	α-humulen	0.24	0.13	0.10	0.12	0.54
13.193	germacren D	0.72	0.48	0.28	0.33	0.59
13.782	artemisia triena	-	-	0.11	-	--
13.609	α-terpinen	0.12	0.08	0.16	0.08	0.60
14.466	cariofilenoxid	0.86	1.02	0.78	0.83	1.72
14.522	Δ-gurjunen	0.14	-	0.15	0.08	0.23
15.236	t-cadinol	0.45	0.03	-	-	-

TABELUL III

Compoziția procentuală, pe grupe de componente, a uleiurilor volatile din flori de *Lavandula sp.*

Conținut (%)	Probe					Standard în aromaterapie (%)
	LAA	LAP	LAH	LAM	LHB	
Hidrocarburi monoterpenice	19.67	10.70	14.22	12.96	11.12	**7-13**
Monoterpenoli	34.10	48.72	40.25	34.39	44.81	**30-40**
Monoterpenesteri	25.57	24.13	34.46	31.34	24.00	**40-50**
Monoterpencetone	2.33	1.54	0.42	3.27	2.23	-
Monoterpenoxizi	0.82	3.20	0.53	1.40	0.37	**până la 1.5**
Monoterpenaldehide	0.23	0.29	0.14	0.74	-	-
Sescviterpene	7.44	4.89	4.71	4.49	8.56	**până la 8**
Compuşi alifatici	0.37	1.48	0.94	1.04	0.51	-
Compuşi aromatici	1.46	1.12	1.01	1.98	1.30	-
Cumarine	-	0.05	-	0.05	-	-

Față de cele prezentate, trebuie să admitem că uleiul volatil separat din florile de *Lavandula hybrida* Rev. (care furnizează aşa-numita lavandină) şi *Lavandula angustifolia* ssp. *pyrenaica* se diferențiază de celelalte trei (LAA, LHB şi LAM), care se încadrează, conform definiției produsului, în categoria *L.angustifolia* Mill. Comparând uleiul de lavandină distilat din florile de *L. hybrida* Rev. de la Galați, cu datele cerute în aromaterapie pentru acest produs, vom constata din nou (1), că acesta nu corespunde prevederilor. Dacă uleiul de lavandină de uz terapeutic trebuie să aibă un conținut de 35-45% monoterpenesteri (preponderent acetat de linalil), 30-40% monoterpenoli (preponderent linalool), hidrocarburi monoterpenice 5-10%, monoterpencetone 4.5-5.5% (preponderent camforă), oxizi 2.5-3.5% (preponderent 1,8-cineol) şi 2% sescviterpene, concluzionăm că LAH corespunde în privința primelor două grupe de compuşi terpenici volatili, dar hidrocarburile monoterpenice sunt supradimensionate, iar cetonele, oxizii şi sescviterpenele, subdimensionate.

Dacă se urmăreşte **calitatea farmaceutică** a uleiului volatil obținut din florile de lavandă oficinală (*Lavandula angustifolia* ssp. *angustifolia*), ținând cont de prevederile specificate în Ph Eur. ed. a 6-a (5), se constată că nicio probă nu corespunde întrutotul din acest punct de vedere (tab. IV).

TABELUL IV

Calitatea farmaceutică a uleiului volatil obținut din probele de *L.angustifolia* ssp. *angustifolia*

Component (%)	Prevederi Ph Eur. ed. a 6-a	LAA	LHB	LAM
limonen	< 1	1.32	0.97	1.42
cineol	< 2.5	0.82	-	1.17
3-octanona	0.1-2.5	0.21	0.09	0.53
camfor	< 1.2	0.47	1.35	0.24
linalool	20-45	24.35	28.06	26.90
acetat de linalil	25-46	18.23	13.12	17.38
4-terpineol	0.1-6	-	2	3.75
acetat de lavandulil	> 0.2	3.74	9.46	8.42
lavandulol	> 0.1	-	0.80	1.46
α-terpineol	< 2	5.20	6.71	4.42

CONCLUZII

Fracțiunile volatile separate din cele 2 subspecii şi 2 varietăți de cultură ale speciei *Lavandula angustifolia*, precum şi de la specia *Lavandula hybrida* s-au dovedit a fi foarte diferite din punct de vedere chimic. Nici unul dintre uleiurile volatile de lavandă nu a prezentat toți parametrii de calitate impuşi pentru utilizarea în aromaterapie, cea mai importantă abatere fiind subdimensionarea conținutului în monoterpenesteri la circa 1/2-1/3 din valoarea standard. De asemenea, uleiul volatil din probele oficinale de lavandă nu corespunde întrutotul cerințelor de calitate impuse de ediția în vigoare a Farmacopeei Europene. Conținutul mic în esteri monoterpenici ar putea fi explicat prin faptul că temperatura medie zilnică în perioada de înflorire este mai mică în zona Galați-România prin comparație cu cea din zona mediteraneană. Totodată, orașul Galați este situat la o altitudine de 42 m deasupra nivelului mării, în timp ce uleiul de lavandă folosit în aromaterapie se obține de la plante cultivate la 800-1200 m deasupra nivelului mării. În aceste condiții, când începând cu data de 01.05.2011, Comunitatea Europeană impune respectarea condițiilor de admisibilitate a produselor vegetale de uz farmaceutic în funcție de alinierea lor la standardele europene, înainte de a le aduce pe piața farmaceutică, producătorii noştri de uleiuri volatile ar trebui să le controleze din punct de vedere chimic, chiar dacă ştiu sigur că au respectat prescripțiile privind proveniența botanică şi organul de plantă (menționat în definiție) în cursul prelucrării.

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CONSTRUCŢIA ŞI CARACTERIZAREA UNUI ELECTROD MEMBRANĂ SELECTIV PENTRU DETERMINAREA LISINOPRILULUI

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CONSTRUCTION AND CHARACTERISATION OF A SELECTIVE MEMBRANE ELECTRODE FOR THE ASSAY OF LISINOPRIL (Abstract) : In order to analyze lisinopril using a selective membrane electrode, the electro-active compound was obtained and included in the selective membrane. The new analysis method was developed and validated. **Material and method** : The electro-active compound was obtained through the precipitation of lisinopril in acidic media using silicowolframic acid solution. The membrane was obtained by mixing the electro-active compound with polyvinylchloride using tetrahydrofurane as solvent. The solution was evaporated in order to obtain a thick membrane, which was then attached at one end of a polyvinylchloride tube. The internal Ag/AgCl reference electrode was inserted in that tube. The assembly was filled with a lisinopril internal solution. The electrode was characterized by establishing the following characteristics : electrode slope, selectivity, optimal pH range, response time and life-time period. The developed method was validated. **Results** : The method showed a good linearity in the range of 10^{-7} and 10^{-2}M (the correlation coefficient r = 0.9991). The detection limit (LD) was $8,66 \times 10^{-8}$M and the quantification limit (LQ) was $7,8 \times 10^{-7}$M. There were established the precision (RSD = 1.73%) and the accuracy (mean recovery was 99.92%) **Conclusions** : The experimental results demonstrated a good sensibility. **Key words** : LISINOPRIL, MEMBRANE-SELECTIVE ELECTRODE, POTENTIOMETRY, VALIDATION

Lisinoprilul face parte din clasa inhibitorilor enzimei de conversie a angiotensinei, fiind indicat în tratamentul hipertensiunii arteriale şi insuficienţei cardiace congestive.

Au fost obţinuţi şi studiaţi relativ puţini electrozi membrană selectivi pentru analiza compuşilor din această clasă, precum lisinoprilul, enalaprilul şi ramiprilul (1,2,3).

Lucrarea de faţă prezintă construcţia şi caracterizarea unui electrod membrană selectiv, precum şi metoda de determinare potenţiometrică a lisinoprilului ce foloseşte acest electrod (4,5,6).

MATERIAL ŞI METODĂ

Reactivi şi aparatură :

- soluţie apoasă, stoc de lisinopri10^{-2}M ;
- soluţii de lucru de concentraţii între 10^{-3} M şi 10^{-9}M obţinute prin diluţia cu apă distilată a soluţiei stoc de lisinopril ;
- soluţie internă de referinţă – soluţie de lisinopril 10^{-5}M preparată în soluţie saturată de AgCl ;
- soluţii tampon acetat pH = 4,0 şi 5,0 (7) ;
- soluţii apoase de KNO_3 0,01M, 0,1M, 1M, 1,5M ;

- electrod de calomel ;
- potenţiometru/pH-metru Hanna Instruments 300.

Sinteza complexului lisinopril-acid silicowolframic

La un volum de 20 mL soluţie apoasă de lisinopril 1% s-au adăugat 10 mL HCl 2 M, iar apoi în porţiuni mici şi sub agitare soluţia de acid silicowolframic 5% în exces, până la precipitarea completă. După un repaus de aproximativ o oră, precipitatul format a fost separat prin filtrare, folosind un creuzet filtrant G_4 şi a fost spălat cu o soluţie saturată de precipitat şi cu apă distilată. Precipitatul a fost uscat în exicator la vid, la temperatura camerei, până la masă constantă.

Prepararea membranei selective

O cantitate de 0,01 g complex lisinopril-acid silicowolframic s-a dizolvat în 6,2 g soluţie de clorură de polivinil 5% şi s-au adăugat 0,68 ml dioctilftalat cu rol de plastifiant. Din această soluţie s-au introdus 1,5 mL în interiorul unui inel din inox cu diametrul interior de 30 mm, aşezat pe o placă din sticlă şi lipit în exterior cu soluţie de polietilenă în tetrahidrofuran. După evaporarea solventului, în interiorul inelului a rămas o membrană polimerică ce a înglobat materialul electroactiv.

Construcţia electrodului membrană selectiv

Membrana obţinută s-a aplicat la capătul unui tub de policlorură de vinil utilizând ca adeziv soluţia de policlorură de vinil 5% în tetrahidrofuran şi s-a lăsat să se usuce timp de 24 de ore. În corpul electrodului cu membrana selectivă ataşată s-a introdus soluţia internă de referinţă în care a fost imersat un fir de Ag acoperit cu AgCl. Ca soluţie internă de referinţă s-a utilizat o soluţie de lisinopril de concentraţie 10^{-5}M realizată într-o soluţie saturată de clorură de argint, pentru a mări durata de viaţă a electrodului de referinţă intern prin reducerea solubilităţii clorurii de argint depusă pe firul de argint ce intră în construcţia electrodului membrană selectiv.

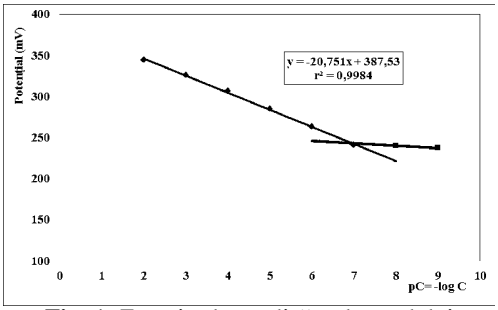

Fig. 1. Funcţia electrodică a electrodului lisinopril–selectiv

Caracteristicile funcţionale ale electrozilor membrană selectivi

Celula electrochimică formată din electrodul membrană ion–selectiv de construcţie proprie ca electrod indicator şi electrodul de calomel cu rol de electrod de referinţă extern a fost imersată în soluţia probei de analizat ce conţine lisinopril şi circuitul a fost închis cu ajutorul milivoltmetrului electronic cu rezistenţă internă mare (potenţiometru/pH-metru Hanna Instruments 300).

Efectul pH-ului s-a determinat prin măsurarea potenţialului la trei nivele de concentraţie : 10^{-2}, 10^{-4}, 10^{-7}M lisinopril ; modificarea pH-ului s-a realizat prin adăugare de soluţii tampon.

Influenţa tăriei ionice s–a studiat prin determinări pe seturi de soluţii de lisinopril, folosind soluţii de KNO_3 de diferite concentraţii pentru a ajusta tăria ionică. S-au comparat valorile pantelor dreptelor de calibrare obţinute prin metoda regresiei liniare şi s-a stabilit concentraţia soluţiei de KNO_3 ce a determinat o tărie ionică constantă pentru care panta a avut cea mai mare valoare.

Timpul de răspuns şi timpul de viaţă au fost evaluate prin înregistrarea valorile potenţialelor funcţie de timp.

Selectivitatea electrodului a fost testată din punctul de vedere al interferenţei ionii K^+, Na^+, CH_3COO^- şi NO_3^- deoarece aceştia au fost introduşi în soluţiile probelor de măsură în cazul ajustării pH-ului şi a tăriei ionice. Selectivitatea a fost apreciată prin coeficientul de selectivitate K.

TABELUL I
Valorile potenţialului de electrod pentru valori diferite ale tăriei ionice

pC = lgC	Soluţie KNO_3 0,01M	Soluţie KNO_3 0,1M	Soluţie KNO_3 1M	Soluţie KNO_3 1,5 M
2	310,6	321,9	344,6	344,1
3	295,4	303,5	326,4	326,7
4	280,2	287,6	307,2	306,4
5	265,6	270,3	290,1	289,4
6	250,7	253,9	264,3	263,6
7	235,6	236,3	240,8	240,2
Panta	**-14,96**	**-16,97**	**-20,64**	**-20,62**

TABELUL II
Valorile coeficienţilor de selectivitate calculate

Nr. crt.	Interferent	E(I)	E(II)	P	Coeficient de selectivitate, K
1	Na^+	306,8	136,2	20,75	$1,884 \cdot 1010^{-06}$
2	K^+		128,4		$1,031 \cdot 10^{-06}$
3	CH_3COO^-		115,5		$3,805 \cdot 10^{-07}$
5	NO_3^-		112,8		$3,088 \cdot 10^{-07}$

Funcţia electrodică a fost stabilită astfel: s-au efectuat măsurători de potenţial pe o serie de soluţii de lisinopril de concentraţie 10^{-9} până la 10^{-2}M. S-a trasat curba de calibrare a electrodului reprezentând potenţialul în funcţie de valoarea pC = – lg [lisinopril]. S-au determinat domeniul de răspuns liniar, panta electrodului şi potenţialul standard condiţional al electrodului.

Validarea metodei

Pentru determinarea liniarităţii s-au preparat, prin diluţii succesive cu apă distilată, soluţiile de lucru de concentraţii cuprinse în intervalul 10^{-2}–10^{-9}M. S-au citit valorile potenţialului folosind aceeaşi celulă electrochimică. Datele obţinute s-au prelucrat prin regresie matematică şi s-a trasat curba de calibrare. Metoda se consideră liniară dacă coeficientul de corelaţie este mai mare decât 0,998.

Precizia şi exactitatea au fost evaluate astfel: au fost analizate probe într-un domeniu de concentraţie de ±25% faţă de concentraţia de interes ce are valoarea de 10^{-4}M (10^{-3}M, 10^{-4}M, 10^{-5}M). În cazul preciziei se efectuează 2 seturi de probe pentru a evalua repetabilitatea metodei şi precizia intermediară. Pentru fiecare valoare a concentraţiei au fost efectuate un număr de trei determinări. Precizia şi exactitatea se exprimă prin deviaţia standard relativă (RSD) a valorilor obţinute care trebuie să fie mai mică de 2%.

Limitele de detecţie şi cuantificare s-au calculat utilizând metoda grafică (8,9,10).

REZULTATE ŞI DISCUŢII

Funcţia electrodică

Panta electrodului pentru domeniul liniar este P = –20,751 mV/decadă de concentraţie, iar potenţialul standard condiţional al electrodului determinat de pe curba de calibrare, prezentată în fig. 1, este $E_0' = + 387,53$ mV.

Influenţa tăriei ionice: valorile potenţialului de electrod înregistrate la diferite valori ale tăriei ionice sunt prezentate în tab. I.

Tăria ionică (constantă) pentru care panta a avut cea mai mare valoare s-a realizat prin adăugare de soluţie **KNO_3 1M**.

Efectul pH-ului: s-a stabilit că domeniul de pH optim este cuprins între 4,0 şi 5,0. Măsurătorile de potenţial pentru curba de calibrare şi determinarea potenţiometrică a lisinoprilului au fost efectuate după

TABELUL III
Liniaritatea metodei

Lisinopril pC = –lg C	Potenţial (mV)				
	Seria I	Seria II	Seria III	Seria IV	Media
2	343,5	342,6	344,6	345,1	343,95
3	326,6	325,2	326,1	325,9	325,95
4	305,2	306,6	305,4	306,8	306,00
5	285,8	286,4	284,9	286,5	285,90
6	264,2	266,2	265,2	267,0	265,65
7	240,7	243,2	242,3	241,4	241,90
Coeficient de corelaţie şi regresie **r = 0,9991, r^2 = 0,9983** Eroare standard = 1**,7298** Intercept = **386,3** Panta = **-20,32**					

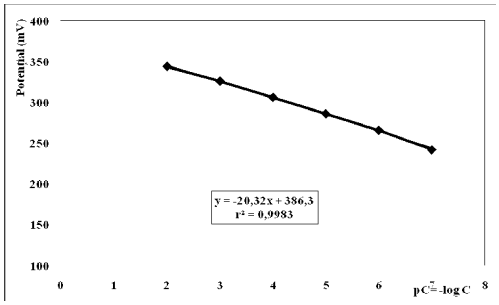

Fig. 2. Dreapta de calibrare

ajustarea valorii pH-ului la valoarea 4,0 prin adăugare de **soluţie tampon acid acetic-acetat de sodiu pH = 4,0.**

Timpul de răspuns este de aproximativ 15 secunde. Stabilitatea valorilor potenţialului de electrod este bună pentru o perioadă de aproximativ 2 luni, după care valoarea pantei electrodului începe să se modifice datorită degradării treptate a membranei selective.

Selectivitatea electrodului: valorile coeficienţilor de selectivitate sunt cuprinse în tab. II.

Valorile coeficientului de selectivitate, K, sunt foarte mici ceea ce indică că prezenţa ionilor testaţi în probe nu influenţează calitatea determinărilor.

Prin prisma acestor rezultate obţinute, procedura adoptată pentru determinarea cantitativă a lisinoprilului cu ajutorul noului electrod membrană selectiv a fost următoarea: din soluţia stoc de lisinopril 10^{-2}M se prepară prin diluţii succesive cu apă distilată soluţiile de lucru, de concentraţii în intervalul 10^{-3} - 10^{-7} M; din fiecare soluţie de lisinopril se măsoară un volum de 10 mL, se aduce în pahare Berzelius de 25 mL şi se adaugă un volum de 1,5 mL soluţie tampon pH = 4,0 şi 1,5 mL KNO_3 1M. Se citesc valorile potenţialului folosind electrodul de construcţie proprie şi electrodul de calomel (electrod de referinţă).

Validarea metodei

Liniaritatea metodei: s-a studiat liniaritatea pentru domeniul de concentraţie 10^{-7}–10^{-2}M. Datele obţinute sunt evaluate statistic (tab. III), iar prelucrarea acestora conduce la obţinerea dreptei de calibrare (fig. 2).

Domeniul de concentraţii în care electrodul prezintă un răspuns liniar este cuprins între **10^{-7} M şi 10^{-2} M**. Dreapta de calibrare calculată prin regresie matematică este:

$$\mathbf{E = -20,32 \times pC + 386,3}$$
$$\mathbf{(r^2 = 0,9983)}$$

Limita de detecţie şi Limita de cuantificare

Limita de detecţie are valoarea pC = 7,062 **($LD = 8,66 \times 10^{-8}$M)**

Limita de cuantificare are valoarea pC = 7,10 **($LC = 7,80 \times 10^{-8}$M)**

Precizia metodei: se constată că atât pentru fiecare set de date separat, cât şi pentru ambele seturi de date luate împreună, deviaţia standard relativă are o valoare mai mică de 2% (**RSD = 1,79%),** deci metoda de analiză este precisă (tab. IV)

Exactitatea: din valorile experimentale

TABELUL IV
Precizia şi exactitatea metodei

Conc. (mol/L)	Repetabilitatea metodei		Precizia intermediară		Exactitatea metodei	
	E (mV)	Regăsire %	E (mV)	Regăsire %	E (mV)	Regăsire %
10^{-3}	325,2	98,05	325,6	102,60	325,4	100,30
	325,5	101,44	325,2	98,05	325,3	99,17
	325,3	99,17	325,4	100,30	325,2	98,05
10^{-4}	305,1	100,54	305,3	102,85	305,0	99,41
	304,9	98,29	304,9	98,29	305,2	101,69
	305,3	102,85	305,1	100,54	305,1	100,54
10^{-5}	284,6	98,53	284,9	101,93	284,7	99,65
	284,8	100,79	284,7	99,65	284,6	98,53
	284,9	101,93	284,6	98,53	284,9	101,93
Date statistice	**Medie** = 100,17% **RSD** = 1,73%		**Medie** = 100,30% **RSD** = 1,84%		**Medie** = 99,92% **RSD** = 1,32%	

(tab. IV) se constată că pe domeniul de concentraţie studiat regăsirea medie este de 99,92%, iar deviaţia relativă standard (RSD = 1,32) este mai mică de 2% ; deci metoda de analiză propusă este exactă.

CONCLUZII

A fost construit şi caracterizat un electrod membrană selectiv ce utilizează ca material electroactiv complexul lisinopril-acid silicowolframic.

Electrodul membrană selectiv a fost caracterizat prin stabilirea funcţiei electrodice, efectul pH-ului, influenţa tăriei ionice, timpul de răspuns şi timpul de întrebuinţare, precum şi selectivitatea electrodului.

Metoda de analiză a fost validată, stabilindu-se : liniaritatea metodei (pe domeniul 10^{-2}M-10^{-7}M), coeficientul de corelaţie (r = 0,9991), limita de detecţie ($8{,}66\times10^{-8}$M), limita de cuantificare ($7{,}8\times10^{-8}$M), precizia metodei (RSD = 1,73%) şi exactitatea metodei (regăsire medie = 99,92%, RSD = 1,32%).

În concluzie, metoda de analiză propusă este simplă, uşor de executat şi sensibilă. De asemenea metoda este liniară, precisă şi exactă.

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EVALUAREA POSIBILITĂŢILOR DE REFACERE A ECHILIBRULUI ESTETIC DENTO-PARODONTAL PRIN MJLOACE PROTETICE: FAŢETE CERAMICE *VS.* COROANE DE ÎNVELIŞ METALO-CERAMICE

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CLINICAL EVALUATION OF THE POSIBILITIES OF RESTORING THE DENTAL AND PERIODONTAL ESTHETICS USING VENEERS *VS.* METAL CERAMIC CROWNS (Abstract): Achiving the esthetic balance is one of the most important aims of the restorations used for the anterior area of the dental arches. **Aim**: To evaluate the posibilities of veneeers and metal ceramic crowns to restore dental and periodontal esthetics. **Material and method**: We have evaluated 90 restorations, 40 veneers and 50 metal ceramic crowns, analysing the following parametres: gingival index, bleeding index, palque index, also restorations margins index, presence/absence of secondary decays, marginal integrity index and the aspect of the rstorations surfaces. The study also evaluates patient staisfaction using questionares. **Results**: Even the veneers seem to be more indicated to restore dental and periodontal esthetics, the esthetic outcome depends mainly on the way the clinician evaluates and manages the tissues and less on the technique used (all ceramic or metal ceramic). **Key words**: VENEERS, METAL CERAMIC CROWNS, DENTAL ESTHETIC, PERIODONTAL ESTHETICS

Coroanele metalo-ceramice, deşi eficiente din punctul de vedere al refacerii funcţionalităţii unităţilor odonto-parodontale, prezintă o serie de dezavantaje cum ar fi necesitatea realizării unei preparaţii ce presupune îndepartarea unei cantităţi importante din structura dentară, necesitatea plasării subgingvale a marginii vestibulare ale preparaţiei atunci când se realizează la nivelul dinţilor arcului anterior maxilar, existenţa infrastructurii metalice care alterează capacitatea porţelanului de a reda translociditatea naturala a dinţilor şi uneori a culorii acestora. Faţetele ceramice rezolvă o serie dintre neajunsurile coroanelor de înveliş metalo-ceramice deoarece necesită preparaţii minime, de obicei doar la nivelul smalţului, permit refacerea morfologiei şi esteticii dentare, presupun amplasarea pragului preparaţiei la nivel supragingival, deci prezintă o serie de avantaje incotestabile. În aceeaşi masură însă indicaţiile lor trebuie stabilite cu discernământ, utilizarea lor fiind o varintă optimă doar în anumite cazuri, deci nu pot substitui sub nici o formă în totalitate coroanele de înveliş.

SCOPUL STUDIULUI

În cadrul acestui studiu ne-am propus realizarea unei comparaţii între coroanele de înveliş metalo- ceramice şi faţetele de porţelan din punctul de vedere al:

- Conservării stării de sănătate parodontală
- Capacităţii de resturare a echilibrului estetic
- Conservării rezultatelor în timp

MATERIAL ŞI METODĂ

Am evaluat astfel 90 de restaurări protetice, dintre care 40 au fost faţete de porţelan (grup I) şi 50 au fost coroane de înveliş metalo-ceramice (grup II).

Din grupul nr I au facut parte 8 barbaţi şi 12 femei cu vârste cuprinse între 27 şi 56 ani iar din grupul nr II au facut parte 10 barbaţi şi 15 femei, cu vârste cuprinse între 24 şi 62 ani.

Aceste două grupuri au fost divizate la rândul lor în subgrupuri în funcţie de vechimea restaurărilor protetice :

- Între 0 şi 6 luni
- Intre 7 luni şi 24 luni
- Mai vechi de 24 luni

Toţi pacienţii incluşi în studiu au fost selectaţi astfel încât să nu prezinte afecţiuni care să influenţeze starea de sănătate parodontală. Au fost astfel excluşi pacienţii care urmau tratamente cu ciclosporine, betablocante, anticoncepţionale, antiinfamatorii şi pacienţii cu afecţiuni cronice (ex : diabet). Am exclus din studiu pacienţii fumători.

În vederea evaluării stării de sănătate parodontala precum şi a gradului de refacere funcţională a unităţilor odonto-parodontale măsuratorile au fost efectuate de către 3 evaluatori. Aceştia au urmat acelaşi protocol de lucru şi au examinat aproximativ acelaşi număr de pacienţi. Calibrarea evaluatorilor a avut în vedere explicarea detaliată a protocolului de examinare cu realizarea unor determinări prealabile începerii studiului. Pentru calibare şi verificarea acurateţii/reproductibilităţii datelor s-au făcut şi evaluări încrucişate (fiecare evaluator a facut determinări la câte 6 pacienţi din celelalte loturi de studiu), concordanţa datelor culese având o rata de 97%.

Evaluarea stării de sănătate parodontală a presupus următoarele determinări :

- Investigarea profunzimii spaţiului biologic parodontal. Măsurarea s-a realizat după metoda clasică, în şase puncte : DV, V, MV, MP, P, DP.
- Determinarea indicelui de sângerare gingivală
- Determinarea indicelui de placă bacteriană

Pentru evaluarea profunzimii spaţiului biologic parodontal precum şi pentru determinarea indicelui de sângerare s-au utilizat sonde parodontale gradate . Determinarea indicelui de placă s-a realizat cu ajutorul revelatorilor de placa bacteriană.

Investigarea gradului de refacere funcţională a unităţilor odonto-parodontale a presupus analiza următorilor parametri :

- Indicele de localizare a marginilor restaurării protetice (1) :
- Prezenţa /absenţa cariilor secundare
- Gradul de integritate marginală a restaurărilor
- Aspectul suprafeţei restaurărilor protetice : structură, culoare, formă

Ultimii trei parametri au fost apreciaţi utilizând criteriile Ryge de evaluare clinică (2). Pentru acest parametru s-au luat în considerare numai suprafeţele vestibulare ale restaurărilor.

Pentru determinarea gradului de satisfacţie din punct de vedere estetic precum şi a gradului de confort, fiecare pacient a completat un chestionar unde a raspuns la următoarele întrebări :

1. Cum apreciaţi din punct de vedere estetic tratamentul protetic efectuat : 1- Excelent ; 2- Bun ; 3- Acceptabil ; 4- Inacceptabil
2. Cât de confortabile vi se par coroanele/faţetele realizate : 1- Excelent ; 2- Bun ; 3- Acceptabil ; 4- Inacceptabil

Datele au fost interpreatate din punct de vedere statistic utilizând teste t perechi, datele obţinute în cadrul celor două grupuri de studiu fiind analizate cu ajutorul testelor Krusall – Wallis. Pentru evaluarea modificărilor în timp din cadrul subgrupelor definite la nivelul celor două grupe de studiu am utilizat analiza de variabilitate a datelor.

REZULTATE

Am evaluat în total un nr de 90 de res-

TABELUL I

Rezultatele investigării stării de sănătate parodontală

Indice/tip de restaurare	Vestibular		Palatinal	
	Interproximal	Central	Interproximal	Central
Profunzimea spaţiului periodontal				
➢ FP	2,28	1,50	2,12	1,42
➢ CCM	2,52	1,67	2,48	1,73
Indicele de sângerare				
➢ FP	0,40	0,22	0,39	0,17
➢ CCM	0,64	0,25	0,77	0,60
Indicele de placă				
➢ FP	0,93	0,82	1,89	1,62
➢ CCM	0,89	0,75	0,48	0,25

taurări protetice, 40 de faţete de porţelan (FP) şi 50 de coroane de înveliş metalo--ceramice (CCM)

Pentru faţetele de porţelan vechimea medie a restaurărilor a fost următoarea :

0-6 luni : 2,6 luni

7-24 luni : 12,9 luni

< 24 luni : 35,5 luni

Pentru coroanele de înveliş metalo-ceramice vechimea medie a restaurărilor a fost următoarea :

0-6 luni : 2,5 luni

7-24 luni : 15,6 luni

< 24 luni : 38,7 luni

Rezultatele investigării stării de sănătate parodontală sunt prezentate în tabelul I.

Procentul situsurilor prezentând o profunzime a spaţiului periodontal mai mare de 3 mm a fost mai mic în cadrul lotului de faţete de porţelan. Frecvenţa pungilor parodontale de 4 mm a fost de 1% la nivelul suprafeţelor vestibulare ale faţetelor, de 4% în cazul feţelor vestibulare ale CCM şi de 2% în cazul faţelor palatinale ale CCM. Nu au existat diferenţe semnificative din punct de vedere statistic între determinările realizate la nivelul situsurilor apreciate la nivelul CCM şi cele evaluate la nivelul FP.

Indicele de sângerare a crescut proporţional cu creşterea indicelui de localizare a marginilor restaurării protetice (fig. 1,2).

Pentru fiecare grupa de indici (0-3) de localizare a marginilor restaurării, indicii de sângerare prezentau valor mai mari în cazul CCM decât în cazul FP.

Nu s-au constatat diferenţe semnificative din punctul de vedere al indicelui de sângerare între subgupele de vechime sau în cadrul aceleiaşi subgrupe de vechime.

Indicele de placă bacteriană a fost în general scăzut pentru faţa vestibulară şi nu a variat important statistic între cele doua grupuri de studiu. Înregistrarea indicelui de localizare a marginilor restaurării protetice a aratat ca 56 % dintre marginile FP au fost plasate juxtagingival iar 21% au fost plasate supragingival.

În cazul CCM 28% dintre marginile restaurărilor protetice au fost dispuse juxtagingival la nivelul feţei vestibulare în timp de 62% au fost plasate subgingval. La nivelul feţelor palatinale ale CCM, 31% dintre acestea erau dispuse juxtagingival şi 49% dispuse subgingival.

Evaluatorii au apreciat calitatea suprafeţelor restaurărilor protetice şi culoarea acestora ca fiind mai bună în cazul FP decât în cazul CCM în cazul restaurărilor incluse în grupa de vechime 0-6 luni (85%α vs 30%α) precum şi a celor incluse în grupa de vechime > 24 luni (75%α vs 35%α).

Nu s-au înregistrat diferenţe semnificative din punct de vedere statistic între cele două grupe de studiu în ceea ce priveşte capacitatea de restabilire a formelor şi contururilor, deşi 35% dintre coroanele analizate şi 32% dintre faţete erau supraconturate ca urmare a preparaţiei insuficiente.

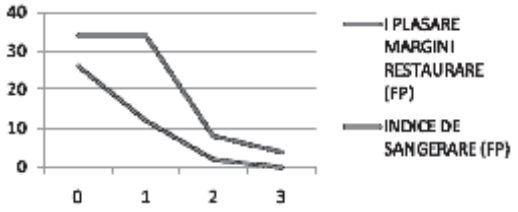

Fig. 1. Variaţia indicelui de sângerare în funcţie de indicele de plasare a marginilor restaurării protetice (FP)

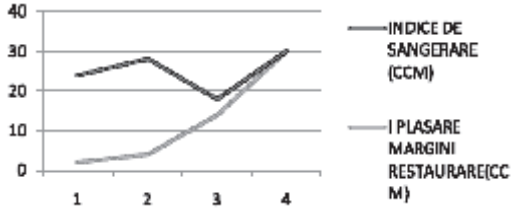

Fig. 2. Variaţia indicelui de sângerare în funcţie de indicele de plasare a marginilor restaurării protetice (CCM)

Nu s-au decelat fracturi nici în cadrul grupului FP nici în cadrul grupulu CCM.

Integritatea marginală a restaurărilor nu a diferit semnificativ din punct de vedere statistic între cele două loturi.

3 CCM au prezentat carii secundare (6%).

În ceea ce priveşte gradul de confort nu s-au înregistrat diferenţe semnificative, pacienţii apreciindu-l cu excelent şi bun. 80% dintre pacienţii incluşi în lotul cu FP cu o vechime mai mare de 24 de luni au apreciat ca excelent rezultatul estetic spre deosebire de 40% dintre pacienţii aceluiaşi subgrup dar trataţi cu CCM.

DISCUŢII

Succesul /eşecul diferitelor metode de restaurare a echilibrului estetic al unităţilor dento-parodontale depinde în cea mai mare măsură de maniera în care clinicianul gestionează suportul dento-parodontal, tehnologia în sine aleasă (integral ceramică sau metaloceramică) având un impact mult mai mic (3).

Studiile (4,5) arată că amplasamentul subgingival al marginilor restaurărilor protetice are efecte negative asupra stării de sănătate parodontală şi implicit asupra rezultatelor din punct de vedere estetic. În momentul în care clinicianul decide nivelul de amplasarea marginilor restaurării protetice trebuie să ia în calcul practic două aspecte :

- capacitatea de a menţine starea de sănătate parodontală
- plasarea marginilor restaurării să fie suficient de profundă dar în acelaşi timp să conserve dimensiunile spaţiului biologic parodontal.

Studiul a arătat creşterea proporţională a indicelui de sângerare în corelaţie cu indicele de plasare a marginilor restaurărilor protetice. Cu cât marginile restaurărilor protetice au fost lasate mai apropae de marginea gingivală liber cu atât riscul de apariţie a gingivitei a fost mai mare.

În aceeaşi măsură o altă cauză a alterării stării de sănătate parodontală a fost prepararea insuficientă a suprastructurii organice fapt care a condus la supraconturare atât în cazul faţetelor de porţelan cât şi în cazul coroanelor de înveliş.

În cadrul lotului de studu s-au constatat diferenţe semnificative între cele două loturi de studiu din punctul de vedere al recesiunii parodontale : 28% în cazul CCM faţă de 20% în cazul FP.

Din punctul de vedere al accentuării gradului de recesiune parodontală în timp , nu s-au înregistrat diferenţe semnificative între cele două loturi de studiu .

Determinarea profunzimii pungilor parodontale a relevat valori mai mari ale acestora în cazul CCM. Nu s-au înregistrat însă profunzimi mai mari de 4 nmm în nici unul dintre cele două loturi de studiu.

Din punctul de vedere al refacerii formelor, conturilor, culorii, structurii şi texturii suprafeţelor, rata de succes în cazul faţetelor de porţelan a fost mai mare decât în cazul coroanelor de înveliş metalo-ceramice (89% vs74%). Cauzele acestor rezultate sunt multiple.

Din punctul de vedere al formelor şi contururilor s-a constat o tendinţă mai mare a preparaţiei insuficiente în cazul coroanelor de înveliş fapt care a condus la restaurări supraconturate (6).

În aceeaşi măsură amplasarea coroanelor de înveliş metalo ceramice între structuri dentare naturale este un demers estetic mult mai dificil decât amplasamentul faţetelor integral ceramice, mai ales în cazul restaurărilor expuse integral iluminării directe cum sunt cele din zona arcului anterior maxilar (7). Existenţa infrastructurii metalice conduce la diferenţe decelabile vizual între gradul de transluciditate al dinţilor naturali şi suprastructurile protetice metalo-ceramice, fapt care conduce consecutiv la percepţia diferită a culorilor, formelor, contururilor şi chiar al texturii si structurii suprafeţelor restaurărilor. Acest deficit nu există în cazul faţetelor ceramice (8) .

Un alt motiv al alterării stării de suprafaţă este acela al ajustărilor realizate în cabinet la nivelul coroanelor de înveliş metalo-ceramice, fapt care a condus la alterarea stării de suprafaţă şi deci a luciului acesteia. Consecutiv se modifică comportamentul luminii la acest nivel şi deci percepţia culorilor, formelor şi dimensiunilor (9).

Deşi nu s-au determinat diferenţe importante din punct de vedere statistic între cele două loturi de studiu din punctul de vedere al integrităţii marginilor restaurărilor protetice, margile CCm par a fi mai suseptbile la carie secundară decât cele ale FP (5% faţă de 0%).

Din punctul de vedere al satisfacţiei pacienţilor faţetele de porţelan par a fi preferabile CCM. Acest fapt se poate datora pe de o parte rezultatelor estetice optime precum şi preparaţiei minim invazive şi faptului ca restaurarea nu ocupă decât faţa vestibulară a dinţilor.

CONCLUZII

Faţetele de porţelan par a avea, datorită exigenţelor de preparare şi avantajului pe care le prezintă datorită structurii integral ceramice, cel mai mic impact asupra stării de sănătate parodontală .

În acelaşi timp în cazul CCM cu cât marginea acestora se apropie de marginea gingivală liberă cu atât indicele de sângerare creşte şi este mai mare decât în cazul faţetelor de porţelan amplasate la acelaşi nivel.

CCM sunt mai susceptibile la carii secundare faţă de FP.

Potenţialul de refacere a formelor, dimensiunilor, culorii, structurii şi texturii suprafeţelor este mai mare în cazul faţetelor de porţelan, mai ales în cazul în care se are în vedere restaurarea incisivilor centrali maxilari. Amplasarea restaurărilor protetice între structuri dentare naturale face foarte dificilă crearea iluziei naturalului cu ajutorul CCM. De aceea este indicat ca în cazurile în care situaţia clinică permite să se prefere FP ca mijloc terapeutic.

Din punctul de vedere al satisfacţiei pacienţilor faţetele de porţelan par a fi preferabile CCM, diferenţa dintre cele două loturi de studiu fiind cu atât mai mare cu cât intervalul de timp creşte (80% satisfacţie în cazul FP la 24 luni faţă de 40% în cazul CCM).

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NOUTĂŢI

EVALUAREA ALGORITMILOR FARMACOGENETICI PENTRU STABILIREA NECESARULUI DE WARFARINĂ ÎN CAZUL PACIENŢILOR JAPONEZI

Warfarina este unul dintre cele mai prescrise anticoagulante orale, folosită pentru profilaxia şi tratamentul bolilor tromboembolice. Stabilirea dozei necesare fiecărui pacient pentru a se încadra în intervalul terapeutic vizat, se face de regulă prin tatonare ; doza respectivă variind semnificativ de la un pacient la altul (de pana la 10 ori) în funcţie de vârstă, dietă, rasă şi genotip. Din aceasta cauză, cel puţin în primele luni de la începerea terapiei anticoagulante, pacienţii prezintă un risc crescut de anticoagulare excesivă ce poate duce la apariţia unor accidente hemoragice fatale. Pentru a se reduce cât mai mult posibil acest risc au fost elaboraţi o serie de algoritmi de dozare a acestui anticoagulant, majoritatea incluzând factori clinici, variabile demografice precum şi variaţii moleculare ale genelor implicate în realizarea efectelor warfarinei (VKORC1 – gena ce codifică subunitatea 1 a complexului vitamina K epoxid reductaza şi respectiv CYP2C9 – ce codifică enzima responsabilă de metabolizarea warfarinei). In anul 2007, FDA-ul a impus introducerea în prospectul warfarinei a informaţiilor relevante din punct de vedere farmacogenetic, iar recent, International Warfarin Pharmacogenetics Consortium (IWPC) a elaborat, în urma unui studiu la care au participat 5052 de pacienţi, un algoritm farmacogenetic de dozare a warfarinei : 5,4952–(0,2614 × [varsta în decade]) + (0,0087 × [înălţimea în cm]) + (0,0128 × [greutatea în kg]) – (0,8677 × [VKORC1 A/G]) – (1,6974 × [VKORC1 A/A]) – (0,9357 × [CYP2C9*1/*3]) – (2,3312×[CYP2C9*3/*3]) + (1,1816 × [statusul enzimatic]) – (0,5503 × [statusul amiodarona]) = radacină pătrată din doza săptămânală de warfarină. Performanţa acestui algoritm a fost testată de către cercetătorii japonezi utilizând 200 de pacienţi împărţiţi în 3 categorii : sub tratament cu doză scăzută de warfarină (≤10,5 mg/săptămână), sub tratament cu doză crescută de warfarină (≥31,5 mg/ săptămână), şi sub tratament cu doză intermediară de warfarină (între 10,5 şi 31,5 mg/ săptămână). Acest experiment a demonstrat reproductibilitatea performanţei algoritmilor IWPC în cazul celor 3 categorii de pacienţi japonezi, şi în mod special, în cazul pacienţilor sub tratament cu doză scăzută de warfarină (≤10,5 mg/săptămână), în cazul cărora procentajul supra-estimării dozei a fost semnificativ mai mic când doza de warfarină a fost stabilită folosindu-se algoritmul farmacogenetic (32%) decât ăn cazul folosirii algoritmului clinic (64%), reducându-se astfel semnificativ riscul anticoagulării (Takeuchi F, Kashida M, Okazaki O. Evaluation of pharmacogenetic algorithm for warfarin dose requirements in Japanes patients. *Circ J* 2010 ; 74 (5) : 977-982).

M. Macovei

RESEARCHES OF THE NITRATES AND NITRITES IN SOME WELL WATERS FROM RURAL AREA IN CORRELATION WITH METHOGLOBINEMIA MORBIDITY

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RESEARCHES OF THE NITRATES AND NITRITES IN SOME WELL WATERS FROM RURAL AREA IN CORRELATION WITH METHOGLOBINEMIA MORBIDITY (Abstract): Ground water is a major source of public supply for the rural population and in some areas, it is only source of available water. It is well known the toxic action of nitrate upon organisms, by formatting methemoglobin and N-nitroso compounds. **Material and methods**: In this study it was investigated the presence of nitrates and nitrites in same well waters, mountain and hill areas in Neamţ district. The chemical parameters were carried out by following standard methods. The statistical analysis includes descriptive statistics of the water quality parameters. **Results**: It is noticed high values of the nitrate concentrations, over Maximum Admissible Concentration (MAC), explained by excessive use of agricultural synthetic fertilizer and telluric nitrogen. It was registered a number of 38 cases of methemoglobinemia in small children with artificial nutrition, all cases from hill area. The maximum methemoglobin level was 60% and the minimum value of nitrate concentration in the water samples was 396 mg NO_3^-/L. **Conclusions**: We established a direct correlation between the level of methemoglobin and the nitrate concentrations in water samples. It is recommended the use well waters with maximum 48 mg/L nitrates content. **Key words**: WELL WATER, METHEMOGLOBINEMIA, NITRATES

OPTIMIZED METHOD FOR OBTAINING CHITOSAN NANOPARTICLES LOADED WITH XANTHINE DERIVATIVE

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OPTIMIZED METHOD FOR OBTAINING CHITOSAN NANOPARTICLES LOADED WITH XANTHINE DERIVATIVE (Abstract): The use of nanotechnology is the current trend for drug delivery approaches. **Aim**: This research was focused on the development of an optimized method for preparation and characterization of chitosan nanoparticles (CSNPs) and xanthine derivative loaded CSNPs. **Materials and methods:** In order to develop appropriate method different parameters such as the concentration of chitosan and tripolyphosphate as well as the stirring speed were varied. The aggregation of the nanoparticles (NPs) was prevented by using Tween 80 as a surfactant. **Results:** Following the CSNPs optimization process nanoparticles in the range of 231-244 nm were obtained. The optimized method was used for the loading of xanthine derivative in the CSNPs. The particle size of CSNPs loaded with xanthine derivative was 267 nm and the loading efficiency was ~ 86%. In presence of the surfactant, the NPs size decreases slightly from 267 nm to 193 nm with increasing the concentration of Tween 80. The CSNPs loaded with xanthine derivative remain stable after 72 h of storage. **Conclusions:** The CSNPs and xanthine derivative loaded CSNPs obtained after the optimization method by modifying several independent variables can be characterized as having small size and a good stability over time. Given these important features CSNPs are promising materials for targeted drug delivery system. **Keywords:** NANOPARTICLES, IONIC GELATION, CHITOSAN, TRIPOLYPHOSPHATE.

Drug carrier systems constitute an important research field due to some characteristics such as the capacity to encapsulate bioactive compounds, cross biological barriers and to protect macromolecules such as proteins, peptides from degradation in biological media and to deliver drugs to a target site with following controlled release (1). Nanogels (nanometer sized hydrogel particles) have attracted growing interest over the last several years in terms of their potential for applications in pharmaceutical fields, like drug delivery systems for improve the pharmacokinetic profile of drugs such as poor gastro-intestinal absorption, low drug solubility, and rapid metabolism. In the last years have been important developments of several synthetic as well as natural polymers and has researched the possibility of their use for pharmaceutical applications (2).

Natural polymers can be generally divided into four different categories, including polysaccharides, proteins, nucleic acids, and lipids. One polysaccharides large used is chitosan (CS) and it is defined as polymeric carbohydrate structures composed of repeating monosaccharide units linked by glyosidic bonds (3).

Recently CS, the second most abundant natural polymer after cellulose, has attracted great attention in pharmaceutical and biomedical fields because of its advantageous biological properties such as: widely found in nature, biocompatibility, nontoxicity and biodegradability (4, 5). The manufacturing process of CS particles as carriers for drug delivery system, especially in terms of nanoparticles (NPs), has widely been studied (6). Several different techniques are usually employed to obtain NPs from CS including ionic gelation emulsion, synthesis with carboxymethyl cellulose, formulation using glutaraldehyde, synthesis with alginate and polymerization with poly (hydroxyethyl methacrylate). Among them the ionic gelation technique (IGT) has attracted considerable attention because it uses nontoxic cross-linker, organic solvent free and has convenient and controllable benefits (7, 8). IGT using syringe dropping is usually employed to prepare chitosan nanoparticles (CSNPs), in which sodium tripolyphosphate (TPP) solution (pH 7-9) was dropped into the acidic solution (pH 4-6) of CS. Despite of the easy procedures, it is uneasy to control the process for reproduction (9). The obtaining of CSNPs by IGT involve electrostatic interactions between the positively charged primary amino groups of chitosan and negatively charged groups of polyanions employed as cross-linkers to determined pH values, behaving as a metastable thermodynamic system which is highly sensitive to variations in ionic strength. For this reason appropriate choice of CS amount, the time spent in the reaction, the rate of addition and concentration of cross-linker agent TPP, the stirring speed applied during the reaction, pH of the solution are factors that affect the properties of CSNPs: particle size, long-term stability during storage, drug loading and release efficacy. It can be concluded that preparation process of CSNPs could be manipulated by varying the preparation conditions. TPP is the most frequently non-toxic polyanion used as cross-linker agent for preparation CSNPs (2).

It is know that NPs have a high tendency to aggregate after a storage period as a result of increasing the surface area in NPs formulations (10). During the preparation process of CSNPs the mechanical energy associated with the reaction stirring speed may exceed the electrostatic repulsion energy between nanoparticle positive surface charges, thus eventually triggering aggregation tendency. This represents a recurrent problem in the formation of single nanoparticles. Tween 80 decreases the surface tension at the interface between materials so this agent can be used mainly to limit aggregation of NPs and therefore acts in the reducing of their sizes (11). Therefore, developing CSNPS with small particle size is the key to improve its encapsulation efficacy and other functionalities.

This paper aims to describe the protocol for obtaining CSNPs in order to be used as drug delivery systems for xanthine derivatives.

MATERIAL AND METHODS

Materials. Chitosan low molecular weight (CSLMW), TPP and Tween 80 were purchased from Sigma Aldrich. Chitosan

had a molecular weight (M_w) of 190,000-375,000), viscosity of 200 cps and degree of deacetylation (DD) of 75-85% as indicated by the supplier. All other reagents and solvents were of high grade purity and were obtained from commercial suppliers.

Methods

Nanoparticles formulation

CSNPs were prepared *via* IGT proposed by Fabregas et al. (10). In order to optimize the method different concentrations of CSLMW – 0.05%, 0.1%, 0.25% w/v were chosen. Briefly, CSLMW was dispersed in acetic acid 2% (v/v) and the mixture was left under stirring overnight at room temperature to obtain clear CS gel. Then 2 mL of TPP aqueous solution (0.1% and 0.5%) were added drop wise to 0.25 mL CSLMW solution (0.05%, 0.1% and 0.25%) with a micropipette under 700 rpm and stirring speed of 1000 rpm. The conversion of the solution from clear to opaque appearance indicated nanoparticles formation. The final suspension was stirred for 2 h to obtain an efficient reticulation and then centrifuged. The NPs (sediment) could be observed from the bottom of centrifuge tube. The supernatant was removed and CSNPs were dispersed in distilled water for further characterization. The size and distribution of the CSNPs were monitored using a dynamic light scattering set-up equipped with a Brookhaven BI-9000 AT digital correlator (Brookhaven Instrum. Corp, N.Y.) at the temperature of 25^0C. For improving the CSNPs diameter and for avoiding the aggregation, the Tween 80 (10 μL and 20 μL) was added to a sample of 0.25 mL CSLMW gel (0.05%, 0.1% and 0.25% w/v) under stirring for 30 min. The particle size distribution was reported as polydispersity index (PDI) from the DLS analyzer, in which PDI <1 represented the good polydispersion (12). Particle size and polydispersity measurements were run in triplicate.

Chitosan nanoparticles loaded with xanthine derivative

The xanthine derivative (2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl]hydrazono}-5-benzylidene-3-(4-brom-phenyl)-thiazolidine-4-one) (13) (2 mg) was dissolved in 5 mL of glacial acetic acid and the solution was mixed with 20 μL Tween 80. Then, 0.25 mL CSLMW gel (0.05% w/v) was added and the mixture was stirred for one hour. Afterwards, 2 mL of TPP solution (0.5% w/v) were added dropwise slowly using syringe dropping method and the suspension was stirred for another 2 h. The conversion of the solution from clear to opaque appearance indicated nanoparticles formation. The particles formed were separated by centrifugation. The supernatant was removed and spectrophotometrically analyzed for loading efficiency evaluation, and xanthine derivative-loaded CSNPs were dispersed in distilled water for further characterization. Particle size and polydispersity measurements were run in triplicate.

Loading efficiency (LE)

The amount of xanthine derivative in the CSNPs was evaluated using UV spectrophotometric method (UVIKNO XL, BIOTECH Instruments). After centrifugation of the xanthine loaded CSNPs suspension, the supernatant was spectrophotometrically analyzed at 280 nm for evaluating the content of xanthine derivative in the TPP solution.

The loading efficiency (%) was calculated using the following formula:

$$LE\ (\%) = C_1/C_0 \times 100$$

where: C_0 = initial concentration of the xanthine derivative (mg/ml); C_1 = concen-

tration of the xanthine derivative in the TPP solution (mg/ml) (14).

RESULTS AND DISSCUSION

Chitosan nanoparticles formulations

Chitosan (CS) undergoes nucleophilic interaction with the cross-linking agent (TPP) resulting in CSNPs formation. Chitosan with a pKa of 6.3 is polycationic and presents NH_3^+ sites when dissolved in acidic medium (acetic acid). When sodium tripolyphosphate ($Na_5P_3O_{10}$) is dissolved in water it dissociates to give both hydroxyl and phosphoric ions. In the present study, the pH of TPP was selected to be 9.0 and at this pH both hydroxyl and phosphoric ions are and may compete with each other to interact with the NH_3^+ of chitosan (13). The hydroxyl ions get linked to the amino groups by deprotonation. The chemical structure of chitosan (a), tripolyphosphate (b) and xanthine derivative (c) are presented in figure 1.

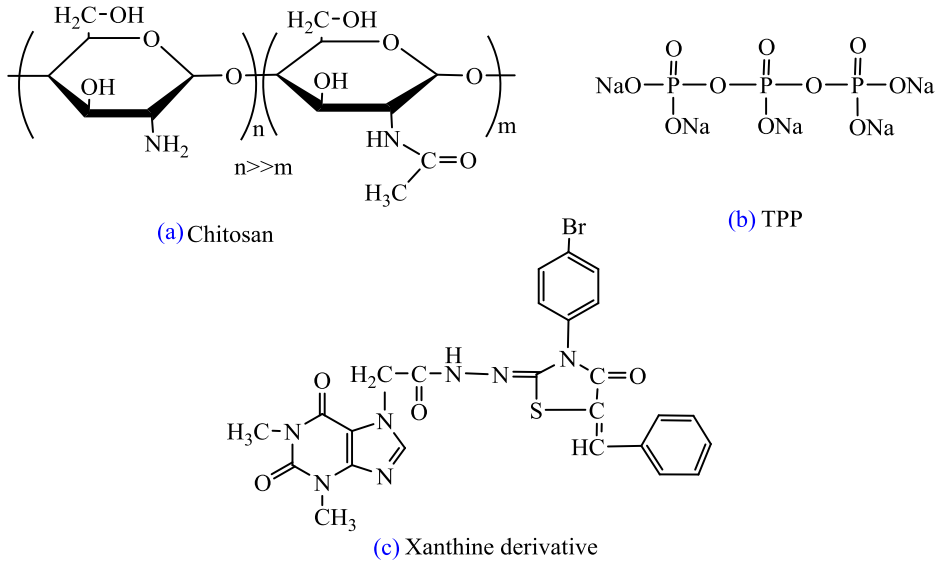

Fig.1 The chemical structure of chitosan (a), tripolyphosphate (b), xanthine derivative (c)

The size of the particles according to different concentration of CSLMW, TPP and stirring speed during ionic gelation are shown in tab. I. By varying these parameters NPs in the range of 231-963 nm were obtained.

The increase in CS concentration was associated with an increase in particle size due to the large number of amino groups that are able to interact as reported in the literature. As can be seen in table I, the average diameter of NPs was about 200 nm when CS concentration was 0.05% w/v while the size increased more than four times when the concentration of CS was 0.25% w/v. At the same time it was noticed that at the higher concentration of TPP the particle size decreased with increasing cross-linking degree of the CSNPs which adopt a more compact and stable structure. The stirring speed during NPs formation also influences their size; thus by increasing the speed from 700 rpm to 1000 rpm there was a decrease in the NP size. NPs have a high surface area and this may lead to very high aggregation after long periods

of storage. After 72 h of storage, the aggregation tendency was more pronounced, the NPs size being higher comparative with the initial size. The PDI changed from 0.179 to 0.517 for NPs stored less than 48 h, indicating good dispersity for all CSNPs.

TABLE I

The size of CSNPs obtained by ionic cross-linking method

Conc. of CSLMW solution (%)	Conc. of TPP solution (%)	Stirring speed (rpm)	Particle average size initial (nm)	Particle average size after 48 h (nm)	Particle average size after 72 h (nm)
0.05	0.1	700	394 ± 19	431 ± 21	651 ± 23
		1000	380 ± 15	400 ± 31	571 ± 27
	0.5	700	244 ± 12	281 ± 12	576 ± 27
		1000	231 ± 24	274 ± 15	438 ± 19
0.1	0.1	700	685 ± 28	743 ± 28	946 ± 25
		1000	623 ± 14	716 ± 26	903 ± 22
	0.5	700	486 ± 28	547 ± 37	739 ± 29
		1000	415 ± 12	521 ± 24	891 ± 32
0.25	0.1	700	963 ± 11	1892 ± 33	2301 ± 30
		1000	912 ± 21	1806 ± 29	2048 ± 37
	0.5	700	875 ± 15	1145 ± 32	1708 ± 26
		1000	821 ± 18	1100 ± 27	1643 ± 35

CSNPs-xanthine derivative and loading efficiency

Because particle size is the most important characteristic of NPs systems in terms of drug release, for drug loaded CSNPs the following formulation was chosen: CSLMW 0.05%, TPP 0.5% under 1000 rpm stirring speed during ionic gelation. The size of CSNPs loaded with xanthine derivative obtained without Tween 80 is slightly increased (267 nm), but the size decreases in the presence of Tween 80 as shown in Table II. Also seen was a similar LE% after variation in surfactant concentration which means that the entrapment efficiency was not affected.

TABLE II

The size of CSNPs-xanthine derivative and the loading efficiency

Xanthine derivative (mg)	Tween 80 (µL)	Initial average particle size (nm)	Average particle size after 72 h (nm)	PDI	LE (%)
5	-	267 ± 18	468 ± 11	0.26 ± 0.7	86.10 ± 2.8
5	10	214 ± 12	221 ± 20	0.17 ± 1.4	86.50 ± 3.1
5	20	193 ± 23	195 ± 17	0.52 ± 0.9	86.20 ± 2.4

CONCLUSIONS

The aim of this study was to develop and optimize a method for preparation of CSNPs in order to be used as delivery system for xanthine derivative. Some parameters such as concentration of CS, TPP, Tween 80 and the stirring speed during ionic gelation are very important in

NP formation process. The method was optimized by modifying these independent variables in order to obtain small size NPs, and the optimized method was used for loading the xanthine derivative. The optimized formulation led to xanthine loaded CSNPs with the size in the range 193-214 nm depending on surfactant concentration, without agglomeration effects and with an adequate percentage of loading efficiency.

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EXPERIMENTAL RESEARCHES ON ACUTE TOXICITY OF A *BIDENS TRIPARTITA* EXTRACT IN MICE - PRELIMINARY INVESTIGATIONS

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EXPERIMENTAL RESEARCHES ON ACUTE TOXICITY OF A *BIDENS TRIPARTITA* EXTRACT IN MICE - PRELIMINARY INVESTIGATIONS (Abstract): Plants take up an important place in traditional medicine and scientific research confirmed properties about their use as alternative therapy. *Bidens tripartita*, commonly known as Three-lobe Beggarticks, Three-part Beggarticks, Trifid Bur-marigold, is a flowering plant in the genus Bidens, family Compositae, subfamily Asteroideae. Evaluation of the chemical composition of this plant has revealed the presence of flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins. Aim: Theoretical data investigation regarding *Bidens tripartita* plant and experimental researches on acute toxicity of an original extract in mice. **Material and methods:** The vegetal product of *Bidens tripartita* used for study was obtained by maceration and extraction in alcohol, and its chemical composition was determined. Acute toxicity of the alcoholic extract of *Bidens tripartita* was assessed by median lethal dose (LD50) calculation, using a limit dose test of up- and- down procedure at a limit dose of 2000mg/kbw after intraperitoneal administration in mice. **Results**: In the alcoholic extract of *Bidens tripartita* different active principles were identified: tannins, anthracene derivatives, triterpenes, coumarins, antocyanosides. The toxicity of plant product was evaluated by different characteristic signs for the mouse which can be retained as toxicity elements of the extract. Using the intraperitoneal route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death. Single-dose intraperitoneal LD50 value of the alcoholic *Bidens tripartita* extract in mice was 4038 mg/kg. No macroscopic changes were seen in the organs of mice that died following extract administration. Histopathological lesions were not found in all examined organs. **Conclusions**: The obtained LD50 value classifies the study plant extract as slightly toxic according to Hodge and Sterner toxicity scale. We determined the low toxic dose at a rate of 4038 mg of body weight for the alcoholic extract of this medicinal plant. These results suggest that the alcoholic extract of *Bidens tripartita* is relatively safe toxicologically when administered intraperitoneally, and this product could be used with some degree of safety to continue the investigation for *in vivo* biocompatibility evaluation. **Keywords**: *BIDENS TRIPARTITA*, LETHAL DOSE 50, MICE.

Adaptogens are naturally occurring substances found in rare plants and herbs, which appear to increase body ability to adapt to stress and changing situations (1).

The exact mechanism through which an adaptogen works is unclear so far, as it seems to be caused by the effects of different chemical compounds. In general, adaptogens are all potent antioxidants and they are reported to improve endurance and produce effects associated with stress reduction, for instance, improved sleep and enhanced physical performances (2).

The main effects of adaptogens are represented by: an increased availability of energy during the day, a reduction of stress feelings, increased endurance, greater mental alertness, and deep and restful sleep (3). Also, adaptogens significantly accelerate the recovery process after illness. They can have individual effects, depending on general health, constitution, specific nutrient deficiencies, and medical conditions present in an individual (4).

Bidens tripartita (B. tripartita), commonly known as Three-lobe Beggarticks, Three-part Beggarticks, Trifid Bur-marigold, is a flowering plant in the genus *Bidens*, family *Compositae*, subfamily *Asteroideae*. It is found along streams and ditches, and flowers during late summer and autumn.

Literature data reported that *B. tripartita* contains a significant amount of flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins (5).

In traditional medicine, infusions of *B. tripartita* are widely used in the treatment of catarrhal rhinitis, angina, acute respiratory infection, and as an anti-inflammatory in colitis and gout (6). It also proved antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic, sedative and sudorific effects (1,7).

MATERIAL AND METHODS

The study consisted in obtaining an alcoholic extract of *Bidens tripartita* plant, establishing its composition and determining the lethal dose 50% (LD50) after acute intraperitoneal administration in mice.

Collection and processing of plant material. The plant material for the study was represented by an alcoholic extract of *Bidens tripartita L.* plant. Plants were collected during flowering stage in July and August 2009, 2010, 2011 from the Ciric area, Iasi district, where it grows naturally. This medicinal plant species was taxonomically identified and authenticated by botanical specialists.

The vegetal product of *Bidens tripartita* used for the study was obtained by maceration and extraction in alcohol, and its chemical composition was determined.

The plants were washed in water and dried in laboratory using continuous ventilation away from sun light and dust. Then, they were cut into smaller pieces and ground into a coarse powder with a blender. This procedure ensures proper penetration of the extracting solvent into the cell to facilitate the release of the flower's active ingredients. The vegetable product was kept in closed plastic bag. The powder was dissolved in absolute chloroform (96%) and re-extracted seven (times (repeated maceration). The suspension was filtered by filter paper and dried using a Rota vapour. Then, a jet air was used to remove the solvent smell. After complete dryness, the product was extracted by the addition of ethanol seven times (repeated maceration) following the same procedure of evaporation. Finally, the extract was weighed by digital balance, stored in bottles and kept in the laboratory refrigerator for further research (8).

Animals. Swiss white male mice (30-40 g) were used. The animals were housed under standard laboratory conditions (rela-

tive humidity 55-65%, room temperature 23.0±2.0°C and 12 hours light: dark cycle (lights on at 6:00 a.m.)). The animals were fed with standard diet and water *ad libitum*, except during the time of experiments. Before the experiment, mice were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

Acute toxicity investigation. This study was designed to evaluate the *Bidens tripartita* extract acute toxicity *in vivo*, after intraperitoneal administration in mice.

Median lethal dose (LD50) is the dose, given all at once, which causes the death of half the number of test animals. LD50 is one way to measure the short-term toxic potential of a product (9). Establishing of LD50 in lab animals is very important to estimate the degree of acute toxicity of a tested substance (12).

The acute toxicity of the alcoholic *Bidens tripartita* extract was assessed by LD50 calculation, using a limit dose test (up-and- down procedure) at a limit dose of 2000 mg/kg bw of each vegetal extract after intraperitoneal administration in mice (five animals per group) (OECD-OCDE 425 Guide).

LD_{50} determination of *Bidens tripartita* extracts was estimated where all doses were expressed in terms of extract weight/ animal weight. Preliminary experiments were done to determine the minimal dose that kills all animals (LD100) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose being injected intraperitoneally in a group of five animals. The mice were observed for 14 days and symptoms of toxicity and mortality rates in each group were recorded and LD50 was calculated.

The toxicity of plant product was evaluated by different characteristic signs for the mouse which can be retained as toxicity elements of the extract. Using the intraperitoneal route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death (11).

The arithmetic method of Karber with the following formula was used:

$$LDL50 = LD100 - \sum(axb) / n$$

a = the difference between two successive doses of administered substance

b = the average number of dead animals in two successive groups

n = the number of animals in a group,

DL100 = lethal dose 100% (representing the dose, given all at once, which causes the death all test animals).

Graphic curve was realized with experimental data to have LD01, LD50, LD99. Much more, two ratios: LD99/LD50 and LD50/LD01 were calculated and compared.

The determined LD50 value allowed us to find out the level of toxicity of the plant product on the toxicity scale suggested by WHO/IPCS (2002) representing an adaptation of the Hodge and Sterner scale (1980) (1).

Data were statistically analyzed with SPSS software for Windows version 17.0 and ANOVA one-way method. The results were expressed as mean of LD01, LD50 and LD99 ± S.E.M. (standard error of mean).

Experimental protocols were implemented according to recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues. Each animal was used only once, and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the ex-

periment (13).

RESULTS AND DISCUSSION

Phytochemical analysis of the vegetal extract of *Bidens tripartite*, consisting of simple chemical tests, detected the presence of different active principles, especially tannins, anthracene derivatives and triterpenes (tab. I).

TABLE I

Active principles identified in the extract of *Bidens tripartita* plant

Extract	Active substances
Ethanolic	tannins, anthracene derivatives, triterpenes, coumarins, antocyanosides

The toxicity of *Bidens tripartita* extract was evaluated according to different signs for the mouse, which can be retained as toxicity elements of the vegetal product: decrease in spontaneous behavior normal manifestations and exploration instinct, somnolence, lack of appetite, depression, respiratory distress, and death.

The LD50 value of *Bidens tripartita* extract administered intraperitoneally in a single dose was estimated to be 4038 mg/kbw in mice (fig.1)

DL01 and DL99 of the alcoholic *Bidens tripartita* extract injected intraperitoneally in mice were determined to be 2500 mg/kbw and 4950 mg/kbw, respectively.

The calculation of DL99/DL50 ratio = 1.22 and DL50/DL01 ratio = 1.61 showed comparable values expressing the steadiness of regression line for mortality in relation to dose and validate the biologic significance of the results.

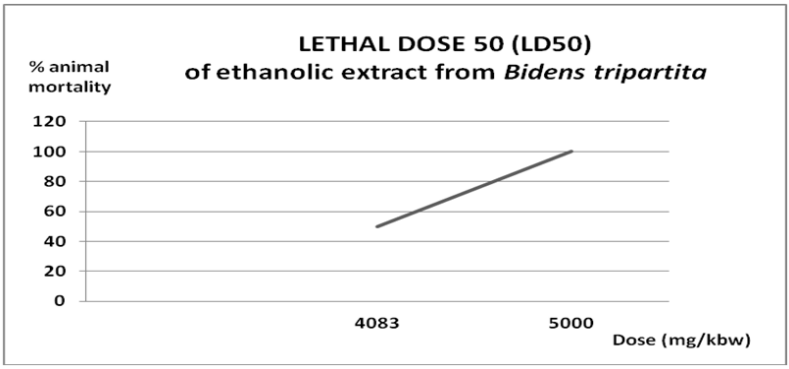

Fig. 1. Curve of the general acute toxicity of alcoholic extract from *Bidens tripartita*

TABLE II

Hodge and Sterner Toxicity Scale (1980)(1)

Toxicity rating	Commonly used term	LD50 (rat, oral)
1	Extremely Toxic	less than 1 mg/kg
2	Highly Toxic	1-50 mg/kg
3	Moderately Toxic	50-500 mg/kg
4	Slightly Toxic	500-5000 mg/kg
5	Practically Non-toxic	5000-15000 mg/kg

According to Hodge and Sterner toxicity scale, the LD50 value of the alcoholic *Bidens tripartita* extract is in the slightly toxic category (tab. II). For the future researches the retained dose of the alcoholic extract from *Bidens tripartita* for mouse administration is 1/20 LD50, that is around 201.9 mg/kg bw.

CONCLUSIONS

These results suggest that the alcoholic extract of *Bidens tripartita* is relatively safe toxicologically when administered intra-peritoneally, and this product could be used with some degree of safety to extend the researches for *in vivo* biocompatibility evaluation.

This study is a preliminary investigation on acute toxicity of an alcoholic extract from *Bidens tripartita* offering an outset to continue the researches regarding the effects of this vegetal product on biochemical parameters, immune system, and oxidative stress in lab animals.

ACKNOWLEDGEMENTS

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STAREA DE SUPRAFAȚĂ A RESTAURĂRILOR FIXE ŞI INFLUENȚA ASUPRA PARODONȚIULUI

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SURFACE ASPECT OF FIXED RESTAURATIONS END PARODONTAL INFLUENCES (Abstract): Any new class of materials requires a new cutting technology which, unless complied with properly, may negatively impact on the advantages and performance of the material. The modifications appeared as a result of the technological processes in the structure or surface aspect of the materials not only affects the mechanical resistance of the restorations but also casts doubts on their biological qualities. **Aim**: This study evaluates the impact of biomaterials involved in fixed restorations on the periodontal architecture, bearing extremely important connotations in the long run. **Material and methods**: The "in vitro" testing was conducted on culture cells for the cytotoxic effect of certain restorative materials – metallic alloys used in prosthetic restorations, composite materials, in collaboration with the Virology Laboratory of the Public Health Institute.The tested materials were metallic alloys, composite materials and acrylic resins used for the construction of standard sized plates (out of each material) in order to avoid the differences that might arise from the technological process. Artificial saliva processed to reach a pH=7 was prepared in the Biophysics Laboratory of UMF Iasi. Material samples and the saliva inoculated with these were tested. –p. **Results and discussions:** The cytotoxic effect of the tested materials on the celular cultures takes on extremely diverse forms, from discrete morphological modifications of the cells with regard to the size, shape, internal structure (for the noble and semi-noble alloys) up to the partial stripping-off of the celular film, the modification of density and coloration. In the case of the witness of non-inoculated culture, *the testing results* showed the presence of a continuous film, with cells having the same size, transparency and colouring, with an unaltered polyhedral contour, with visible nuclei, an image also kept in the case of the saliva witness. **Conclusions.** The involvement of restaurative materials in triggering, maintaining and aggravating a periodontal pathology indicates the capital role played by the dentist in the identications of lesions during measures by avoiding or excluding etiological agents before an obvious lesions occurs in the process of active dispensarization**. Key words**: BIOCOMPATIBILITY, DENTAL BIOMATERIALS, PERYODONTIUM, FIXED RESTAURATIONS

Numărul mare şi diversificarea în ritm accelerat a materialelor restaurative cu o compoziție chimică din ce în ce mai sofisticată, creşte riscul apariției unor fenomene toxico-iritative, alergice şi de altă natură, ceea ce a suscitat din partea specialiştilor un interes crescut privind comportarea lor în diverse condiții de exploatare.

Proprietățile mutagene ale unor materiale stomatologice reprezintă un pericol pentru sănătatea omului şi introducerea unei strategii logice a testării devine o necesitate, deşi metodologia de investigare este încă limitată (5).

Testele de toxicitate, au avantajul că se verifică materialele ca atare dar şi componentele acestora, ceea ce permite eliminarea substanțelor accentuat toxice într-o fază timpurie (4).

Orice clasă nouă de materiale necesită o tehnologie nouă de prelucrare care, dacă nu este respectată, poate compromite avantajele şi performanțele materialului.

Modificările rezultate în urma proceselor tehnologice în structura sau aspectul de suprafață a materialelor nu numai că influențează în mod negativ rezistența mecanică a restaurațiilor dar pune la îndoială şi calitățile biologice ale acestora. Aşa se explică apariția unor reacții parodontale sau mucozale de vecinătate. Experiența clinică atrage atenția asupra unor astfel de reacții adverse posibile la materialele restaurative (aliaje metalice şi materiale acrilice, materiale compozite (2).

În ultimele decenii a devenit posibilă testarea biocompatibilității diferitelor materiale stomatologice pe animale de experiență sau *in vitro* pe culturi celulare, metoda fiind utilizată atât pentru materiale ca atare cât şi pentru elementele ce le compun. Aplicând cele mai simple tehnici se poate permite contactul materialului cu celula vie, rezultatele fiind în funcție de toxicitatea materialului.

Scopul acestui studiu urmăreşte evaluarea impactului biomaterialelor folosite in restaurarile fixe asupra arhitecturii parodontale, cu implicatii importante pe termen lung.

MATERIAL ŞI METODE

S-a efectuat testarea „in vitro" pe culturi de celule a efectului citotoxic al unor materiale restaurative-aliaje metalice utilizate în restaurările protetice, materiale compozite, în colaborare cu Laboratorul de Virusologie al Institutului de Sănătate Publică Iaşi. Au fost testate aliaje metalice, materiale composite şi răşini acrilice din care s-au realizat plăcuțe de dimensiuni standard pentru a evita diferențele ce ar putea decurge din procesul tehnologic.

Ca suport celular pentru testarea acestor biomateriale s-a folosit: linia de celule epiteliale tip HeLa - pentru materialele compozite şi acrilice; fibroblaşti din ser de viței proaspăt - pentru aliajele metalice. Mediile utilizate pentru creşterea şi întreținerea culturilor au fost livrate de Institutul Cantacuzino, Bucureşti. Saliva artificială adusă la un pH =7 a fost preparată în Laboratorul de Biofizică UMF Iaşi, sterilizată prin autoclavare 20 de minute şi controlată din punct de vedere bacteriologic.

Fragmentele de material au fost introduse în recipiente sterile cu salivă şi păstrate la temperatura camerei, de unde, la intervale de 3, 6 şi 18 zile au fost recoltate probe (0, 2 ml) în vederea testării efectului citotoxic. Câte 5 eşantioane din probele de salivă, au fost inoculate în tuburi de culturi celulare, ulterior mediul de creştere a fost înlocuit cu cel de întreținere.

Ca martor şi element de comparație s-au folosit tuburile inoculate cu salivă artificială şi cele neinoculate.

Efectul citotoxic s-a urmărit macroscopic şi microscopic proliferarea sau liza celulară survenită, refringența, continuitatea filmului celular sau tulburarea lanțului adeziune - multiplicare şi aderare de pereții vasului. Lipsa aderării, cu formarea filmului celular pe pereții vasului a reprezentat

semnul morții celulare, iar tehnicile histologice au permis evaluarea microscopică.

REZULTATE ŞI DISCUȚII

Efectul citotoxic al materialelor testate asupra culturilor celulare îmbracă aspecte din cele mai variate, de la modificări morfologice discrete ale celulelor privind mărimea, forma, structura internă (pentru aliaje nobile şi seminobile) până la desprinderea parțială a filmului celular, modificarea densității şi a colorației (4).

TABELUL I

Testarea citotoxicității materialelor inoculate în salivă pe culturi de celule

Materiale	3 zile	6 zile	18 zile
Salivă artificială inoculată cu compozit autopolimerizabil	+ 1/5	+2/5	0/5
Salivă artificială inoculată cu compozit fotopolimerizabil	0/5	+1/5	0/5
Salivă artificială inoculată cu acrilat termopolimerizabil	0/5	+1/5	0/5
Salivă artificială inoculată cu acrilat autopolimerizabil	**+2/5**	**+++4/5**	**++3/5**
Salivă artificială inoculată cu aliaj nobil	0/5	0/5	0/5
Salivă artificială inoculată cu aliaj seminobil Pd - Ag	0/5	+1/5	0/5
Salivă artificială inoculată cu aliaj oțel inoxidabil Cr-Co	0/5	0/5	0/5
Salivă artificială inoculată cu aliaj Cr-Ni	+1/5	+1/5	0/5
Cr-Co Soft	0/5	0/5	0/5
Salivă neinoculată	0/5	0/5	0/5
Martor cultură	0/5	0/5	0/5

În cazul martorului cultură neinoculată, rezultatele testărilor au arătat prezența unui film continuu, cu celule de aceeaşi mărime, transparență şi colorabilitate, cu contur poliedric nealterat, cu nuclei vizibili, imagine ce se păstrează şi la martorul salivă (tab. I, fig. 1).

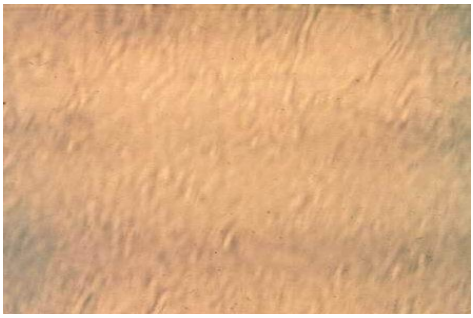

Fig. 1. Martor celular normal

Nici una din cele 5 probe nu a arătat modificări esențiale la 3 săptămâni, filmul celular fiind confluent, cu celule cu contur poligonal ce prezentau o uşoară modificare de volum dar fără picnoză, cu nuclei clari, ovalari, cu structura fină a cromatinei. Creşterea în culturile martor a început imediat în zilele următoare inoculării şi a progresat treptat.

Analiza tuburilor cu salivă inoculată cu material acrilic termopolimerizabil şi compozite fotopolimerizate a indicat lipsa toxicității în primele zile ale testării, un efect redus de toxicitate remarcându-se la unul din tuburi la 6 zile de testare, efect ce a dispărut la 18 zile (tab. I, fig. 2).

Reacția citolitică remarcată la compozitele autopolimerizabile poate fi atribuită monomerului eliberat (1 tub la 3 zile şi 2 la 6 zile), iar la cele fotopolimerizabile (1 tub) la evaluarea la 6 zile, reacția poate fi pusă pe seama polimerizării incomplete (tab. I, fig. 3).

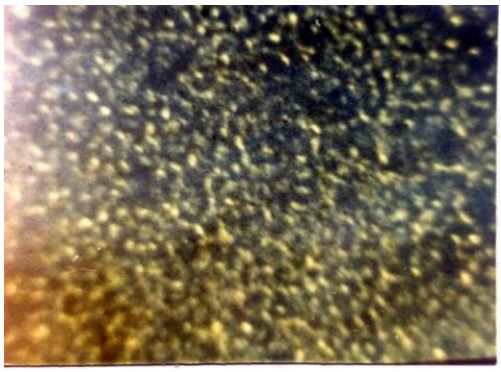

Fig. 2. Film celular continuu x10

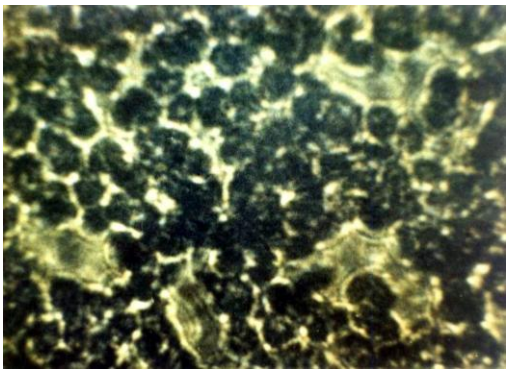

Fig. 3. Reacție citolitică citolitică uşoară X20 - compozit

Reacție citolitică intensă la acrilatele şi compozitele autopolimerizabile, cu distrucția şi chiar desprinderea filmului celular, mai redus în primele 3 zile (2 respectiv 1 tub) şi mai intens la 6 zile (4 respectiv 2 tuburi) şi la 3 săptămâni, când doar acrilatul autopolimerizabil continuă să abă efecte citotoxice (3 tuburi) (tab. I, fig. 4).

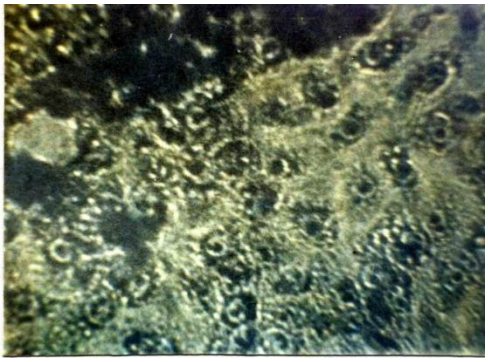

Fig. 4. Reacție citolitică intensă 20 X acrilat autopolimerizabil

Toxicitatea aliajelor evaluată după răspunsul citotoxic în mediu de cultură şi viabilitatea celulară apreciată prin continuitatea filmului celular, ne indică o viabilitate mai crescută la aliajele nobile, unde filmul celular apare continuu, neîntrerupt, cu celule fără refringență sau rare celule refringente la aliajele seminobile, dar cu o reducere a refringenței celulare şi afectarea proliferării acestora, în prezența ionilor de cupru (tab.I, fig.5).

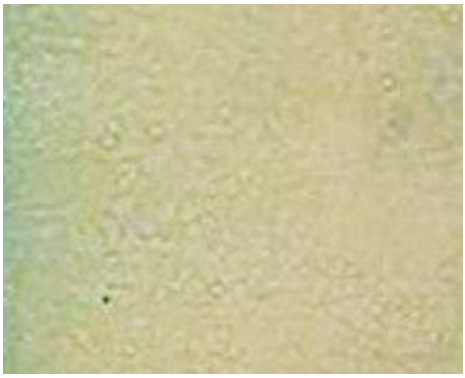

Fig. 5. Aliaj aur, film celular continuu, neîntrerupt, cu celule fără refringență

La Pallidor filmul apare cu rare celule refringente (fig. 6), în timp ce la Palliag imaginile arată goluri cu celule ce se desprind, fără refringență şi balonizate (fig. 7).

La aliajele de Cr-Co (hard) (fig. 8) şi Cr-Co-Mo (fig.9) suspensia şi filmul celular evidențiază un aspect şi un contur celular nemodificat.

La Cr-Co soft şi Cr-Co suspensie, se observă celule ce se desprind, dar cu tendință de refacere (fig. 10, 11).

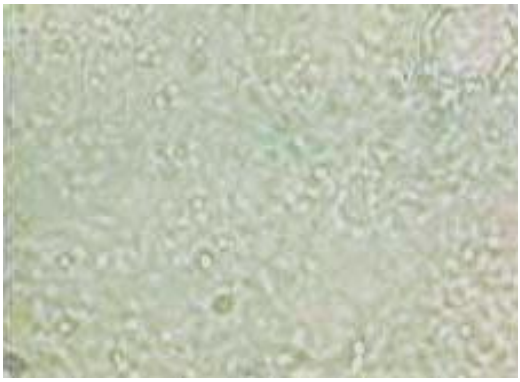

Fig. 6. Palidor - film continuu, cu rare celule refringente.

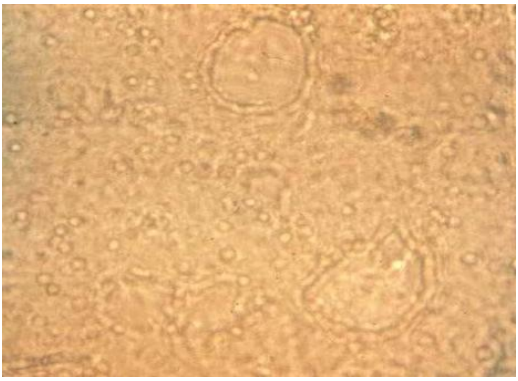

Fig. 7. Palliag - film cu celule ce se desprind

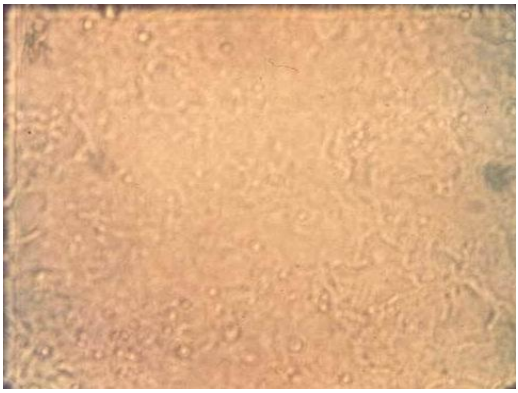

Fig. 8. Cr-Co Hard- Suspensie aspect și contur celular nemodificat

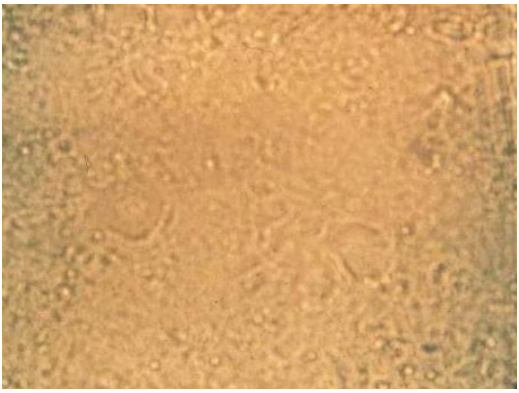

Fig. 9. Suspensie Cr-Co-Mo - film neîntrerupt cu celule normale

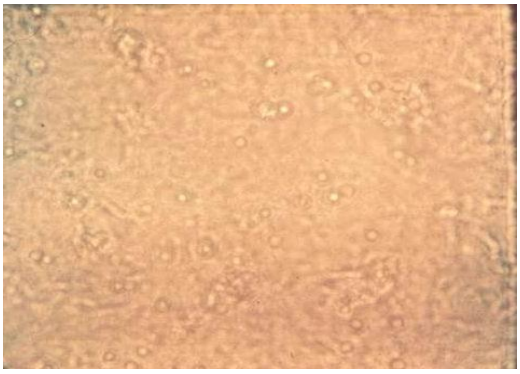

Fig. 10. Cr-Co soft + Ni - film întrerupt cu celule refringente, ce se desprind dar se refac

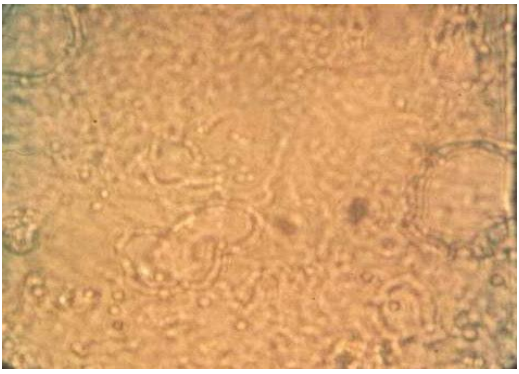

Fig. 11. Cr-Co Suspensie – celule ce se desprind dar se refac

Rezultate asemănătoare au fost obținute prin introducerea concomitentă în tuburi, odată cu însămânțarea suspensiei de cultură celulară a fragmentelor din materiale (5).

Urmărind gradul de creştere a filmului celular şi modificările morfologice apărute, viabilitatea celulelor în prezența materialului comparativ cu martorul, se constată modificări asemănătoare celor din experimentul cu saliva inoculată.

Aliajele, în principal acelea pe bază de cupru pot elimina în salivă ioni şi alți produşi de coroziune, în concentrații care uneori pot afecta viabilitatea şi proliferarea celulară (6, 7). Efectul toxic asupra culturilor celulare demonstrează trecerea ionilor metalelor de bază în saliva artificială şi acțiunea lor toxică asupra culturilor de celule.

În tuburile cu fragmente din acrilat termopolimerizabil şi compozite fotopolimerizabile aliaje nobile, seminobile şi Cr-Co în primele 48 de ore de incubare la 37°C s-a produs creşterea filmului celular, care examinat la microscop înainte şi după colorare arată formarea unui monostrat confluent de cultură celulară, exceptând zona în care este depus fragmentul, unde pare să apară un lizereu de celule mai aglomerate şi turtite, iar modificări discrete să se remarce la 6 zile, demonstrând gradul redus de toxicitate.

Modificări evidente de citotoxicitate s-au remarcat la fragmentele de acrilat autopolimerizabil şi aliajelor pe bază de Cu, dar chiar din primele zile de incubare, când distrucția filmului celular s-a produs nu numai la nivelul contactului, ci şi la distanță, ce ar indica fie toxicitatea materialului prin el însuşi, fie prin componentele sale eliberate în mediul de cultură, cum este cazul monomerului, cuprului, nichelului

Modificările discrete constatate la fragmentele de compozit fotopolimerizabil, aliaje nobile, seminobile, Cr-Co pot fi puse pe seama traumatismului fizic la contactul celule - material şi diferențiate de efectul citotoxic. Rezultatele testărilor sunt în concordanță cu datele din literatură.

Testarea citocompatibilității aliajelor Fe-Cr-Ni şi Cr-Co în studii recente indică absența efectului citotoxic la perioade scurte de timp dar remarcă efecte citotoxice la aliajul Cu-Al-Ni (8, 9).

Craig et al.(8), testând efectele aliajelor dentare pe bază de Cu asupra liniilor celulare gingivale au constatat că prezența ionilor de Cu şi Ni poate determina o reducere a proliferării şi a vitalității celulare cu o scădere a tolerabilității biologice şi un risc de citotoxicitate ridicat în mediul bucal, ceea ce atrage atenția asupra indicării cu discernământ a acestor materiale sau chiar a evitării utilizării lor.

Efectuarea testului de citotoxicitate este pretențioasă şi foarte riguroasă, începând cu elaborarea probelor şi terminând cu interpretarea rezultatelor obținute pe culturi de celule din care un procent cu atât mai mare îşi pierde vitalitatea cu cât biomaterialul este mai toxic.

CONCLUZII

Studiul nostru a arătat modificări evidente de citotoxicitate la acrilatul autopolimerizabil şi la aliajele pe bază de Cu, chiar din primele zile de incubare, când distrucția filmului celular s-a produs nu numai la nivelul contactului, ci şi la distanță, ceea ce ar indica fie toxicitatea materialului prin el însuşi, fie prin componentele sale eliberate în mediul de cultură, cum este cazul monomerului, cuprului, nichel

Compozitul fotopolimerizabil, aliajele nobile şi seminobile au determinat modificări discrete ce pot fi puse pe seama trau-

matismului fizic la contactul celule - material şi diferențiate de efectul citotoxic.

Efectul ionilor de Cu şi Ni din aliajelor dentare pe bază de Cu asupra liniilor celulare gingivale, poate determina o reducere a proliferării şi vitalității celulare cu o scădere a tolerabilității biologice şi un risc de citotoxicitate ridicat în mediul bucal.

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NOUTĂȚI

CHLAMYDIA TRACHOMATIS - NOI MODALITĂȚI DE INVESTIGARE

Infecțiile cu *Chlamydia trachomatis* sunt dintre cele mai frecvente infecții de la nivelul tractului genital deşi mecanismul prin care aceasta se adaptează țintei (celulele epiteliale endocervicale) nu este clar înțeles. Pentru a-şi asigura supraviețuirea intracelulară aceasta secretă factori de virulență insuficient investigați. Studiul de față foloseşte linii celulare HeLa şi linii celulare de celule epiteliale endocervicale pentru a pune în evidență proteinele bacteriene secretate în timpul creşterii induse de ampicilină. În mod specific se observă o scădere a CPAF (chlamydial protease like activity factor) în citosolul celulelor expuse ampicilinei în condițiile în care CPAF produs de *Chlamydia trachomatis* este constant. Pe de altă parte, expresia CopN şi Tarp este scăzută sugerând că *Chlamydia trachomatis* răspunde la expunerea la ampicilină prin alterarea selectivă a expresiei genelor care codifică secretia proteinelor. De asemenea, se observă o acumulare de vezicule în celulele cu infecție persistentă. Aceste rezultate susțin ipoteza că atât expresia genelor cât şi secreția factorilor de virulență sunt implicați în adaptarea bacteriei la micromediul tractului genital în cazul infecțiilor persistente (Wang J, Frohlich K, Buckner L et all. Altered protein secretion of *Chlamydia trachomatis* in persistently infected human endocervical epithelial cells. *Microbiol* 2011: l 157, 2759-2771).

Enache Ecaterina

SINTEZA ŞI CARACTERIZAREA UNOR NOI DERIVAŢI AI RUTOZIDULUI CU ACID 7-AMINO-CEFALOSPORANIC

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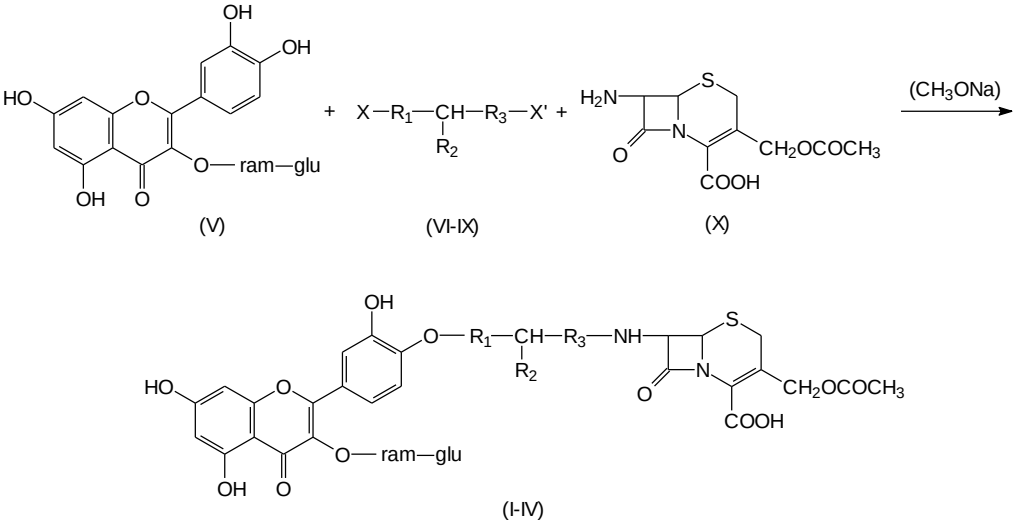
SYNTHESIS AND CHARACTERIZATION OF SOME NEW RUTIN AND 7-AMINO-CEPHALOSPORANIC ACID DERIVATIVES (Abstract): Many studies show that flavonoids have a numerous biological properties, antimicrobial effects included. It is also known that rutin is able to increase the antibacterial activity of other compounds. **Material and methods:** Starting from these facts, we synthesised some water soluble rutin derivatives treating rutin with 1, 3-dichloro-2-propanol, 1-bromo-3-chloro-propane, 2-dibromethane, and dibrommethane, and than with 7-amino-cephalosporanic acid. The antimicrobial activity of the new compounds was determined by disc diffusion method. **Results:** Molecular formula, weight, yield, melting points and solubility of the new derivatives have been determined. Elemental analysis and spectral analysis (UV and IR) confirmed the structure of new compounds. **Conclusions.** These derivatives are water-soluble and have a good antimicrobial activity, both on Gram-positive and Gram-negative bacteria, comparable with that of tetracycline. **Key words:** FLAVONOIDS, RUTIN, CEPHALOSPORIN, ANTIMICROBIAL ACTIVITY

Multitudinea de studii întreprinse până în prezent a stabilit faptul că flavonoidele prezintă numeroase proprietăți biologice (antioxidantă, antiinflamatoare, antialergică, antimicrobiană, antivirală, antitumorală, etc.) exercitate prin variate mecanisme de acțiune. În multe cazuri, flavonoidele sunt capabile de protecție împotriva unor afecțiuni cronice şi degenerative, dintre care amintim cancerul, bolile cardiovasculare, disfuncții ale sistemului imunitar, etc. Mai mult decât atât, există dovezi că rutozidul ar potența acțiunea antimicrobiană a unor compuşi cunoscuți a avea astfel de proprietăți (1-9).

MATERIAL ŞI METODĂ

Pornind de la aceste premize, ne-am propus obținerea unor derivați ai rutozidului cu acidul 7-amino-cefalosporanic, prin tratarea rutozidului cu 1,3-dicloro-2-propanol, 1-brom-3-cloro-propan, 2-dibrom-etan şi dibrom-metan, în mediu de metoxid de sodiu, şi apoi cu acidul 7-amino-cefalosporanic (fig. 1). Derivații I-IV au fost obținuți după aceeaşi tehnică de lucru: 0,01 mol (6,64 g) rutozid (V) se introduc într-un flacon Erlenmayer cu dop rodat, peste care se adaugă 180 ml metoxid de sodiu (preparat prin reacționarea a 0,01 mol (0,23 g) cu metanol absolut). Amestecul de reacție se supune agitării magnetice la temperatura camerei,

timp de 60 de minute. Se adaugă derivaţii halogenaţi (0,01 mol 1,3-dicloro-2-propanol (VI), 1-brom-3-cloro-propan (VII), 2-dibrom-etan (VIII), respectiv, dibrom-metan (IX)) şi se continuă agitarea magnetică timp de 2 ore. Înainte de adăugarea acidului 7-amino-cefalosporanic s-a verificat pH-ul amestecului de reacţie, situat în jurul valorii 7-8. În final, se adaugă 0,01 mol acid 7-amino-cefalosporanic (X) şi amestecul de reacţie se agită timp de 12 ore. Soluţia obţinută se lasă în repaus 24 ore şi apoi se toarnă în porţiuni mici, sub agitare peste 400 ml izopropanol. Precipitatul format se filtrează şi se usucă în etuva termostatată, la 37°C, timp de 12 ore. Produsul brut se purifică pe coloană (Ø 0,5 cm, 15 cm lungime) cu Silicagel H (dimensiunea particulelor 10-40 μm), debitul soluţiei în alcool 50° fiind de 0,25-0,30 ml/min.



ram = ramnoză; glu = glucoză

Nr. compus	X	X’	R_1	R_2	R_3
I	Cl	Cl	CH_2	OH	CH_2
II	Br	Cl	CH_2	H	CH_2
III	Br	Br	CH_2	H	-
IV	Br	Br	-	H	-

Fig. 1. Sinteza derivaţilor de rutozid

Analiza elementală C, H, N s-a realizat cu un aparat “Exeter Analytical” CE-440; spectrele IR ale derivaţilor obţinuti au fost efectuate prin metoda comprimării în pastile de KBr (în raport 1/30), înregistrarea fiind realizată cu ajutorul unui aparat FT/IR "Jasco 670 Plus", în domeniul 500-4000 cm^{-1}; spectrele UV au fost înregistrate utilizând un spectrofotometru UV-VIS “Hewlett-Packard” HP 8453 E şi softul de firmă “HP-CHEMSTATION”. S-au folosit soluţii apoase de concentraţie 10 μg/ml, solventul de compensare fiind apa bidistilată.

Testările microbiologice s-au făcut pe mediu agarizat, tip Mueller-Hinton (Oxoid) şi pe agar Sabouraud, utilizând ca microorganisme test: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* meticilin rezistent (meti- R), *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, o tulpină de *Klebsiella pneumoniae* 1, producătoare de betalactamaze cu spectru extins (BLSE), *Candida albicans* ATCC 10231, *Candida sake* şi *Candida glabrata*. Tulpinile utilizate în teste fac parte din colecţia de microorganisme a Disciplinei de Microbiologie, Facultatea de Farmacie, U. M. F. "Gr. T. Popa", Iaşi.

Activitatea antimicrobiană a compuşilor I-IV s-a studiat prin metoda difuzimetrică. Din cultura peste noapte, incubată la temperatura optimă (30°C), s-a preparat o suspensie cu densitatea corespunzătoare tubului 0,5 din scala turbidimetrică Mc Farland. Suspensia s-a încorporat în proporţie de 1/10 în mediu Mueller-Hinton topit şi răcit la 50°C. Din amestec, după omogenizare, s-au repartizat câte 25 ml în plăci Petri cu diametrul de 12 cm. Pe suprafaţa fiecărei plăci, s-au dispus, după solidificare, rondele de hârtie de filtru impregnate cu derivaţii de testat. Rezultatele au fost citite după menţinerea plăcilor la termostat, la 37 grade Celsius, timp de 24 ore, pentru bacterii şi la 24 grade Celsius, timp de 48 de ore, pentru *Candida spp*. S-a măsurat cu şublerul şi s-a înregistrat diametrul zonelor de inhibiţie. S-a comparat activitatea antimicrobiană a probelor cu activitatea tetraciclinei (30 μg/disc), cotrimoxazolului (25μg/ disc), sulfamidei (200 μg/ disc) şi, în cazul fungilor, cu cea a nistatinei (100 μg/ disc).

REZULTATE ŞI DISCUŢII

Derivaţii I-IV au fost obţinuţi cu randamente satisfăcătoare (82-90%); aceştia se prezintă sub formă de pulberi cristaline galbene, higroscopice, solubile în apă, etanol 50°, dimetilsulfoxid şi dimetilformamidă la rece, etanol 96° şi metanol la încălzire, parţial solubile în propanol, de asemenea la cald şi practic insolubile în izopropanol, dioxan, eter etilic, acetona, benzen şi cloroform; soluţiile apoase au un pH ≅ 7.

Compuşii au fost caracterizaţi prin stabilirea formulelor şi maselor moleculare, a denumirilor chimice conform normelor IUPAC, determinarea punctelor de topire şi calculul randamentelor, iar structura lor a fost confirmată prin analiză elementală C,H,N şi analize spectrale în IR şi UV, după cum urmează:

3-[[6-O-(6-deoxy-α-L-manopiranozil) - β - D - glucopiranozil] - oxi] - 2 -(3, 4 - dihidroxifenil) – 5 – hidroxi - 7-(oxi-1-(β-hidroxi- propil)3-(3 - (acetiloximetil)-7-amino-8-oxo-5-tia-1-azabiciclo[4.2.0]oct-2-enil-2-carboxil)) - 4H-1-benzopiran-4-ona (I)

$C_{40}H_{46}N_2O_{22}S$; Mr = 938.86; Randament: 89,75; p.t.° (punct de topire) = 186-191°C; Analiza elementală (C,H,N) (Calculat / Experimental): 51,17; 4,93; 2,98/51,11; 4,88; 2,91; IR (cm^{-1}): 3423 (NH), 3333 (OH legate), 2923 (CH), 1733 (-C=O beta-lactamic), 1669 (C=O pe nucleu aromatic), 1611 (structură aromatică), 1512 (C=C aromatic), 1364, 1300, 1221, 1063 (C-O-C), 817 (substituenţi aromatici); UV-VIS (λ_{max} (nm)): 257, 355.

3-[[6-O-(6-deoxy-α-L-manopiranozil) - β - D - glucopiranozil] - oxi] - 2 -(3, 4 - dihidroxifenil) – 5 – hidroxi - 7-(oxi-1-propil- 3 – (3-(acetiloximetil)-7-amino-8-oxo-5-tia-1-azabiciclo[4.2.0]oct-2-enil-2-carboxil)) - 4H-1-benzopiran-4-ona (II)

$C_{40}H_{46}N_2O_{21}S$; Mr = 922.86; Randa-

ment: 82,00; p.t.° = 198-205°C; Analiza elementală (C,H,N) (Calculat / Experimental): 52,05; 5,02; 3,03/51,98; 4,97; 3,09 ; IR (cm^{-1}): 3430 (NH), 3341 (OH legate), 2933 (CH), 1753 (-C=O beta-lactamic), 1660 (C=O pe nucleu aromatic), 1626 (structură aromatică), 1521 (C=C aromatic), 1369, 1311, 1231, 1078 (C-O-C), 812 (substituenţi aromatici); UV-VIS (λ_{max} (nm)): 266, 357.

3-[[6-O-(6-deoxy-α-L-manopiranozil) - β - D - glucopiranozil] - oxi] - 2 -(3, 4 - dihidroxifenil) – 5 – hidroxi - 7-(oxi-1-etil-2-(3 - (acetiloximetil)-7-amino-8-oxo-5-tia-1-azabiciclo[4.2.0]oct-2-enil-2-carboxil)) - 4H-1-benzopiran-4-ona (III)

$C_{39}H_{44}N_2O_{21}S$; Mr = 908.83; Randament: 85,67; p.t.° = 143-148°C; Analiza elementală (C,H,N) (Calculat / Experimental): 51,54; 4,87; 3,08/51,49; 4,82; 3,03; IR (cm^{-1}): 3419 (NH), 3322 (OH legate), 2943 (CH), 1768 (-C=O beta-lactamic), 1677 (C=O pe nucleu aromatic), 1620 (structură aromatică), 1527 (C=C aromatic), 1370, 1320, 1244, 1076 (C-O-C), 818 (substituenţi aromatici); UV-VIS (λ_{max} (nm)): 266, 357.

3-[[6-O-(6-deoxy-α-L-manopiranozil) - β - D - glucopiranozil] - oxi] - 2 -(3, 4 - dihidroxifenil) – 5 – hidroxi - 7-(oxi-metil- 3 - (acetiloximetil)-7-amino-8-oxo-5-tia-1-azabiciclo[4.2.0]oct-2-enil-2-carboxil) - 4H-1-benzopiran-4-ona (IV)

$C_{38}H_{42}N_2O_{21}S$; Mr = 894.81; Randament: 90,10; p.t.° = 129-137°C; Analiza elementală (C,H,N) (Calculat/Experimental): 51,00; 4,73; 3,13/50,97; 4,70; 3,15; IR (cm^{-1}): 3434 (NH), 3330 (OH legate), 2947 (CH), 1758 (-C=O beta-lactamic), 1666 (C=O pe nucleu aromatic), 1623 (structură aromatică), 1519 (C=C aromatic), 1366, 1323, 1239, 1067 (C-O-C), 811 (substituenţi aromatici); UV-VIS (λ_{max} (nm)): 267, 358.

Diametrele zonelor de inhibiţie (în mm), corespunzătoare derivaţilor testaţi, sunt prezentate în tabelul I.

TABELUL I

Activitatea antibacteriana a derivatilor I-IV. Diametrul zonei de inhibitie (mm)

Nr. compus	*S. aureus* ATCC 25923	*S. aureus* meti-R	*S. lutea* ATCC 9341	*B. cereus* ATCC 14579	*B. subtilis*	*E. coli* ATCC 25922	*Ps. aeruginosa*	*K. pneumoniae* 1, producatoare (BLSE)
I	30	15	30	0	32	15	0	20
II	30	15	30	0	31	17	0	21
III	30	15	25	0	32	17	0	23
IV	29	14	30	0	31	10	0	15
Tetraciclina 30 μg	26	16	30	20	25	17	0	30
Cotrimoxazol 25 μg	17	20	30	19	20	21	0	21
Sulfamida 200 μg	15	0	25	20	15	15	0	14

Se constată că derivaţii I-IV sunt activi asupra tulpinilor microbiene utilizate în testări, cu excepţia *B. cereus* ATCC 14579 şi *Pseudomonas aeruginosa*; activitatea antimicrobiană este comparabilă cu cea a tetraciclinei. Nu prezintă activitate antifungică.

CONCLUZII

Au fost obţinuţi 4 derivaţi semisintetici originali ai rutozidului, prin tratarea acestuia cu 1,3-dicloro-2-propanol, 1-brom-3-cloropropan, 2-dibrom-etan, respectiv, dibrommetan, în mediu de metoxid de sodiu, şi apoi cu acidul 7-amino-cefalosporanic.

Noii compuşi au fost obţinuţi cu randamente satisfăcătoare (82-90%); aceştia se prezintă sub formă de pulberi cristaline galbene, higroscopice, solubile în apă; soluţiile apoase au un pH ≅ 7.

Compuşii au fost caracterizaţi prin stabilirea formulelor şi maselor moleculare, determinarea punctelor de topire şi calculul randamentelor.

Structura chimică a derivaţilor nou-sintetizaţi a fost confirmată prin analiză elementală C,H,N şi analize spectrale în IR şi UV.

Noii derivaţi prezintă o activitate antimicrobiană asemănătoare cu cea a tetraciclinei.

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ENDODONTIC RETREATMENT IN CASE OF FAILURE. CASE REPORT

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ENDODONTIC RETREATMENT IN CASE OF FAILURE. CASE REPORT (Abstract): In medical practice, clinicians come across an increased number of endodontic treatments, which, like other dental treatments, can fail. The increase in the number of endodontic treatment resulted in an increased number of failures, their management raising complex and serious endodontic problems. The endodontic retreatment of a failure is required by the increased desire to preserve the tooth on the dental arch, thus preventing the need for dental extraction that may have adverse consequences in terms of functional and psychological effect on patients. This article presents two clinical cases that required endodontic retreatment in order to avoid the complications that could ultimately require tooth extraction. The teeth in question (a mandibular first molar and an upper central incisor) having a special importance, and a physiognomic role, the endodontic retreatment consisted in covering them in porcelain fused to the metal crowns. **Keywords:** ENDODONTIC FAILURE, RETREATMENT, INCOMPLETE OBTURATION, CROWN.

Endodontic retreatment is a procedure which is done on a tooth previously endodontically treated, but whose current condition requires further endodontic treatment to achieve a successful result.

Despite the fact that the success rate of endodontic treatment is high, each clinician must be prepared to endodontically retreat the endocanalicular system, if initial treatment failes (1).

Several specialists showed that 30-50% of their clinical work is represented by endodontic retreatments.

Endodontic retreatment repeats steps of root canal treatment by coronary approach, its objective being the cleaning of the canals of irritating agents, most of them beeing microorganisms, which have survived the previous treatment or have infiltrated after therapy. Therefore, endodontic retreatment is consistent with the biological rationale of the root canal therapy and is preferable whenever possible.

CASE REPORT

Case 1: L.O. patient, a woman, showed up in the dental office complaining of pain in the right mandibular arch. Following clinical and radiological examination, it was noted that in the right mandibular region, at the level of 4.5 and 4.7 tooth, there were present two decays that required resolution only by the preparation of simple cavities filled with restorative materials. But the radiological exam showed us that, at the level of 4.6, an incorrectly performed

endodontic treatment due to the root canal filling being incomplete (fig. 1), but fortunately, has not revealed the presence of periapical pathology. Although mesial root of the tooth shows a slight curvature, suggesting a cause of endodontic treatment failure, distal root is common. Across all three canals the endodontic treatment was incomplete. We started by removing the coronary filling using a high speed spherical bur. Penetration holes into root canals were highlighted and we have performed the removing of their material fillings. Canal filling material was removed and infected root canals were disinfected using antiseptic and medicinal substances. Initially, we've resorted to a temporary root canal filling and the distal canal was filled with no problems. In an attempt to perform the treatment of the two mesial canals, their curvature constituted an obstacle. The direction of mesio-lingual canal was detected by radiological examination using a reference needle (fig. 2), ultimately being successful in achieving endodontic retreatment despite the root curvature (fig. 3), using a material based on calcium hydroxide. As a final coronal restoration a porcelain fused to metal crown was made that restores the integrity of the mandibular arch (fig. 4, 5, 6). Endodontic retreatment done on time allowed remission of clinical symptoms and also prevented the occurrence of periodontal complications. The first mandibular molar, which is an important tooth, has been saved successfully.

Fig. 1. Incompete root canal filling -4.6

Fig. 2. Needle pointing false path - 4.6

Fig. 3. Final endodontic retreatment - 4.6

Fig. 4. Coronary restoration - intermediate aspect - 4.6

Fig. 5. Coronary restoration - final aspect - 4.6

Fig. 6. Coronary restoration - final aspect

Case 2: A similar case of failure of endodontic treatment, is the one of T.L. patient, female, who when she came in the dental office was complainig of pain in the right upper incisor-1.1 (fig. 7). After radiological examination (fig. 8) we can see an incomplete root canal filling, what is the cause of patient's symptoms. During the stages of root canal treatment, the removing of material fillings was performed and endodontic retreatment was done thoroughly, the final result beeing illustrated in figure 8b. As coronal restoration, as in the previous case, a physiognomic porcelain fused to metal crown was done (fig. 9, 10). As a result, we prevented the loss of this front tooth, which is very important in the patient's physiognomy. Following endodontic retreatment, after a period of monitoring, there was a reduction in periapical lesion size.

Fig. 7. Initial aspect - 1.1

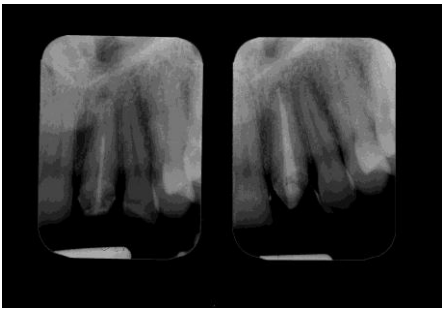

Fig. 8. a. Incomplete filling -1.1.
b. Final endodontic retreatment - 1.1

Fig. 9. Coronary restoration - intermediate aspect - 1.1

Fig. 10. Coronary restoration - final aspect - 1.1

DISCUSSION

As said by Mao Tse Tung, "The foundation of success is failure".

In situations when we diagnose a failure of initial endodontic treatment, there are three possible variants of solving the existing problem. The first and most indicated solution is represented by endodontic retreatment. The second option is represented by apical surgery followed by retrograde sealing of the root canal, while the last option, which is not desirable, is represented by dental extraction. The latter is necessary if the first two options can not be achieved.

The first option - non-surgical endodontic retreatment is preferable in case of endodontic treatment failures, when gaining acces to the root canals is possible. During endodontic retreatment we should do cleaning, shaping, and three-dimensional filling, with an inert filling material in all previously obturated root canals.

Some studies conducted over time, showed the presence of persistent apical periodontitis in case of endodontically treated teeth, in a proportion of 45% (2,3). The cause of persistent apical periodontitis is represented mainly by microorganisms that have survived primary endodontic treatment. This problem can be resolved by orthograde endodontic retreatment (4). During the stages of endodontic retreatment, the total removing of root filling materials is essential, so it will be possible to eliminate all residual microorganisms and to create favorable conditions for periradicular healing (5).

Studies were done on the success rate of endodontic retreatment, looking at the presence or absence of periapical lesions. Holland and colleagues have analyzed seventeen such studies (6). The conclusion is that, in case of periradicular lesions's absence, the success rate is higher, while in the cases where lesions were present, the success rate varies from a minimum of 31 , 8% to a maximum of 85%.

Friedman et al. (7) show a success rate of 100% for the endodontic retreatment of cases without periapical lesions, which suggests that in cases where infection is not present, and retreatment is done by skilled clinicians, success rate can be very high. Also Sjogren and colleagues (8), performing endodontic retreatments on 173 roots in the absence of periapical lesions, obtained a success rate of 98%. If the periapical lesion is present before starting endodontic retreatment, the success rate can be estimated at not more than 70% (7).

The second approach, apical surgery, tries to block microorganisms within the canal, through its apical seal. Leaving debris and microorganisms into the root canals is contrary to biological reasoning. Thus, surgery is a secondary alternative if endodontic retreatment is not feasible, or is contraindicated, or has failed probably due to microorganisms that invaded the periapical tissue.

If in a given situation the tooth is impossible to restore, the extraction will be recommended and an alternative replacement will be examined.

CONCLUSIONS

It is very difficult to define failure of endodontic treatment. A clear and precise definition of what success or failure is, can be said that there was not among clinicians (9).

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NOUTĂȚI

STATUSUL TIROIDIAN LA PACIENȚII CU RETARD MENTAL: REZULTATELE STUDIULUI TOP-R

Un status tiroidian modificat poate afecta dezvoltarea cerebrală ducând până la retard mental. Studiul multicentric olandez TOP-R (Thyroid Origin of Psychomotor Retardation) a măsurat parametrii tiroidieni într-o cohortă de 946 pacienți diagnosticați cu retard mental de etiologie neprecizată. Rezultatele au indicat că majoritatea celor investigați aveau aceşti parametri în limitele considerate normale de laboratorul utilizat pentru testare. Împărțirea cohortei în două grupuri în funcție de utilizarea medicației antiepileptice a relevat scăderea valorilor tiroidiene pentru pacienții cărora li se administrează aceste medicamente în regim cronic (T4: 102.1±1.2 vs. 83.9±1.2 nmol/L, P<1x10(-24); FT4: 18.0±0.2 vs. 16.1±0.2 pmol/L, P<1x10(-9); T3: 1.72±0.02 vs. 1.57±0.02 nmol/L, P<1x10(-9) şi rT3: 0.37±0.01 vs. 0.27±0.01 nmol/L, P<1x10(-28) pentru pacienți fără vs pacienți cu medicație antiepileptică). Prevalența hipo şi hipertiroidismului diagnosticat de novo a fost de 5,2% respectiv 2,2%. Aceste rezultate întăresc ipoteza conform căreia medicația antiepileptică influențează nivelul hormonilor tiroidieni şi subliniază necesitatea investigării cauzelor genetice ale retardului mental în raport cu statusul tiroidian. (Visser WE, de Rijke YB, van Toor H, Visser TJ. Thyroid status in a large cohort of patients with mental retardation: the TOP-R (Thyroid Origin of Psychomotor Retardation) study. *Clin Endocrinol* (Oxf). 2011; 4:45-53).

Teodora Alexa

CERCETĂRI PRIVIND COMPOZIŢIA CHIMICĂ A ULEIULUI VOLATIL SEPARAT DIN FRUCTE DE ANASON DULCE CULTIVAT ÎN ROMÂNIA

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CHEMICAL COMPOSITION OF ESSENTIAL OIL OBTAINED FROM ROMANIAN FENNEL FRUITS (Abstract): For therapeutical purposes, fennel (*Foeniculum vulgare* Mill.), an important aromatic plant, is used for its expectorant, antispasmodic, carminative and diuretic properties. The chemical composition, especially of the volatile fraction, depends on the origin of the plant, and the ecological and environmental conditions in which it grows. **Aim**: To determine the chemical composition of the volatile fractions obtained from fennel fruits (*Foeniculum vulgare* Mill.) available on the Romanian market. **Material and methods**: The essential oils obtained from dried fruits by hydrodistillation have been characterized by using gas chromatography and mass spectroscopy (GC-MS). **Results:** In all oils, the main components were: trans-anethole (9.72-56.57%), fenchone (7.72-34.66%) and estragole (4.69 - 49.04%). **Conclusions**: The essential oil from fennel samples showed an inadequate chemical profile for aromatherapy purposes. **Key words**: ESSENTIAL OILS, *FOENICULUM VULGARE*, CHEMICAL COMPOSTION

Originar din bazinul Mării Mediterane, anasonul dulce sau feniculul (*Foeniculum vulgare* Mill.) este o binecunoscută plantă medicinală şi condimentară folosită în întreaga lume, fiind cultivată pe scară largă în toată Europa, Asia şi în unele zone din Africa şi din America de Sud.

Principalele efecte terapeutice atribuite feniculului sunt cele expectorante, carminative, spasmolitice, galactagoge, estrogen-like, vermifuge şi, în ultimul timp, antioxidante. Aceste efecte sunt în mare măsură determinate de prezenţa în fructe a uleiului volatil (1, 2, 3).

Cel mai adesea, fructele de fenicul sunt recomandate în tratamentul unor tulburări dispeptice sau al unor afecţiuni respiratorii superioare. De asemenea, aceste fructe sunt componente ale unor produse farmaceutice destinate calmării colicilor infantile sau ale unor siropuri pediatrice antitusive.

În industria farmaceutică, uleiul de fenicul constituie cea mai importantă sursă de obţinere a trans-anetolului, iar în industria alimentară, se utilizează la aromatizerea produselor de panificaţie, a preparatelor din carne, a diferitelor sosuri sau a băuturilor alcoolice şi nealcoolice.

Este cunoscut faptul că tipul şi cantitatea componentelor active, prezente în drogul vegetal, în special pentru fracţiunea volatilă, variază în funcţie de o serie de

factori endogeni şi exogeni, precum: subspecia, condiţiile pedoclimatice în care se dezvoltă planta ori modalitatea de uscare şi de obţinere a fracţiunii volatile (1, 3).

Oficinale sunt uleiurile volatile obţinute de la *Foeniculum vulgare* Mill. ssp. *vulgare* var. *dulce* (care conţine 1-10% fenconă, 3-10% estragol şi 80-90% t-anetol) respectiv, de la *F. vulgare* ssp. *vulgare* var. *vulgare*, care pe lângă 50-75% t-anetol, mai conţine 2-5% estragol şi 12-33% fenconă (4).

Plecând de la acest aspect, ca şi de la faptul că între proporţia/tipul de componente active şi acţiunea biologică a produsului vegetal există o relaţie de directă proporţionalitate, ne-am propus studiul comparativ al fructelor de fenicul care circulă pe piaţa farmaceutică românească, din perspectiva fracţiunii lor volatile în scopul stabilirii compoziţiei chimice şi evidenţierii posibilelor diferenţe între acestea. Fructele de fenicul oferite în farmacii provin de la var. *dulce*.

MATERIAL ŞI METODĂ

Cele 7 mostre de fenicul supuse cercetărilor au fost constituite din produs vegetal (recoltă 2007) provenit din zone diferite ale României (Ardeal, Banat, Muntenia, Moldova).

Studiul fitochimic al probelor investigate s-a realizat prin analiza chimică cantitativă şi calitativă (GC/MS) a fracţiunii volatile.

Izolarea şi determinarea cantitativă a fracţiunii volatile s-a realizat prin hidrodistilarea timp de 4 ore a materialului vegetal uscat. Conţinutul în ulei volatil al produsului vegetal s-a exprimat procentual (ml ulei volatil la 100 g produs vegetal).

Compoziţia chimică a uleiului volatil a fost stabilită prin analiza gaz-cromatografică cuplată cu spectrometrie de masă (GC-MS) în următoarele condiţii:

Gaz-cromatograf HP 5890 series II; **Spectrometru de masă** HP 5971; Coloană **cromatografică** capilară (cross linked methylsilicone gum): HP-5 MS (30 m x 0,25 mm x 0,25 μm); Faza mobilă heliu 1 ml/min; **Temperatura injector** 260ºC; **Temperatura detecto**r 280ºC; **Regim de temperatură**: iniţial 35ºC, apoi creştere cu 10ºC/min. de la 35ºC la 260ºC (2 min. constant); **Volum injectat**: 0,1 μl ulei volatil. Identificarea substanţelor din probele analizate gaz-cromatografic s-a făcut pe baza comparaţiei între spectrul de masă din vârful peak-ului şi spectrele din biblioteca Wiley (5).

REZULTATE ŞI DISCUŢII

Urmărind aspectul organoleptic al celor 7 mostre de fructe de fenicul (fig. 1) am putut constata că mărimea, greutatea şi culoarea lor variază, unele dintre ele fiind impurificate cu fragmente de tulpină (probele 2, 5, 6).

Caracteristicile macroscopice ale fructelor de anason dulce şi randamentele la extracţia uleiurilor volatile sunt prezentate în tabelul I.

Pentru probele investigate există o variaţie importantă şi destul de largă a conţinutului în ulei volatil (2,6-9,52%); cu excepţia probelor de la Oradea şi Râmnicu Vâlcea, toate celelalte corespund cerinţelor FR X (min. 3,5% ulei volatil) (7).

Rezultatele analizei GC-MS a fracţiunilor volatile investigate sunt redate în tabelul II.

Compuşii majori identificaţi în toate probele au fost trans-anetolul, fencona şi estragolul, caracteristici uleiului volatil de fenicul; acestora li s-a adăugat limonenul, care în proba de la Vâlcea ajunge la 19%.

1

2

3

4

5

6

7

Fig. 1. Aspectul macroscopic al fructelor de fenicul
Probe: 1. Oradea; 2. Orăştie; 3. Râmnicu Vâlcea; 4. Ploieşti; 5. Bucureşti; 6. Timişoara; 7. Secuieni Neamț.

TABELUL I

Caractere macroscopice ale fructelor de fenicul şi conţinutul (%) în ulei volatil

Proba	Caractere macroscopice			Culoare	Miros/Gust	ml ulei volatil/100 g fructe uscate
	Dimensiuni (mm)		Greutate g/100 fructe			
	L= lungime	l = lățime				
Oradea (1)	5	2	0.557	cafenie	slab aromatic/ dulceag	2.60
Orăştie (2)	5.2	1.7	0.892	cafeniu-roşcată	aromatic/ dulceag, arzător	6
Rm. Vâlcea (3)	6.4	2.7	0.327	verde	aromatic camforat/ dulceag	3
Ploieşti (4)	5.7	2.2	0.892	cafenie	puternic aromat/ dulceag-arzător	8.80
Bucureşti (5)	4.1	1.5	0.446	cafenie	aromatic/ dulceag-arzător	7
Timişoara (6)	5.9	2.1	0.889	cafenie	puternic aromat/ dulceag-arzător	9
Secuieni-Neamț (7)	6	2.3	0.665	cafenie	puternic aromat/ dulceag-arzător	9.52

TABELUL II

Componenți identificați în uleiurile volatile de anason dulce

TR (min.)	Compus	(%)						
		1	2	3	4	5	6	7
6.29	α -pinen	4.81	5.57	3.36	6.50	5.30	4.91	3.80
6.54	alloocimen	-	-	-	-	-	-	0.92
6.94	sabinen	0.20	0.22	0.47	0.23	0.20	0.59	0.23
7.01	β-pinen	0.47	0.53	0.75	0.59	0.46	-	0.49
7.18	β-mircen	1.50	2.11	1.26	2.01	1.75	1.87	1.56
7.46	l-felandren	0.48	0.87	0.48	0.79	0.60	0.67	0.69
7.90	limonen	4.70	4.71	18.99	5.25	4.46	4.32	6.11
8.37	γ-terpinen	1.09	1.36	2.09	1.34	1.39	1.20	2.02
8.70	α -terpinolen	-	-	-	-	0.20	-	-
8.75	cis-sabinen hidrat	-	-	-	-	-	0.25	-
9.23	fenchona	34.66	24.33	7.72	25.70	23.54	22.25	15.68
9.44	α -tuiona	0.64	0.49	-	0.27	0.42	0.17	-
9.65	camfen	-	-	0.20	-	-	-	-
9.91	pulegona	-	-	0.85-	-	-	-	-
9.93	camfor	2.03	1.43	-	1.29	1.41	1.19	0.72
10.41	terpineol-4	-	-	-	-	-	0.17	-
10.82	estragol	6.40	7.30	49.04	7.83	5.77	4.69	14.97
11.75	carvona	-	-	1.06	-	-	-	-
11.93	anisaldehida	-	-	0.90	-	-	-	-
12.55	trans-anetol	38.58	48.76	9.72	45.77	52.94	56.57	51.68
13.60	p-metoxifenilacetona	0.31	1	-	0.37	-	-	0.80
14.97	germacren -D	-	-	0.09	-	-	-	-
15.44	Δ-cadinen	-	-	0.07	-	-	-	-
16.73	trans-izodilapiol	-	-	0.21	-	-	-	-

Comparând aceste valori cu prescripţiile existente în Farmacopeea Europeană ed. a 5-a (în vigoare la momentul respectiv) (8), situaţia se prezintă conform celor redate în tabelul III.

După cum reiese din datele prezentate, nici una din probele de fructe nu a furnizat un ulei volatil care să se încadreze în cerinţele Farmacopeei Europene în ceea ce priveşte conţinutul în fenconă, estragol şi trans-anetol. Faţă de reglementările menţionate, aromaterapia (6) vine şi ea cu o serie de precizări şi cerinţe, dar acestea nu sunt îndeplinite, după cum vedem, de nici una din probele de fructe analizate.

Dacă surplusul de estragol (peste 4%, cu excepţia fructelor de la Râmnicu Vâlcea al căror ulei volatil conţine 49%) nu impietează asupra efectului expectorant, mucolitic, antiinflamator, gastro-şi bronhospasmolitic, psihostimulant, conţinutul ridicat în fenconă al uleiurilor volatile obţinute din fructele incluse în studiu aduce în sine, la administrare internă, un efect neurotoxic. Substanţa intră în categoria psihotropelor, care în doze mici sunt psihostimulante (prin modificările de metabolism cerebral pe care le induce) dar care, în doze mari dezvoltă neurotoxicitate; totodată, fencona este şi avortivă.

TABELUL III

Compoziţia probelor de ulei de anason dulce (recolta 2007) şi valori indicate în Ph. Eur. 5

Proba	Proveniența	%		
		Fencona	**Estragol**	**Trans-anetol**
1	Oradea	34.66	6.40	38.58
2	Orăştie	24.33	7.30	48.76
3	Rm. Vâlcea	7.72	49.04	9.72
4	Ploieşti	25.70	7.83	45.77
5	Bucureşti	23.54	5.77	52.94
6	Timişoara	22.25	4.69	56.57
7	Secuieni-Neamț	15.68	14.97	51.68
Prevederi Ph. Eur. 5		max. 7.5	max. 10	min. 80
Cerințe aromaterapie		0.5-5	max. 4	55-85

CONCLUZII

Dacă fructele de anason dulce sunt destinate extracţiei uleiului volatil la scară industrială, anterior acesteia trebuie analizată compoziţia chimică şi conformitatea acesteia cu cerinţele Farmacopeei Europene în vigoare.

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ENZYME-ASSISTED PURIFICATION OF DENATURED ATELOCOLLAGEN, USING PRONASE AS SCAVENGING AGENT

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ENZYME-ASSISTED PURIFICATION OF DENATURED ATELOCOLLAGEN, USING PRONASE AS SCAVENGING AGENT (Abstract): Collagen liquid-crystal characteristics can be exploited in advanced applications, like microelectromechanical systems (MEMS), collagen-silica biohybrids or intelligent substrates for tissue engineering. Such applications necessarily require high purity of the collagen colloidal solutions, in terms of macromolecular unitary composition, which is equivalent to almost zero poydispersity. In this work a protocol for the enzyme-assisted removal of non-triple helical polypeptide entities in atelocollagen solutions was conceived, using pronase as scavenger, in the presence of poly(ethyleneglycol) (PEG 10000) as a crowding agent, trimethylamine-N-oxide dihydrate, (TMAO) as kosmotropic agent and anhydrous sodium sulfate as anti-chaotrope salt. Unusually high enzyme concentration (3 : 100 w/w 5 U/mg Pronase : total dry protein) imposes the triple-helix integrity protection, which was achieved by means of protective adjuvants. The adjuvant agents mixture comprising the crowder, the kosmotrope and the anti-chaotropic salt, was formulated according to the Design of Experiments (DOE) principles. The crowding agent represents the key factor in modulating pronase hydrolytic action upon atelocollagen substrate. The optimal adjuvant mixture tested in order to confirm the model validity had the composition: 0.675 PEG, 0.200 TMAO, 0.125 Na_2SO_4 (mass fractions). The proposed protocol is suitable for purifying medium and large quantities of atelocollagen previously solubilized through an alkali-enzyme technique. **Key words**: ATELOCOLLAGEN, PURIFICATION, ENZYME-ASSISTED, PRONASE, CROWDING AGENTS, KOSMOTROPES

As a strong disymmetric biopolymer and having a remarkable intrinsic stiffness, type I collagen self-associates to give chiral nematic phases (liquid-crystals), especially in confined spaces arising from protective concentrations of pure colloidal protein acidic solution, exceeding 80 mg/mL DM. The resulting mesomorphic state offers an important support in a wide variety of applications, mostly in the so-called "Lilliput structures and technologies", e.g. in generating microelectromechanical systems (MEMS) (1). An interesting series of hybrid materials, such as collagen-silica biohybrids, can be also constructed based on collagen aggregates templates (2). This kind of advanced applications however requires medium to large amounts of highly pure, triple-helical intact collagen, in native state or telopeptide deprived, able to participate in long-range self-organizing interactions. Besides, the biomedical applications require non-immunogenicity and morpho-structural stability under *in-vivo* conditions.

All atelocollagen-obtaining techniques starting from natural sources lead to a large polydispersity of solubilized molecules characteristics, both at laboratory scale and at plant scale, at most. "Screening" such a complex colloidal system, to isolate minimally denaturated, intact triple-helical atelocollagen macromolecules, can be done by

TABLE I
The restricted mixture design matrix and the measured responses

The structure of the mixture design matrix						
Number of mixture components: 3			Imposed restrictions to mixture components			
Design degree: 3			Variable		Lower value	Upper value
Design type: extreme vertices			PEG		0.6000	0.9000
Design augmenting: central point, axial points			TMAO		0.0500	0.2000
A priori model: third order, full cubic			Na_2SO_4		0.0500	0.2000
Exp. no.	Order in the experiment	Design point (Fig. 1)	Mixture components (mass fractions)			Measured response
			PEG	TMAO	Na_2SO_4	Total nitrogen, %
1.	10	1	0.6000	0.2000	0.2000	0.071
2.	3	2	0.7500	0.0500	0.2000	0.069
3.	5	3	0.9000	0.0500	0.0500	0.064
4.	12	4	0.7500	0.2000	0.0500	0.067
5.	9	5	0.8250	0.0500	0.1250	0.068
6.	4	6	0.8250	0.1250	0.0500	0.066
7.	7	7	0.6750	0.2000	0.1250	0.069
8.	1	8	0.6750	0.1250	0.2000	0.072
9.	6	9	0.7500	0.1250	0.1250	0.063
10.	2	10	0.6750	0.1625	0.1625	0.061
11.	8	11	0.7500	0.0875	0.1265	0.065
12.	13	12	0.8250	0.0875	0.0875	0.060
13.	11	13	0.7500	0.1625	0.0875	0.065

various physicochemical methods. This paper focuses on a procedure, feasible at large scale, able to scavenge atelocollagenic sols by enzymatically degrading all the denaturated protein molecules, resulting in a mixture of "undamaged" atelocollagen molecules and of easily ultrafiltrable polypeptides. The protocol can be applied in a stand-alone purifying operation, or can be adjusted, to enhance the clasic pepsin – papain solubilizing techniques of collagenic sources.

In the main, the proposed scavenging technique is based on the limited cleavage ability of proteolytical enzymes, other than collagenase, to hydrolyze the peptide bonds inside the native helical region of collagen macromolecule, and even of chemicaly non-altered α-chains decoupled from the triple helix. The only portions of fibrillar collagen that can be split up or divided by usual proteases are the telopeptides, placed at both ends of the macromolecule. Telopeptides accelerate the linear and lateral assembly of collagen fibrils, both *in vivo* and *in vitro*, but do not provide fibrillogenesis competency by themselves. So, they are unessential in supramolecular self-assembly (3) or in long-range physicochemical interactions of atelocollagen (4), but are involved in nucleation (5), the first step in supramolecular structuring. Because telopeptides represent the sites of collagen immunogenicity (6), they must be removed in all xenogenic biomedical applications.

Commercial preparations marked as Pronase represent complex mixtures of endopeptidases (serine proteases and metalloproteases) and exopeptidases (carboxypeptidases and aminopeptidases) isolated from

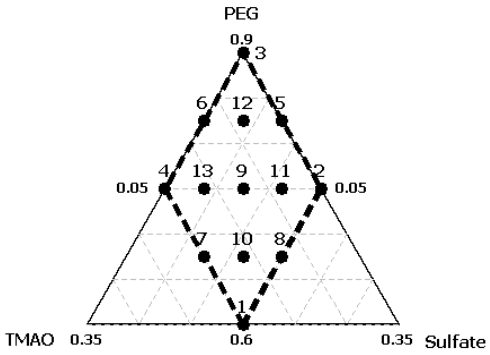

Fig. 1. The experimental design of tertiary adjuvants mixture

Streptomyces griseus. The product is free of nucleases, but comprises alkaline phosphatases and 20% calcium acetate (7). It exerts proteolytic activity upon both denatured and native proteins, which in general are broken down into individual amino acids. Pronase inhibiting and inactivating is very difficult, because of its complex composition, but all included proteases can be removed by ultrafiltration from atelocollagen solution, due to the fact that the molecular weights of individual components range between 20 and 60 kDa. The optimum pH range is between 7.0 and 8.0, but the preparation is very stable in the pH range of 6.0 to 9.0. Outside the pH range 4.0 to 10.0, pronase is unstable. The optimum temperature lies in the range of 40 to 60°C. Even in dilute solutions, pronase is not very temperature sensitive; it loses activity at 80°C, an unfeasible temperature in collagen processing.

Treating alkali-enzyme solubilized collagen with Pronase guarantee the spliting-off of both telopeptides (5), with minimal impact on triple helical domain, in the context of complete hydrolysis of denaturated forms of collagen and of macromolecular subfragments detached from the triple-helix (alpha and beta chains). Comparing to the enzymes used in the solubilizing step, tipically pepsin or papain, pronase cuts the telopeptides in the closest position to the helical region (4). This kind of action is important in the obtaining hypoimmunogenic atelocollagen, but slows down all the assembly processes.

Supplemental precautions have to be taken when working with Pronase on collagen colloidal solution, in order to modulate the proteolytic action, and to limitate parasitic cleavage of peptide bonds. PH lower than optimal and higher ionic strength are the simplest ways to modulate Pronase action, but cannot guarantee the avoidance of unwanted hydrolysis. To prevent extended attack on intact atelocollagen macromolecules, which can intervene both before and after the main telopeptide cleavage action, some kosmotropes and macromolecular crowding agents, together with anti-chaotropic salts must be added to the starting colloidal solution, during a preliminary conditioning step. The kosmotrope and the crowding agent prevent the destructuring of telopeptide- deprived triple-helix, during the final solution processing (pumping, settling/decanting, filtering). According to some authors (8), in dilute solutions and in the presence of crowding agents, the hydrolytic or degradative reactions are likely to be somewhat hindered. The antichaotropic salt preserves the spatiality of solubilized atelocollagen in the presence of the calcium acetate, an active lyotropic salt introduced in the solution by the Pronase product, during the whole period before ultrafiltration, when the calcium acetate is removed together with the antichaotropic salt. Because the majority of proteases included in Pronase require calcium ions to become active, a highly soluble, non-lyotropic calcium salt must be added in the conditioning step.

MATERIALS AND METHODS

The primary atelocollagen solution was obtained starting from newborn pigskin, pursuing a 52-operation plant-scale proce-

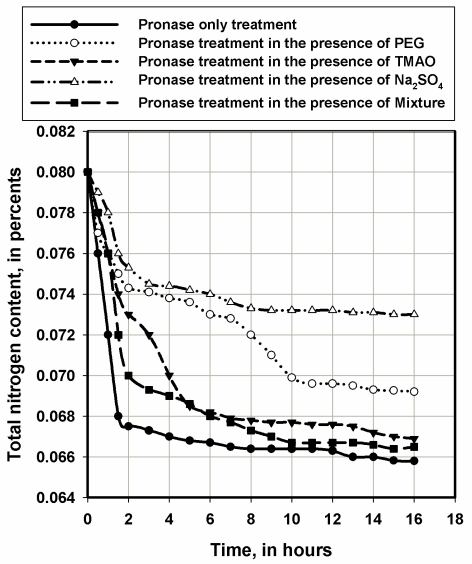

Fig. 2. Time evolution of total nitrogen content in the processed atelocollagen sol

dure, based on alkali pre-treatment, papain solubilization / refining and acid resolubilization processes, resulting in a concentrated impure, acidic atelocollagen solution, with slightly variable characteristics from one batch to another (1.6 ÷ 1.8% DM, 1.2 ÷ 1.35% protein content, aprox. 75 g protein / 100 g DM, pH 3.1 ÷ 3.3, global isoelectric pH range 4.12 ÷ 4.16, no sediment after 30 minutes centrifugation at 8000 g RCF).

Pronase was purchased from Fluka (Pronase E, Protease from Streptomyces griseus, P 81750, CAS 9036-06-0, 5 U / mg).

The crowding agent – 10 kDa poly(ethylene glycol) (Fluka P 81280, CAS 25322--68-3) was choosen as crowding agent, because it makes a good compromise between confining (9) and crystallizing (10) effects induced in protein solutions.

The kosmotrope (trimethylamine-N-oxide dihydrate, TMAO, Fluka P 92277, CAS 62637-93-8) was choosen from the nonionic class, due to its minimal electrostatic interference with collagen; its main effect is the decrease of dielectric constant near the surface of triple-helix, by a thinning effect

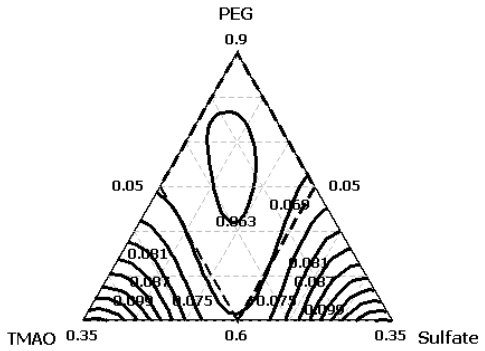

Fig. 3. The dependence of total nitrogen content of Pronase processed atelocollagen sol on the adjuvants mixture composition.

upon the attached double layer. As a consequence, the intra- and inter-catenary hidrogen bridges increase their satability, in the virtue of long-range spatial ordering of the solvent (the water). TMAO is the most efficient in the nonionic kosmotrope series (surpassing betaine, sarcosine, proline, trehalose, sucrose, glycerol and sorbitol) (11). At high concentrations (over 0.4 M) (12), kosmotropes induce collagen precipitation. Supplementary, they slow down the enzyme hydrolysis. This is why TMAO molar fraction in atelocollagen solution must be strictly controlled.

The adjuvant agents mixture comprising the crowder, the kosmotrope and the anti-chaotropic salt (sodium sulfate), was formulated according to the Design of Experiments (DOE) principles. Figure 1 and table 1 summarize the experimental design matrix.

The general experimental procedure was designed in order to identify and quantify the factors affecting the scavenging of atelocollagenic sols by removing the protein forms susceptible to pronase hydrolyze. To achieve rapid hydrolytic effects, a high Pronase to substrate ratio was used (3 : 100 w/w 5 U/mg Pronase : total dry protein), comparing to ratios usually prescribed (1 : 100, according (13)). The proposed scavenging protocol comprised the following steps:

1. diluting the raw atelocollagen sol to 0.5 % DM (below 0.4 % dry protein), to reduce viscosity ;
2. removing low molecular protein fractions, by ultrafiltering the neutralized atelocollagen sol (pH 7.5) over a 100,000 NMWL membrane ; this step is not necessarily required, but it will accelerate the overall scavenging process ;
3. sol conditioning with the adjuvants mixture (crowding agent, kosmotrope, anti-chaotrope salt ; 8% w/v mixture in a composition accordingly the experimental design matrix) and the calcium salt Pronase activator (0.05 M/dm^3 $CaHPO_4.2H_2O$ solved in the raw or ultrafiltered atelocollagen sol) ;
4. heating the sol at 38°C, under thermostatic control, in a sterilized glass reactor ; if the enzyme treatment cannot be conducted under sterile conditions, 0.01% sodium azide must be added to the sol ;
5. Pronase treatment for 16 hours, at 38°C ;
6. quick Pronase inactivation by pH lowering to 4.6 with H_3PO_4, under gentle agitation ; during acidification, the temperature is lowered to 18°C, by short bursts of carbonic ice ;
7. ultrafiltering the purified sol over a 100,000 NMWL membrane ;
8. acidification of both the purified sol and the ultrafiltered solution to pH 3.2, with HCl.

Samples of the treated sol were drawn hourly and the atelocollagen concentration was determined. Total nitrogen content of processed atelocollagen sol was determined through the Kjeldahl technique (on a VELP DK6 programmable heating digester and a VELP UDK 132 semiautomatic distillation unit), on samples equivalent to 1 g DM protein, resulted from protective evaporation (40°C, 0.02 kgf/cm^2 = 1961.33 Pa ; at this low pressure the boiling point of pure water decreased to approximately 18°C).

The individual adjuvant influence on Pronase action modulating was studied at 75% of its upper limit mass fraction considered in the mixture design matrix, respectively 5.4% w/v PEG, 1.6% w/v TMAO, 1.2% w/v sodium sulfate. The general procedure was obeyed.

The scavenging process efficiency was qualitatively proved by comparing the HPLC chromatograms of the raw and the purified atelocollagen sols. An Agilent Zorbax Bio Series GF-450 gel filtration column (9.4 x 250 mm) was made use of. The lyophilized sol samples were redissolved in sodium phosphate buffer at pH 7.5, up to a concentration of 0.2 mg/mL ; 100 mL of redissolved sol was injected and then eluted with the same buffer, at 1 mL/min flow rate. The UV absorption was recorded at 215 nm. At this wavelength, the detection limit of quasi-native atelocollagen is above 2 mg (in good accordance with (14). The column was calibrated with High Molecular Weight Range SIGMA Marker (PN S8320), reconstituted in 2 ml of the same buffer type to those in which the sample was redissolved (0.2 mg/mL final protein concentration).

RESULTS AND DISCUSSION

The individual effect of adjuvants on Pronase activity modulation is depicted in figure 2, comparing to the hydrolytic efficiency of Pronase-only treatment.

The measured response was the total nitrogen content of treated atelocollagen sol after the first half hour, and then every hour after. The anti-chaotropic salt has the strongest inhibitory effect on Pronase hydrolytic activity, due to ion pairing with lateral aminoacidic functions, resulting in a global increase of apparent hydrophobicity of polypeptide chains, at long range. The enzyme will difficultly spot the target sites on protein backbone, thus restraining its activity. The crowding agent seems to delay the Pronase hydrolytic activity and to limit its extent over atelocollagen substrate, probably

Table II
Coefficients of the mathematical model fitted to experimental data generated by the mixture experiment

The structure of fitted extended full quartic model		
Main effects	PEG	-0.0228
	TMAO	-0.1896
	Na_2SO_4	-0.1725
Interaction effects	TMAO ?Na_2SO_4	32.1943
Extended effects	1 / PEG	0.0935
Overall adequacy of the model, R^2 (the coefficient of multiple determination)		96.66

by increasing the packaging density of macromolecules. The kosmotrope has the minor direct effect on delaying the hydrolysis.

The study of the modulating efficacity of adjuvant mixtures revealed that the protective effect against extensive Pronase attack over atelocollagen triple-helix correctly fit in the experimental region. Figure 3 depicts the surface responses of mixture compositions, as it resulted from the mathematical model resumed in table II. The choosen extended full quartic type aprioric model was fitted using stepwise procedure. In the final accepted model, all individual adjuvants induced negative sign main effects, suggesting that increasing their mass fraction in the mixture will limit the Pronase hydrolytic activity. The major limiting adjuvant is the kosmotrope, but the anti-chaoptopic salt has a significant contribution, too. A strong sinergetic interaction between the kosmotrope and the anti-chaotrope was identified. A statistically significant extended effect, related to PEG component, suggests that the crowding agent represents the key factor in modulating Pronase hydrolytic action upon the atelocollagen substrate. Summation of the linear main effect (-0.0228.PEG) and the nonlinear extended effect (0.0935/PEG) indicates that the PEG mass contribution to the mixture must be optimized, in order to attain an imposed hydrolytic action. The nonlinear 1/PEG term indicates that the mechanism of Pronase activity modulation by PEG crowder is not purely physical *i.e.* governed by spatial confining only, but also involves chemical interactions, at least at high PEG concentrations.

The modelled effects of individual component mass contribution on modulating Pronase activity are depicted in Figure 4. The most influential constituent is PEG; the other two partners seem to act antagonically towards PEG. In order to obtain a maximal Pronase hydrolytic effect, PEG mass contribution to the adjuvant mixture must not exceed 25% of its accepted interval limits (see figure 4, in correlation to the header of table I), but the other two components mass contribution must exceed 75% of their own accepted interval limits. Therefore, based on the informations offered by Cox response trace plot (figure 4), the optimal adjuvant mixture tested in order to confirm the model validity have the composition: 0.675 PEG, 0.200 TMAO, 0.125 Na_2SO_4.

The modulating effect corresponding to the optimal mixture composition is depicted in figure 2. It is obvious that this mixture is able to recover the individual components inhibitory effects.

The confirmation of the scavenging effect of Pronase treatment is revealed in figure 5. Comparing to the raw atelocollagenic

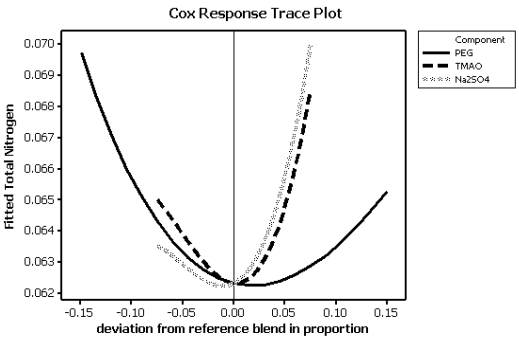

Fig. 4. The individual effects of the mixture components on Pronase activity modulating

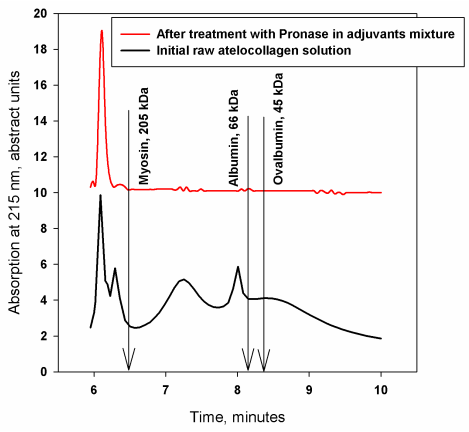

Fig. 5. Comparison between the chromatograms of the raw and the purified atelocollagen sols

sol, after six hours of protected enzyme hydrolysis, the peaks corresponding to molecular weights lower than those of telopeptides-deprived triple-helix dissapeared, but the peak associated to the heavier molecular aggregates preserved its relative amplitude. This confirms the efficiency of atelocollagen protection using optimal mixture of PEG crowder, TMAO kosmotrope and Na_2SO_4 anti-chaotope. The broad peaks, positioned in the raw atelocollagen sol chromatogram, at medium and at low molecular weights, correspond to the denaturated and hydrolyzed collagen forms. These polypeptides were completely hydrolyzed by Pronase.

In figure 5, the position of three known proteins included in the SIGMA marker are shown by arrows, to indicate the range of protein forms in the studied atelocollagen sols. The respective positions, on the time scale are those obtained by the time the Zorbax GF-450 column was calibrated.

CONCLUSIONS

In a protective regime, a certain scavenging effect of Pronase on denaturated atellocolagen colloidal solutions can be achieved.

By sol conditioning with 8% w/v of an optimal mixture including a crowding agent (PEG 10000, at a mass participation fraction of 67.5%), a kosmotrope (TMAO dihydrate, 20% mass fraction) and an anti-chaotropic salt (anhydrous sodium sulfate, 12.5% mass fraction) the Pronase hydrolytic efficacity is minimally altered, but the protective effect over the atelocollagen triple-helix is ensured.

In conditioned sols, Pronase hydrolytically breaks down all the polypeptides resulted from the collagen solubilizing processes, splits up the remaining telopeptide portions, but do not attack the triple-helical domain.

The scavenging protocol described in the paper is able to drastically reduce the polydispersity of atelocollagen colloidal solution, thus offering a way to produce sufficient quantities of stiff, highly self-similar macromolecules to find particular uses in advanced scientific fields, such as in nematic applications, or in scaffolds generation.

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NOUTĂŢI

Denervarea simpatică renală prin cateter (DSRC) o soluţie terapeutică în HTA rezistentă

Progresele recente în tehnologia cateterelor intravasculare au permis punerea la punct a unor catetere purtătoare de sonde de radiofrecvenţă care permit DSRC, metodă lansată în 2009. Primul studiu multicentric randomizat pentru DRSC, denumit Simplicity, a inclus 106 pacienţi cu o HTA rezistentă (definită printr-o TA sistolică - TAS > sau egală cu 160 mm Hg (150 mm Hg la diabetici), tratată cu cel puţin 3 antihipertensive şi confirmată după o supraveghere ambulatorie de 2 săptămâni). În grupul DSRC protocolul terapeutic a constat în 4-6 şedinţe de radiofrecvenţă de putere mică, de-a lungul celor două artere renale. Evaluarea PAS s-a făcut după 6 luni la 103 pacienţi, rezultatele fiind extrem de favorabile : TAS a scăzut în medie cu 32 mm Hg, în timp ce TA diastolică - TAD a scăzut cu 12 mm Hg ($p=0,0001$). TAS şi TAD practic nu au variat în grupul control (+ 1 mm Hg/0 mm Hg ; $p<0,0001$). Şi din punct de vedere al altor criterii DSRC s-a dovedit a fi benefică : TA automăsurată la domiciliu (diferenţă 22/12 mm Hg ; $p<0,0001$), TA măsurată ambulator 24 ore a arătat o scădere medie de 11/7 mm Hg ; atingerea unei valori optime de 140 mm Hg (39% faţă de 6%) ; scădere mai mare de 10 mm Hg a TAS (84% faţă de 35%, $p<0,0001$ în ambele cazuri ; scăderea posologiei (20% fa?ă de 6%). În această serie, totuşi scurtă, de cazuri, efectele secundare ale DSRC au fost limitate la dureri dorsale prezente la un singur pacient şi tulburări vagale ce au necesitat administrare de atropină în 13% de cazuri. (Simplicity HTN-2 Investigators : Renal sympathetic denervation in patients with treatment-resistant hypertension (The Simplicity HTH-2 Trial) : a randomised controlled trial. *Lancet,* 2010, DOI : 10.1016/S0140-6736(10)62039-9. Doumas M et all. : Renal sympathetic denervation : the jury is still out. *Lancet*, 2010, DOI : 10.1016/ S0140-6736(10)62111-3).

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EFICIENŢA TERAPIEI CU OZON ŞI DE STIMULARE CU GEROVITAL H3 CA MIJLOC ADJUVANT AL TRATAMENTULUI COMPLEX ÎN BOALA PARODONTALĂ

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OZONE THERAPY EFFICIENCY AND GEROVITAL H3 AS AN ADDITIONAL WAY TO THE COMPLEX TREATMENT OF PERIODONTAL DISEASE (Abstract): The authors present the methodology of the ozone use and a method of chemical stimulation with Gerovital H3, applied within a complex therapy of the periodontal disease. Its efficiency is argued by the clinical images taken from the oral cavity and radiographic images of the patients with periodontal disease in various evolution stages. **Results**: The images reveal the possibility of stopping the progressive disease and the improvement of the clinical aspect of the marginal periodontium. The X-ray images reveal the possibility of re-mineralization of the alveolar bone and healing processes in certain areas, at different periods of time. **Conclusions**: Favorable results are associated firstly with compliant patients that benefit of treatment planning elaborated after a correct evaluation of the clinical situation. The results prove the strong oxidant and antibacterial effects of ozone as well as the regenerative and biotrophic action of Gerovital H3 in periodontal tissues for patients with early and advanced periodontal disease.
Key words: OZONE, GEROVITAL H3, PERIODONTAL DISEASE

Parodontita marginală cronică este responsabilă de pierderi dentare datorită afectării aparatului de susţinere.

Atunci când dorim reabilitarea funcţională a dinţilor afectaţi trebuie să ne gândim în primul rând la controlul infecţiei bacteriene dar mai ales la repararea defectelor rezultate în urma activităţii bolii (1).

Există numeroase studii care susţin capacitatea de regenerare în urma utilizării diverselor tehnici prin adiţie osoasă şi regenerare tisulară ghidată, însă regenerarea nu este întotdeauna prezentă sau completă. Există şi alte metode de tratament care aduc îmbunătăţiri ale situaţiei clinice precum şi a nivelului osos. Abordările predominant mecanice în ceea ce priveşte tratamentul au dus la rezultate deseori dezamăgitoare datorită neputinţei clinicienilor de a realiza un control adecvat al patogenilor parodontali precum şi a neputinţei sau reavoinţei pacienţilor de a se supune măsurilor necesare pentru prevenirea reapariţiei bolii.

Folosirea agenţilor antiinflamatori şi/sau antiinfecţioşi în tratamentul parodontitei a fost făcută frecvent în mod empiric. Există dovezi care arată că adăugarea unei terapii antiinfecţioase corecte la tratamentul tradiţional poate îmbunătăţi în mod substanţial rezultatele clinice şi poate reduce nevoia de proceduri chirurgicale costisitoare.

Un tratament al parodontopatiei prin mij-

loace care să fie eficiente din punct de vedere al costului - cum este ozonoterapia asociată cu terapia de stimulare chimică cu Gerovital H3 – poate oferi populaţiei, care nu are acces la servicii medicale supraspecializate datorită lipsei de resurse financiare, un mijloc eficient de a menţine o dentaţie funcţională pentru o perioadă lungă de timp. Iată de ce ne-am propus să aplicăm în clinică atât ozonul cu efect antimicrobian în pungile parodontale şi de stimulare a troficităţii la nivelul ţesuturilor parodontale cât şi gerovitalul aplicat prin infiltraţii locale.

MATERIAL ŞI METODĂ

Au fost luaţi în studiu pacienţi cu boală parodontală cronică a adultului aflaţi în diverse stadii de evoluţie la care terapia convenţională de înlăturare a acţiunii factorilor etiologici locali şi generali a fost asociată cu şedinţe de insuflaţie cu ozon în pungile parodontale şi infiltraţii cu Gerovital H3, în cure de tratament.

Expunerea la ozon a pungilor parodontale s-a efectuat în trei şedinţe la interval de o săptămână. In aceeaşi perioadă de timp s-au realizat infiltraţiile cu Gerovital H3. Infiltraţiile au fost efectuate cu soluţii de Gerovital H3 în doză de un mililitru pe şedinţă injectat cu seringi cu ac subţire utilizate pentru injectarea insulinei la diabetici, la interval de 2 sau 3 zile, într-un număr cuprins între 8 şi 12 şedinţe în raport cu gradul de afectare parodontală şi de modalitatea de răspuns al pacientului.

Curele de tratament au fost reluate la 6 luni. La fiecare cură s-a mărit numărul şedinţelor de expunere la ozon cu una, în funcţie de toleranţa individuală.

La începutul fiecărei cure cu Gerovital H3, s-a testat produsul epicutanat sau intradermic, pe antebraţ, pentru a preveni reacţiile alergice.

De la loturile aflate în tratament redăm imagini preluate din cavitatea orală şi imagini radiografice.

Caz clinic nr. 1 :

În fig. 1 se observă aspectul clinic al arcadelor dentare ale unei paciente în vârstă de 42 de ani cu boală parodontală cronică stabilizată. Se observă absenţa proceselor inflamatorii la nivelul mucoasei gingivale după trei cure de tratament. Se observă şi prezenţa cariilor de suprafaţă radiculară oprite în evoluţie şi remineralizate în urma expunerii lor la ozon.

Caz clinic nr. 2 :

În fig. 2 se observă aspectul clinic al arcadelor dentare la un pacient în vîrstă de 54 ani, cooperant în efectuarea tratamentului. Se observă aspectul normal al culorii gingiilor, lipsa depozitelor şi a inflamaţiei, precum şi mineralizarea produsă în urma expunerii la ozon.

Caz clinic nr. 3 :

Imaginile preluate de la cazul clinic nr. 3 reprezintă statusul parodontal la momentul prezentării pacientului P.R. în vârstă de

Fig. 1. Cazul clinic nr. 1

Fig. 2. Cazul clinic nr. 2

Fig. 3. P.R., 53 ani. Aspect radiografic (iniţial)

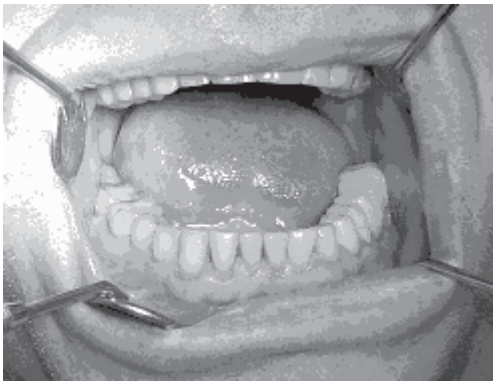

Fig. 5. P.R., 53 ani. Aspect clinic după 5 cure de tratament

53 ani, cu boală parodontală cronică avansată (fig. 3) şi aspectul după cinci cure de tratament, la trei ani de la prezentare (fig. 4).

După un interval de 30 luni se observă o evoluţie favorabilă manifestată prin oprirea evoluţiei bolii, manifestată prin absenţa proceselor inflamatorii, reducerea profunzimii pungilor parodontale şi creşterea densităţii osului alveolar.

Caz clinic nr. 4 :

Imaginile preluate de la cazul clinic nr. 4 reprezintă statusul parodontal la momentul prezentării al pacientei I.J. în vârstă de 55 ani, cu boală parodontală cronică avansată (fig. 6) şi aspectul după cinci cure de tratament, la trei ani de la prezentare (fig. 7).

În momentul prezentării la tratament pacienta prezintă resorbţie osoasă orizontală şi pungi infraosoase asociate cu zone de demineralizare la nivelul arcadei mandibulare şi atrofia crestei alveolare maxilare care afectează stabilitatea protezei superioare. Concomitent cu terapia etiologică

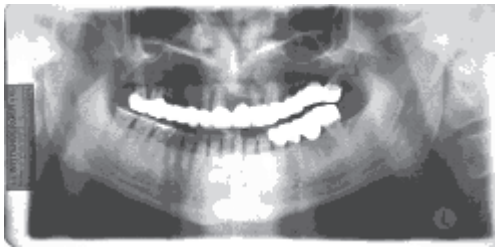

Fig. 4. P.R., 53 ani. Aspect radiografic după 5 cure de tratament

şi de imobilizare a dinţilor arcadei mandibulare, s-au efectuat patru cure de tratament cu ozon în pungi şi infiltraţii cu gerovital. După un interval de 24 luni se observă reducerea profunzimii pungilor osoase şi creşterea densităţii osoase atât la nivelul arcadei mandibulare cât şi la nivelul arcadei maxilare.

Caz clinic nr. 5 :

Pacientul A.M., în vârstă de 42 ani s-a prezentat cu diagnosticul de parodontită marginală cronică profundă. Pe imaginea radiografică (fig. 8) în momentul prezentării se observă liză osoasă orizontală şi verticală asociată cu reducerea densităţii osoase la nivel maxilar şi mandibular. Concomitent cu tratamentul etiologic şi corectiv local, au fost aplicate patru cure de ozonoterapie şi infiltraţii cu Gerovital H3 la intervale de 6 luni. Imaginea radiografică la un interval de 24 luni (fig. 9) arată creşterea densităţii osoase şi reducere profunzimii pungilor osoase. Imaginea radiografică la un interval de 48 luni (fig. 10) evidenţiază refacerea aproape completă a trabeculaţiei osoase.

DISCUŢII

Imaginile preluate de la pacienţii care au cooperat în efectuarea curelor de tratament dovedesc posibilitatea opririi în evoluţie a bolii parodontale cronice a adultului indiferent de faza clinică în care este aplicat tratamentul şi posibilitatea refacerii ţesuturilor parodontale, în special la nivel osos, prin mijloace terapeutice conservative.

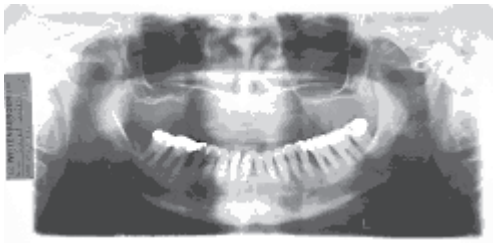

Fig. 6. I.J., 55 ani. Aspect radiografic (iniţial)

Fig. 7. I.J., 55 ani. Aspect radiografic după 4 cure de tratament

Fig. 8. A.M., 42 ani. Aspect radiografic (iniţial)

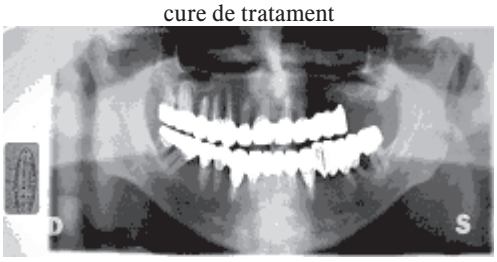

Fig. 9. A.M., 42 ani. Aspect radiografic după 4 cure de tratament

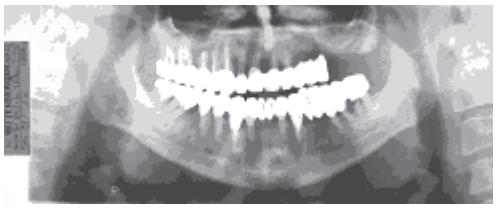

Fig. 10. A.M., 42 ani. Aspect radiografic după 8 cure de tratament

Azarpazhooh şi Limeback constată că, deşi studiile *in vitro* demonstrează potenţialul promiţător al ozonului în stomatologie, studiile clinice necesită metode de măsurare şi analiză standardizate pentru a evalua potenţialul real al ozonului ca modalitate terapeutică în stomatologie (1,2). Aplicarea terapiei cu ozon în pungile parodontale se dovedeşte a fi eficientă în distrugerea florei bacteriene parodontopate (3) şi oferă posibilitatea ţesuturilor parodontale de a se reface, stimulate atât de expunerea la ozon cât şi de curele de tratament cu Gerovital H3. Terapia cu ozon în boala parodontală (4) este eficientă şi lipsită de riscuri dacă este asociată cu precauţii şi măsuri de protecţie. Utilizarea empirică a ozonului a contribuit la limitarea utilizării lui în stomatologie în unele ţări. Există dovezi care scot în evidenţă o sensibilitate deosebită a sistemului bronho-pulmonar la ozon deoarece la acest nivel există cantităţi minime de antioxidanţi, ceea ce face celulele mucozale extrem de vulnerabile la oxidare ; celulele sanguine au însă o capacitate antioxidantă adecvată (1,23 – 1,83 mol/L) şi sunt capabile de a compensa orice doză de ozon cuprinsă între valorile terapeutice (10 – 20 mg/L) (5). În Clinica de Odontologie şi Parodontologie din Iaşi s-a acumulat experienţă în utilizarea ozonului în terapia stomatologică, atât în terapia cariilor simple şi complicate cât şi în boala parodontală - prima lucrare ştiinţifică fiind publicată încă din 1970 (6). Tratamentele au fost începute întotdeauna cu doze mici şi timp de expunere scurt, cu scopul de a induce pacienţilor o adaptare la stresul cronic oxidativ. Studiul de faţă prezintă o serie de cazuri clinice urmărite pe o perioadă de 2-3 ani.

Asocierea curelor de tratament cu ozon cu şedinţe de infiltraţie cu Gerovital H3 a condus la creşterea ratei de succes clinic. Eficienţa Gerovitalului H3 a fost dovedită clinic şi experimental de către Ana Aslan

fiind administrat în numeroase afecţiuni cu scopul de a încetini procesele de involuţie şi de ameliorare a stării pacienţilor suferinzi de maladii cronice degenerative (7,8). Procaina, componentă principală a Gerovitalului H3 nu prezintă toxicitate fiind hidrolizată rapid în ţesuturi, sub acţiunea enzimei procain esteraza, în acid paraaminobenzoic şi dietilaminoetanol, produşi lipsiţi de toxicitate, metabolizaţi imediat şi eliminaţi prin urină (9). Aplicarea lui în diverse forme clinice de boală parodontală este oportună întrucât procesele degenerative sunt prezente şi la nivelul ţesuturilor parodontale (10). Efectele benefice se datorează stimulării troficităţii ţesuturilor parodontale, ameliorării circulaţiei şi metabolismului local, echilibrării tonusului neuro-vegetativ tisular, efecte care conduc la refacerea parţială a zonelor tisulare afectate.

CONCLUZII

Utilizarea ozonului în cadrul tratamentului complex al bolii parodontale conduce la efecte clinice evidente manifestate prin reducerea şi dispariţia sângerării, normalizarea tabloului clinic şi reducerea pierderilor de ataşament parodontal.

Asocierea terapiei parodontale cu şedinţe de infiltraţii cu Gerovital H3 stimulează procesele de regenerare tisulară şi conduce la creşterea troficităţii ţesuturilor parodontale.

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EVALUAREA ACŢIUNII POLIZAHARIDELOR IZOLATE DIN C*HENOPODI BONI HENRICI HERBA* ASUPRA IMUNITĂŢII CELULARE LA GĂINI

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CELLULAR MEDIATED IMMUNITY REGULATION DONE BY C*HENOPODI BONI HENRICI HERBA* POLYZAHARIDES IN HENS. (Abstract) : The immunomodulatory effect of polysaharides from *Chenopodium bonus-henricus* herba was tested on adult hens. **Material and method** : Blood samples were harvested aseptically by punction of brachial vein ; ability of circulating phagocytes was assessed by the carbon particles phagocytosis test *in vitro* ; while reactivity of peripheral lymphocytes was evaluated by lymphocytes transformation assay *in vitro*. Polysaharides were dissolved in saline solution and filtered throughout Millipore. **Results** : Phagocytic indexes (at 15 and 39 minutes) are variable according to polysaharides concentration ; phagocytosis increased at 15 minutes for 40 μg/mL and 60 μg/mL, while for 30 minutes the phagocytic index decreased. In lymphocytes transformation assay, *Chenopodium bonus-henricus* polysaharides, alone, shown no stimulatory effect, and together to the classic mitogen, their influence was variable, but not statistical significant results were seen. **Conclusion** : Polysaharides have inconstant effect on phagocytosis, and mainly inhibitory role on lymphocytes proliferation. **Key words** : C*HENOPODIUM,* CELLULAR MEDIATE IMMUNITY, HENS

SCHIFF BASES OF L-LYSINE: MICROWAVE INDUCED SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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SCHIFF BASES OF L-LYSINE: MICROWAVE INDUCED SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY (Abstract): L-Lysine, also called 2,6-diaminohexanoic acid or alpha-epsilon-diaminocaproic acid is an essential amino acid which recently is being studied for its effects in different conditions, such as: herpes infections, cold sores, osteoporosis, anxiety, migraines etc. **Aim**: This paper is focused on the synthesis of new L-lysine compounds with a potential antioxidant activity. **Material and Methods**: The chemical synthesis is microwave induced and consists in the condensation of H-Lys (Boc)-OMe hydrochloride with various benzaldehyde (3-hydroxy/4-methyl-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde and benzaldehyde) resulting the corresponding Schiff bases. **Results**: The chemical structure of the synthesized compounds was confirmed by spectral methods (IR, ^{1}H-NMR) and the antioxidant potential was evaluated by determining the anti-radical effect using the free radical DPPH and radical cation $ABTS^{\cdot+}$ assays. **Conclusions**: Following the protocol new Schiff bases of H-Lys (Boc)-OMe hydrochloride were synthesized and characterized by physico-chemical and spectral methods. The evaluation of antioxidant potential using *in vitro* methods showed that the compound synthetized by condensation with 3-hydroxy benzaldehyde is the most active. **Keywords**: LYSINE, SCHIFF BASES, ANTIOXIDANT ACTIVITY.

The role of lysine in the prevention and treatment of herpes infections and cold sores has been studied (1). It also increases the intestinal absorption of calcium and decreases its excretion by the kidney, suggesting that it might be helpful in osteoporosis (2). Lysine has also been investigated for its effects on increasing muscle mass, lowering glucose (3), improving anxiety (4) and some case reports suggest that lysine may ameliorate angina (5). Lysine acetylsalicylate has been used to treat pain and to detoxify the body after heroin use (6), while lysine clonixinate has been used to treat migraine headaches and other painful conditions (7).

It has been 150 years since the first Schiff bases (azomethine) were synthesized by *Hugo Schiff* an Italian chemist of German origin who gave the name of this class of substances and which remained in the attention of scientists because of the multitude of

applications (8). Schiff bases have shown a wide range of biological activities such as antifungal, antibacterial, antimalarial, anti-proliferative, anti-inflammatory, antiviral and antipyretic, the azomethine group being present in many natural and unnatural compounds (9). The interest on imines becomes even more pronounced in view of their easy way of synthesis and wide applicability and the presence of an antioxidant property. In order to develop new therapeutic agents derived from the amino acid L-Lysine with antioxidant effects new Schiff bases were synthetized and evaluated for their antiradical properties.

MATERIAL AND METHODS

The aromatic aldehydes (3-hydroxy/4-methyl-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde and benzaldehyde), organic solvents (*p.a.* quality) and the standard reagents used for the antioxidant tests were purchased from Sigma Aldrich Company and Fluka Company and H-Lys-(Boc)-OMe hydrochloride was purchased from Alfa Aesar Company. All solvents and reagents were used without prior purification. For monitoring the reactions TLC silica gel 60 F_{254} plates from Merck Company were used.

Chemistry

Synthesis of Schiff base. The synthesis of azomethine derivatives consists in the condensation of H-Lys (Boc)-OMe·HCl with various aromatic aldehydes (3-hydroxy/4-methyl-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde and benzaldehyde) which results in the corresponding Schiff bases (fig. 1).

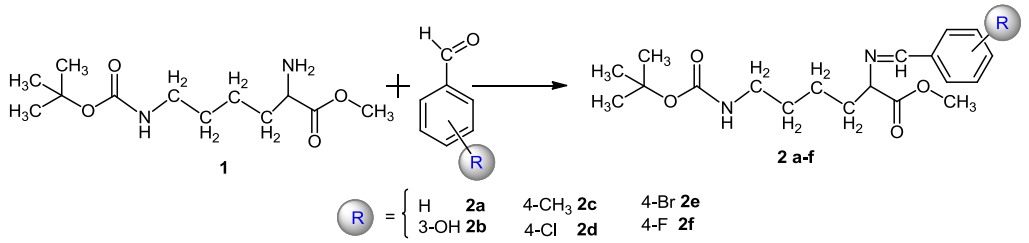

Fig. 1. Scheme of the microwave induced synthesis of lysine Schiff bases

General procedure for the synthesis of lysine Schiff bases:

The microwave induced synthesis of the products started with the suspension in a 5 ml microwave vial of 1 eq. of H-Lys (Boc)-OMe·HCl (MW = 296.79 g/mol) in 2 ml freshly distilled tetrahydrofuran and then 4 eq. of Na_2CO_3 (MW =105.99 g/mol) and 5 eq. of $MgSO_4$ (MW = 120.366 g/mol) were added under continuous stirring. Finally 1.05 eq. of different aromatic aldehydes (3-hydroxy/4-methyl-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde and benzaldehyde) were added and then the vial was put into microwave reactor at 100^0C for 30 min (10, 11). The reaction was monitored by TLC, using as solvent system a mixture of dichloromethane: methanol (8:2). The unreacted benzaldehyde was removed by washing with diethyl ether and petroleum ether. At the end of the reaction, the solution was concentrated in order to remove the tetrahydrofuran.

Characterization by spectral methods. IR spectra of the synthesized compounds were recorded using a Fourier transform spectrometer, ABB-MB 3000 FT-IR MIRacle^{TM} Single Bounce ATR-crystal ZnSe,

and the studied spectral range was in the range of 4000-500 cm $^{-1}$, at a resolution of 4 cm^{-1}, 16 scans being carried out for each sample. The spectra were interpreted using Horizont program MB^{TM} FT - IR. ^{1}H-NMR spectra were registered using NMR Bruker 400 MHz spectrometer (Germany).

Antioxidant assays

DPPH radical scavenging activity. In order to evaluate the antiradical capacity against DPPH radical the samples were dissolved in DMSO to obtain a stock solution concentration of 20 mg/mL. From the stock solution there were taken different volumes (50 µL, 100 µL, 150 µL, 200 µL) which were diluted with methanol in order to obtain 200 µL. The next step consisted in adding 2800 µL methanolic solution of DPPH (0.1 mM, A_{517nm}=1.0±0.05) and the mixture was stirred and let to stand for 30 minutes in the dark, after which the absorbance was read at 517 nm against methanol used as a blank (12, 13) .

Antiradical capacity was calculated as an inhibition percentage (I%) using the formula: I% = ($A_0 - A_s$ / A_0) x 100 wherein, A_0 = the absorbance value of the 0.1 mM DPPH methanolic solution; A_s = the absorbance value of the sample, read at 30 minutes after adding the methanol solution of DPPH. For each compound the effective concentration 50 (EC_{50}) was calculated by linear regression, and vitamin E (20 mg/mL) was used as a positive control. All experiments were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD).

ABTS radical scavenging activity. The generation of the $ABTS^{\cdot+}$ cation radical was performed by treating the aqueous solution of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (7 mM) with potassium persulfate (2.45 mM). The resulting mixture was kept in the dark for 12 to 16 hours to promote the formation of $ABTS^{\cdot+}$ radical, resulting the stock solution. The stock solution of $ABTS^{\cdot+}$ was diluted with ethyl alcohol to obtain a solution with an absorbance of 0.700 ± 0.020 at a wavelength of 734 nm (14).

The samples were dissolved in DMSO to obtain a stock solution with a concentration of 20 mg/mL. From the stock solution different volumes (10 µL, 15 µL, 20 µL, 25 µL) were taken and then diluted with DMSO up to the volume of 25 µL to which 1975 µL of $ABTS^{\cdot+}$ solution were added. After 6 minutes the absorbance of the samples was read at λ = 734 nm in reference to the blank (ethyl alcohol).

The antioxidant activity was expressed as percentage of inhibition (I%) of the $ABTS^{\cdot+}$ radical cation by the following formula: I% = ($A_0 - A_s$ / A_0) x 100 wherein, A_0 = the absorbance value of the ethanolic solution of $ABTS^{\cdot+}$; A_s = the absorbance value of the sample, read at 6 minutes after the addition of $ABTS^{\cdot+}$ solution. For each compound the effective concentration 50 (EC_{50}) was calculated by linear regression, and vitamin E (20 mg/mL) was used as a positive control. All experiments were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD).

RESULTS AND DISCUSSION

Physicochemical and spectral characterization

The Schiff bases of lysine are liquids or glues colored in different shades which vary from colorless to bright brown, very slightly soluble in acetone, ethanol, methanol, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), partially soluble in chloroform, dioxane, and insoluble in distilled water, diethyl ether and petroleum ether. The physicochemical and spectral

characteristics are listed in Table I. The presence of the absorption band characteristic to azomethine bound (-CH=N-) and the appearance of the signal of the proton from the same chemical bound confirms the structure of the synthesized compounds.

TABLE I

Physicochemical and spectral characteristics of lysine Schiff bases

Comp.	R	Physicochemical characteristics		Spectral characteristics	
		Mol. weight	*Yield (%)*	*IR characteristic band (cm^{-1})*	*^{1}H-NMR (250 MHz, CD_3OD, δ ppm)*
2a	H	348.20	85.55	3373 (-NH-), 2978 (CH_{Ar}), 1682 (-CO-NH), 1595 (CH=N)	8.32 (s, 1H, C<u>H</u>=N), 7.75-7.82 (m, 2H, Ar-H), 7.40-7.48 (m, 3H, Ar-H), 3.92-3.97 (m, 1H, C<u>H</u>-$COOCH_3$), 3.72 (s, 3H, $COOC\underline{H_3}$) 2.96 (s, 2H, $C\underline{H_2}$), 1.87-1.80 (m, 2H, $C\underline{H_2}$), 1.54-1.42 (m, 2H, $C\underline{H_2}$), 1.31 (s, 9H, $C(\underline{CH_3})_3$), 1.07-0.98 (m, 2H, $C\underline{H_2}$)
2b	3-OH	364.20	96.43	3251 (-NH), 2976 (-OH), 2947 (CH_{Ar}), 1641 (-CO-NH-), 1597 (-CH=N)	8.26 (s, 1H, C<u>H</u>=N), 7.02-7.17 (m, 4H, Ar-H), 3.92-3.98 (s, 1H, C<u>H</u>-$COOCH_3$), 3,70 (s, 3H, $COOC\underline{H_3}$) 2.98 (t, 2H, $C\underline{H_2}$), 1,75-1.84 (m, 2H, $C\underline{H_2}$), 1.57-1.39 (m, 2H, $C\underline{H_2}$), 1.35 (s, 9H, $C(\underline{CH_3})_3$), 1.26- 1.18 (m, 2H, $C\underline{H_2}$)
2c	4-CH_3	362.22	94.30	3362 (-NH-), 3003 (CH_{Ar}), 1595 (-CO-NH-), 1551 (-CH=N-)	8.26 (s, 1H, C<u>H</u>=N), 7.65 (d, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 3.92-3.98 (m, 1H, C<u>H</u>-$COOCH_3$), 3.70 (s, 3H, $COOC\underline{H_3}$) 2.98 (t, 2H, $C\underline{H_2}$), 2.33 (s, 3H, $C\underline{H_3}$-Ar), 1.80-1.77 (m, 2H, $C\underline{H_2}$), 1.57-1.39 (m, 2H, $C\underline{H_2}$), 1.35 (s, 9H, $C(\underline{CH_3})_3$), 1.30- 0.98 (m, 2H, $C\underline{H_2}$)
2d	4-Cl	382.17	87.80	3373 (-NH-), 2949 (CH_{Ar}), 1647 (-CO-NH-), 1594 (-CH=N-), 813 (C-Cl)	8.30 (s, 1H, C<u>H</u>=N), 7.75 (d, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 3.91-4,02 (m, 1H, C<u>H</u>-$COOCH_3$), 3.69 (s, 3H, $COOC\underline{H_3}$) 2.98 (t, 2H, $C\underline{H_2}$), 1,77-1.80 (m, 2H, $C\underline{H_2}$), 1.54-1.39 (m, 2H, $C\underline{H_2}$), 1.35 (s, 9H, $C(\underline{CH_3})_3$), 1.30-0.98 (m, 2H, $C\underline{H_2}$)
2e	4-Br	426.12	94.47	3362 (-NH-), 2976 (CH_{Ar}), 1643 (-CO-NH-), 1589 (-CH=N-), 864 (C-Br)	8.29 (s, 1H, C<u>H</u>=N), 7.70-7.63 (m, 2H, Ar-H), 7.61-7.52 (m, 2H, Ar-H), 3.91-4.03 (m, 1H, C<u>H</u>-$COOCH_3$), 3.69 (s, 3H, $COOC\underline{H_3}$) 2.98 (t, 2H, $C\underline{H_2}$), 1,72-1.85 (m, 2H, $C\underline{H_2}$), 1.41-1.39 (m, 2H, $C\underline{H_2}$), 1.35 (s, 9H, $C(\underline{CH_3})_3$), 1.15-1.26 (m, 2H, $C\underline{H_2}$)
2f	4-F	366.20	88.38	3373 (-NH-), 2951 (CH_{Ar}), 1595 (-CH=N-), 1095 (C-F)	8.31 (s, 1H, C<u>H</u>=N), 7.79-7.85 (m, 2H, Ar-H), 7.12-7.18 (m, 2H, Ar-H), 3.97-4.03 (m, 1H, C<u>H</u>-$COOCH_3$), 3.70 (s, 3H, $COOC\underline{H_3}$) 2.94 (t, 2H, $C\underline{H_2}$), 1.86-1.78 (m, 2H, $C\underline{H_2}$), 1.53-1.40 (m, 2H, $C\underline{H_2}$), 1.30 (s, 9H, $C(\underline{CH_3})_3$), 1.21-1.10 (m, 2H, $C\underline{H_2}$)

Antioxidant activity

DPPH radical scavenging activity. The method was based on spectrophotometric determination of the capacity of inactivating the stable free radical DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl), purple, by addition of hydrogen with the formation of 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazine, yellow. After analyzing the data from Table II and fig. 2 we can say that the azomethine derivatives resulted from the condensation of H-Lys(Boc)-OMe·HCl with 3-OH-benzaldehyde (2b) were found to be 1.33 times more active than those of H-Lys(Boc)-OMe·HCl. As to the influence of substitution on the aro-

matic ring it was found that the radicals 4-CH_3 (2c), 4-Cl (2d), 4-Br (2e) and 4-F (2f) provided action comparable to that of the starting material. The compounds studied were, however, less active than vitamin E used as positive control.

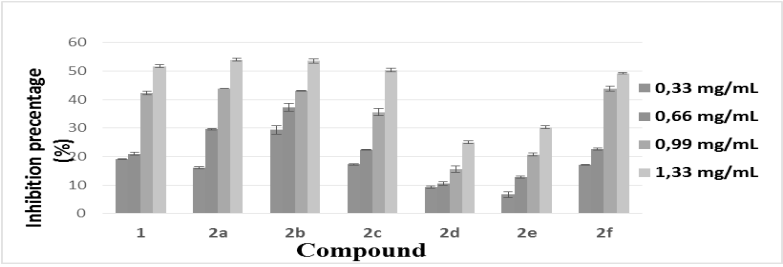

Fig. 2. The inhibition (%) of DPPH radical for 2a-f compounds at different concentrations

TABLE II

EC_{50} values (mg/mL) for the antiradical capacity against DPPH radical of derivatives 2a-f

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
H-Lys(Boc)-OMe·HCl	0.722 ± 0.02	**2d**	2.074 ± 0.02
2a	0.747 ± 0.06	**2e**	2.243 ± 0.03
2b	0.642 ± 0.02	**2f**	1.360 ± 0.14
2c	1.012 ± 0.01	**Vitamin E**	0,062 ± 0.023

* EC_{50} values are the average of three determinations ± standard deviation

ABTS radical scavenging activity. The antioxidants which can reduce the radical cation $ABTS^{\cdot+}$ by electron transfer mechanism are visible by the color variation from blue to yellow. From the results listed in Table III and fig. 3 it was noticed that compound **2b** was the most active synthetized product, being 2.85 times more active than H-Lys (Boc)-OMe·HCl. The azomethine derivatives have an action comparable to that of the starting material and the antiradical capacity against $ABTS^{\cdot+}$ cation radical is directly proportional to the concentration of the respective sample. In comparison with vitamin E used as positive control the studied compounds were less active.

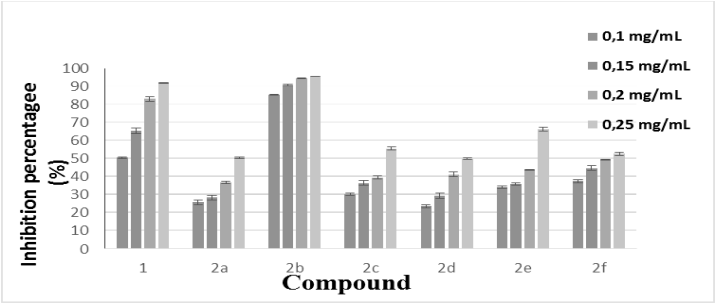

Fig. 3. The inhibition (%) of $ABTS^{\cdot+}$ cation radical for 2a-f compounds at different concentrations

TABLE III

EC_{50} values (µg/mL) for the antiradical capacity against $ABTS^{\cdot+}$ cation radical of derivatives 2a-f

Compound	EC_{50} µg/mL	Compound	EC_{50} µg/mL
H-Lys(Boc)-OMe·HCl	17.24 ± 0.01	**2d**	11.153 ± 0.07
2a	9.126 ± 0.12	**2e**	7.391 ± 0.01
2b	6.034 ± 0.03	**2f**	4.543 ± 0.11
2c	8.243 ± 0.05	**Vitamin E**	5.51 ± 0.21

* EC_{50} values are the average of three determinations ± standard deviation

CONCLUSIONS

In this study new derivatives of amino-acid lysine have been synthesized using microwave and the optimal reaction conditions for obtaining compounds in high yields and with high purity have been established. The synthesized compounds were characterized by their physical constants (yield, molecular formula, molecular weight, solubility in different organic solvents) and their chemical structure was confirmed using spectral methods such as: FT-IR spectroscopy and ^{1}H-NMR. The antioxidant potential was assessed by *in vitro* methods. Most of the studied compounds showed an improved antiradical capacity against $ABTS^{\cdot+}$ radical cation and DPPH radical compared to the starting material, demonstrating a favorable impact of structural modulation on the antioxidant effects of lysine.

ACKNOWLEDGMENTS

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NEWS

HYALURONIC ACID AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS

The aim of this study was to evaluate the adjunctive effect of local application of hyaluronon gel following scaling and root planning in patients with chronic periodontitis. In this randomized split mouth study 33 subjects with chronic periodontitis were evaluated after full mouth scaling and root planning. In the test sites hyaluronon gel was applied immediately after scaling and root planning and one week post-therapy, the control sites were treated with scaling and root planning alone. Bleeding on probing, probing pocket depth, and clinical attachment level were recorded at baseline, 4 and 12 weeks. The results showed a significant reduction in bleeding on probing scores in both the groups at 12 weeks ($p<0.001$). The hyaluronon group showed a greater reduction in bleeding on probing ($p<0.001$). In the hyaluronon group the mean probing pocket depth at baseline was 6.33 ± 0.09 which reduced to 2.49 ± 0.51 at 12 weeks which was statistically significant ($p<0.001$). In the control group the mean probing pocket depth at baseline was 6.09 ± 1.26 which reduced to 4.36 ± 1.29 at 12 weeks which was statistically significant ($p<0.001$). The clinical attachment level measurements showed a significant difference between the groups ($p<0.001$) at 12 weeks post therapy. The conclusion of this study showed that the hyaluronic acid has a beneficial effect on periodontal health in patients with chronic periodontitis. Hyaluronic acid appears to be a suitable candidate as an adjunct to scaling and root planning in chronic periodontitis patients (Rajan P, Baramappa R, Rao NM, Pavaluri AK, PI, Rahaman SM. Hyaluronic Acid as an adjunct to scaling and root planing in chronic periodontitis. A randomized clinical trail. *J Clin Diagn Res* 2014; 8(12): 11-14).

Irina Gradinaru

MULTI-ANALYTIC DETECTION OF ANTIBIOTIC RESIDUES IN HONEY USING A MULTIPLEXING BIOCHIP ASSAY

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MULTI-ANALYTIC DETECTION OF ANTIBIOTIC RESIDUES IN HONEY USING A MULTIPLEXING BIOCHIP ASSAY (Abstract): Contamination of honey may occur through the common use of antibiotics and chemotherapeutics by certain beekeepers to cure or to prevent bacterial or other bee diseases. In order to protect human health the use of antimicrobials in apiculture is usually strictly regulated or banned. LC/MS/MS is currently regarded the tool of choice for analysis of drug residues in animal-derived food. Screening methods (immunoassays and biosensors) are also required and are being developed. **Aim**: The present paper reviews one of the most recent methods for analysis of drug residues in honey and also the abridged method validation according to the criteria set by Commission Decision 2002/657/EC for simultaneous determination of drug residues in honey samples using a test panel which screens for quinolones (QNL), ceftiofur (CEFT), thiamphenicol (TAF), streptomycin (STR), tylosin (TYL) and tetracyclines (TCN). **Material and methods:** A chemiluminescence - based biochip array sensing technique has been applied. Using a multiplex approach, metabolites of CEFT, TAF, QNL, STR, TCN and TYL were simultaneously detected in honey samples. **Results and discussion:** The simultaneous immunoassays showed specificity for the target analytes. The limits of detection were between 1 µg/kg for TAF and 9 *µg/*kg for TCN. Good linearity was obtained, with determination coefficients higher than 0.980. The decision limit (CCα) and the capability of detection (CCβ) were well below established Reference Point for Action (RPA). Method applicability was demonstrated through application to various kinds of honey. **Conclusions:** Biochip array technology allows simultaneous and selective multi-analytic measurement of antimicrobials in honey at levels well below established Reference Point for Action (RPA). The performance of biochip screening method was comparable to that achieved for the confirmatory LC/MS/MS method. **Key words:** HONEY, QUINOLONES, TETRACYCLINES, DRUG RESIDUES, BIOSENSOR.

In apiculture, antibiotics are mainly used for the treatment of bacterial brood diseases, such as American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Streptococcus pluton*). Oxytetracycline was the first antibiotic to be used for the control of American and European foulbrood, but other chemotherapics, such as sulfonamides, macrolides and tylosin, are also used to prevent these diseases (1). In general, the presence of drug residues in honey is banned. Therefore, it is necessary to develop sensitive analytical methods with low limits of detection (LODs) in

order to detect traces of antibiotics in honey. Some surveys show that positive samples can be contaminated by more than one class of antibiotics (2). The development and use of new sensitive multiclass methods are necessary for the simultaneous determination of antibiotics at trace levels in this matrix.

The aim of the present work was the optimization and revalidation of a sensitive method for the simultaneous determination of veterinary drug residues belonging to several classes including CEFT, TAF, QNL, STR, TCN and TYL. The proposed method allows simultaneously screening of parent molecule and major metabolites in honey samples and detects veterinary drugs at low concentration levels (<4 μg/kg), indicating that it can be applied in routine analyses to provide a large amount of data related to the presence of drug residues in honey. The assay was validated according to 2002/657/EC criteria (3). In addition, it was comprehensively evaluated through application to 43 honey samples from diverse geographical regions of Romania, which were also characterized by an accredited LC/MS/MS assay (4). The combined approach allows an effective means of satisfying EU quality criteria specified for screening and confirmatory techniques (2003/181/EC & Regulation (EC) No. 470 /2009).

MATERIAL AND METHODS

Apparatus

A biosensor system, Randox Evidence Investigator™ analyzer (Randox Laboratories Ltd., Crumlin, Co. Antrim) was used for the simultaneous quantitative detection of multiple immunoassays from a single sample (5). The core technology is the biochip, a solid substrate containing discrete test regions (DTRs) of immobilized antibodies specific to different antimicrobials (6).

Reagents

Reagents, were supplied by Randox Laboratories Ltd. (Co. Antrim, Northern Ireland). The *Anti Microbial Array II (EV3524)* was used according to manufacturer's instructions to screen for 6 different types of antibiotics including ceftiofur (CEFT), florphenicol/thiamphenicol (TAF), quinolones (QNL), streptomycin/dihydrostreptomycin (STR), tetracyclines (TCN), tylosin/tilmicosin (TYL). The biochip array allows simultaneously screening of different families of compounds including the parent molecule and major metabolites.

Sample preparation

All honey samples were sourced from retail outlets in Romania. Honey samples determined by LC/MS/MS to be free of drug residues were used as negative controls. Several varieties of honey were examined in the survey. The sample was prepared using a rapid dilution method: weighing 1 g of honey, addition of 9 ml of diluted wash buffer (warmed to 37°C) to ≈1g of a honey sample, rolling for 10 minutes, and dilution with an equal amount of diluted wash buffer, filtration and application on the biochip. To take into account the dilution effect of preparation, the concentration reported were manually multiplied by 20.

Method validation

We evaluated the performance of the biochip method which has been initially validated a few years ago and is qualified to monitor CEFT, TAF, QNL, STR, TCN and TYL residues in honey. The abridged validation procedure applied here has the scope to demonstrate that the method works reliably in our laboratory, is fit for purpose and also follow the European De-

cision 657/2002/EC recommendation to calculate two statistical limits, CCα and CCβ, which allows to evaluate the critical concentrations above which the method reliably distinguish and quantify a substance taking into account the variability of the method and the statistical risk to take a wrong decision. For multi-class methods using biochemical tests the detection capability has to be determined for the representative analyte or the analyte with the lowest cross-reactivity (7).

The analytes chosen for our abridged validation were: ceftiofur, norfloxacin, ciprofloxacin, streptomycin, chlortetracycline, thiamphenicol and tylosin. For this particular biochip binding assay, calibrators are supplied with reagents, and the calibration curves are subsequently generated via the included software. However, given the complicated nature of honey as a sample matrix, all analyses were run alongside matrix-matched calibrants (a honey determined to be free of drug residues via LC/MS/MS, spiked at the appropriate levels). Linearity was then evaluated by spiking blank honey samples with different concentrations of antibiotics from 3 to 10 *μg/kg*. The calibration curves were generated with each of the analytes as standards in the competitive immunoassay. Once the response of these calibrators (measured in Relative Light Units, RLUs) had been determined, a calibration curve was constructed, applying the 4-parameter logistic model

Y= D + ((A-D) / (1+(x/C)^B))

where Y is the response generated, x is the concentration of the analyte, A is the response at zero analyte concentration, B is a slope factor, C represents the inflection point of the calibration curve, and D is the response at infinite analyte concentration.

Precision and accuracy of the method were studied spiking blank samples. Precision of the overall method was studied by performing repeatability (intraday precision) and inter-day precision experiments. Repeatability (intraday precision) was performed spiking blank honey at three concentration levels (10, 20 and 30 μg/kg), using twenty replicates for each concentration level in one day. To evaluate inter-day precision (reproducibility), the same concentration levels were studied, spiking blank honey during five consecutive days. Recovery was studied by analyzing blank samples that were fortified at three concentration levels (10, 20 and 30 μg/kg). Accuracy was estimated through recovery studies.

Decision limit and detection capability

The decision limit (CCα) is defined as the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. The capability of detection (CCβ) is defined as the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β (5% for all substances with permitted or no permitted limit) (3, 7). The Regulatory Limit for unauthorized analytes is the Minimum Required Performance Limit (MRPL) or the Reference Point for Action (RPA). The Screening Target Concentration must be at or less than the MRPL (or RPA). For banned and unauthorized compounds where no MRPL has yet been established, CCβ should be as low as reasonably achievable. Methods for screening should be able to detect the analyte at the CCβ in 95% of the cases (7). For analized drug residues the RPA was stated at 20 μg/kg. This is the reason why we set the Desired Screening Target Concentration =

10 μg/kg. In order to determin the decision limit and detection capability, 20 blank matrix samples were selected. Replicates of these samples were spiked at the Screening Target Concentration = 10 μg/kg. The matrix blank samples and spiked samples were analysed. The decision limits (CCα, where α = 0.05) were calculated from the mean concentration of the 20 spiked samples at Desired Screening Target Concentration plus 1.64 times the corresponding standard deviations. The CCβ was obtained adding to CCα 1.64 times the same standard deviation.

The confirmatory method meet the requirements of Decision 2002/657/EC concerning the performance of method for the determination and confirmation of CEFT, TAF, QNL, STR, TCN and TYL residues in honey samples. Analysis were performed on an Agilent 1100 LC system (Agilent Technologies, USA) coupled to a 4000 Q TRAP mass spectrometer instrument (Applied Biosystems, USA). The following criteria were used for the confirmatory method: the signal-to-noise ratio (S/N) of the characteristic ions selected were > 3, the differentiation of the retention time of analyte and corresponding standard were within ±2.5%, the deviation of the relative abundance of the characteristic ions of the target matter and that of the characteristic ions of the corresponding standard were within ±20% to ±50%.

RESULTS AND DISCUSSION

Good linearity was obtained and determination coefficients were higher than 0.980 (tab. I). Satisfactory results were found, with recoveries higher than 70% for all the assayed compounds at the three concentration levels. Precision of the overall method was studied by performing repeatability (intraday precision) and interday precision experiments. Three concentration levels of the analytes were used and the intra-assay precision was determined from the results of twenty replicates of each level within the same run. The precision expressed as %CV and was always lower than 10% (table I).

The simultaneous immunoassays showed specificity for the target analytes. The limits of detection (LODs) ranged from 1 $\mu g/kg$ for TAF to 9 $\mu g/kg$ for TCN. Biochip array technology allowes simultaneous and selective multianalyte measurement of antimicrobials in honey at levels well below established Reference Point for Action (RPA) (tab. II). To validate the feasibility of the proposed method further, 43 honey samples of different types, from different geographical regions of Romania were purchased from local markets and analyzed. All 43 honey samples were tested and 9 were found positive for drug residues.

TABLE I

Analytical performance of Antimicrobial Array II

Assay	Calibration Range (µg/kg)	Determination coefficient R^2	LOD Honey (µg/kg)	Intra Assay Precision (n=20)
QNL	0 - 10	0.982	5	≤6.8 %
CEFT	0 - 7.5	0.986	3	≤6.8 %
TAF	0 - 5	0.998	1	≤6.8 %
STR	0 – 76	0.987	8	≤4.6 %
TYL	0 - 5	0.991	2	≤5.0 %
TCN	0 - 4.5	0.994	9	≤7.6 %

TABLE II

Decision limit (CCα) and detection capability (CC*β*)

Analyte	Mean conc. Spiked samples (µg/kg) (n = 20)	SD (spiked samples)	1.64*SD	CCα (µg/kg)	CC*β* (*µ*g/kg)
Ceftiofur	10.34	1.29	2.12	12.46	14.57
Norfloxacin	10.76	1.44	2.36	13.12	15.48
Ciprofloxacin	10.85	1.33	2.18	13.03	15.21
Streptomycin	11.35	1.77	2.90	14.25	17.16
Chlortetracycline	11.17	1.43	2.35	13.52	15.86
Thiamphenicol	9.80	1.31	2.15	11.95	14.10
Tylosin	12.03	1.75	2.87	14.90	17.77

TABLE III

Honey samples screening with Biochip Array and confirmation by LC/MS/MS

Sample No.	*Method*	*QNL (µg/kg)*	*CEFT (µg/kg)*	*TAF (µg/kg)*	*STR (µg/kg)*	*TYL (µg/kg)*	*TCN (µg/kg)*
1	Biochip	< LOD	< LOD	< LOD	< LOD	3.4	< LOD
	LC/MS/MS	< LOD	< LOD	< LOD	< LOD	6	< LOD
2	Biochip	10.4	< LOD	< LOD	29	< LOD	< LOD
	LC/MS/MS	Ciprofloxacin 5µg/kg, Norfloxacin 7µg/kg	< LOD	< LOD	22	< LOD	< LOD
3	Biochip	54.9	< LOD	< LOD	34.9	< LOD	< LOD
	LC/MS/MS	Ciprofloxacin 41µg/kg, Norfloxacin 10µg/kg	< LOD	< LOD	28	< LOD	< LOD
4	Biochip	12.7	< LOD	< LOD	< LOD	< LOD	10.7
	LC/MS/MS	Ciprofloxacin 4µg/kg, Norfloxacin 2µg/kg	< LOD	< LOD	< LOD	< LOD	Chlor-tetracycline 8µg/kg
5	Biochip	11.4	< LOD	< LOD	26.6	< LOD	< LOD
	LC/MS/MS	Ciprofloxacin 7 µg/kg, Norfloxacin 3µg/kg	< LOD	< LOD	19	< LOD	< LOD
6	Biochip	< LOD	< LOD	< LOD	17.8	< LOD	< LOD
	LC/MS/MS	< LOD	< LOD	< LOD	18	< LOD	< LOD
7	Biochip	9.4	< LOD	< LOD	< LOD	< LOD	< LOD
	LC/MS/MS	Ciprofloxacin 6µg/kg, Norfloxacin 4µg/kg	< LOD	< LOD	< LOD	< LOD	< LOD
8	Biochip	24.6	< LOD	< LOD	13.4	< LOD	< LOD
	LC/MS/MS	Ciprofloxacin 3µg/kg, Norfloxacin 15µg/kg	< LOD	< LOD	11	< LOD	< LOD
9	Biochip	< LOD	< LOD	< LOD	< LOD	1.9	< LOD
	LC/MS/MS	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

The presence of QNL, CEFT, TAF, STR, TYL and TCN were detected with the Antimicrobial Array II and the corresponding sample was confirmed by LC/MS/MS. The results showed that all samples which showed higher levels of drug residues in the examined honey were also positive in LC/MS/MS analyses (tab. III). Only one sample was positive for tylosin on Biochip Array and found to be negative with LC/MS/MS. For the tylosin in honey at low concentrations, Biochip method was able to determine precisely but it was not detected by LC/MS/MS. This result indicated that the sensitivity of this method is much higher than LC/MS/MS. No matrix interferences produced by endogenous compounds were observed for these analytes.

CONCLUSIONS

The method shows good linearity, sensitivity, specificity, precision (repeatability and intra-laboratory reproducibility), recovery, decision limits (CCα) and detection capabilities (CCβ) below 50% of established Reference Point for Action (RPA). The results of the validation process demonstrate that the method is suitable for application, as a quantitative screening method, in European Union statutory veterinary drug residue surveillance programmes. This biosensor method possesses the potential to be a fit-for-purpose screening technique in the arena of food safety technology. Following comparison with results obtained via the confirmatory technique (LC/MS/MS), the biochip array sensing technique has proven to be suitable for the rapid, simultaneous screening of the antibiotics in honey. This reduces the requirement for valuable LC/MS/MS time in the laboratory, as samples can be screened via this method, with suspected positives put forward to confirmatory testing. Use of microarray sensing techniques may hold much potential for the area of residue determination.

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DETERMINAREA CONCENTRAȚIEI METANOLULUI PRIN GC-MS ÎNTR-UN CAZ DE INTOXICAȚIE LETALĂ

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DETERMINATION OF METHANOL CONCENTRATION BY GC-MS IN A FATAL CASE POISONING (Abstract): Due to the consumption of alcoholic beverages with methanol content above the admitted limit, a woman from Bacău died at the Bacău County Hospital after 9 hours of hospitalization. **Materials and methods**: For the toxicological examination blood, liver, bile, stomach, intestine and kidney samples were analyzed in order to establish the concentration of methanol. Determination of methanol was carried out by gas chromatography coupled with mass spectrometry (GC-MS), analyzing the distillates obtained from blood and organs. We used an Agilent Technologies 7890 A gas chromatograph coupled to an Agilent Technologies 7683 C spectrometer. Helium was employed as mobile phase and a Phenomenex Zebron ZW-Plus polar column represented the stationary phase. Spectra acquisition mode: SIM, mass range followed: 15-500 atomic mass units, interpretation of the obtained results: Agilent Technologies ChemStation software. **Results**: After peak integration on the chromatograms, the following values of methanol concentration were obtained: 1.720 g ‰ in blood, 0.795 g ‰ in liver, 2.661 g‰ in bile, 2.287 g ‰ in stomach, 1.639 g ‰ in intestine and 1.590 g ‰ in kidney; the results have proved a large quantity of ingested methanol. They have also highlighted the absorption and distribution pattern of the methanol within the body. **Key words:** METHANOL, INTOXICATION, GC-MS.

Intoxicațiile metanolice se produc în mod frecvent prin confuzie cu alcoolul etilic, sau prin consumul de băuturi alcoolice ce au o concentrație metanolică peste limita admisă (1g/100 mL etanol anhidru) (1). Metanolul se metabolizează prin oxidare în ficat sub acțiunea alcool dehidrogenazei (AHD), în formaldehidă (lent) şi respectiv la acid formic (rapid) şi metil formiat (2, 3). Simptomele caracteristice intoxicațiilor metanolice sunt tulburările de vedere care apar la 10–15 ore de la ingestie şi acidoza metabolică (3, 4, 5). Intoxicația metanolică este, de cele mai multe ori, mascată de intoxicația etilică, acest fapt ducând la dificultăți de diagnostic (4, 5). În lucrarea de față se propune evidențierea distribuției şi absorbției metanolului în organism față de variația concentrației metanolice determinată prin cromatografie de gaze cuplată cu spectrometria de masă (GC-MS), în cazul unei morți prin intoxi-

caţie cu alcool metilic provenit din băuturi alcoolice.

PREZENTARE DE CAZ

O femeie de 32 de ani este internată în secţia Urgente a Spitalului Judeţean Bacău în data de 21.02.2010 cu diagnosticul "Coma gradul III, şoc toxic în observaţie" cu simptomatologia caracteristică. Examenele complementare respectiv, examenul biochimic al sângelui relevă: amilază -117 U/L, GGT–113 U/L, TGO–331 U/L şi TGP–147 U/L. Din datele de anchetă rezultă că femeia era o consumatoare cronică de băuturi alcoolice. Starea gravă a pacientei, cât şi lipsa de informaţii privind toxicul care a fost ingerat pe fondul consumului de băuturi alcoolice, a dus la o diagnosticare dificilă. Evoluţia a fost gravă cu deprimare respiratorie; pacienta moare prin stop cardio-respirator după 9 ore de la internare. Raportul de necropsie a cadavrului pune în evidenţă: edem cerebral difuz, plămâni cu numeroşi noduli fibroşi cu conţinut cazeos ce evidenţiau leziuni bacilare vechi. Stomacul prezenta mucoasa cu numeroase zone punctiforme hemoragice. Ficatul cu volum mărit, steatozic, pe secţiune cu structura hepatică ştearsă, consistenţă dură de culoare galben violaceu, pancreas cu structură glandulară păstrată, splina şi rinichii prezentând fenomene de stază. Examenul anatomo-patologic microscopic efectuat în cadrul departamentului de Anatomie patologică al I.M.L. Iaşi pune în evidenţă la nivelul creierului discrete focare de necroze neuronale acute şi uşoară vacuolizare a substanţei albe. La nivel pulmonar s-au evidenţiat multiple abcese cazeoase de etiologie tuberculoasă şi hemoragii cu edem alveolar acut. Ficat cu marcate şi difuze leziuni steatozice macro şi microvacuolare asociate cu discretă colestază intracitoplasmatică, rare microfocare necrotico-inflamatorii cu celule mononucleate şi mici arii de fibroză sinusoidală sau pericelulară. Fragmentele de organe au fost prelucrate pentru examenul microscopic prin coloraţia hematoxilină-eozină, Ziehl-Nilssen şi prin coloraţia PAS. Concentraţia alcoolului etilic s-a determinat în cadrul Laboratorului de Toxicologie al S.M.L. Bacău, prin metoda oficială de chimie analitică Cordebard, obţinându-se o valoare de 1,90 g‰.

MATERIAL ŞI METODĂ

Determinarea concentraţiei metanolice se efectuează prin cromatografie de gaze cuplată cu spectrometrie de masă din sânge şi fragmente de organe respectiv, ficat, bilă, stomac, intestin şi rinichi.

Reactivi şi aparatură

Reactivi:

- apă distilată,

- acid succinic 5% (MERCK ISO 9001 Reg. 303.1.0 Lot K38065981).

Aparatură:

- cromatograf de gaze tip Agilent Technologies 7890 A prevăzut cu injector automat tip Agilent Technologies 7683 B Series, cuplat cu un spectrometru de masă tip Agilent Technologies 5975C inert MSD. Faza mobilă, heliul, cu un debit de 1 mL/minut. În urma consultării bibliografiei de specialitate (6, 7, 8) s-a optat pentru folosirea ca fază staţionară a unei coloane cromatografice Zebron – Phenomenex, ZB-WAXplus (60 m x 0,25 mm; 0,25 µm).

- balanţa analitică (AND HR 200), sticlărie de laborator, instalaţie de distilare.

Pregătirea probelor

Probele de sânge şi fragmentele de organe au fost prelucrate prin distilarea unei cantităţi de 5 ml sânge sau 5 g ţesut cu un volum de 45 ml acid succinic 5%, culegându-se un volum de 25 mL distilat. Disti-

latul obținut s-a concentrat prin redistilare la un volum de 15 mL distilat.

Condițiile de lucru pentru GC-MS:

S-a injectat un volum de 0,1 µl distilat cu o rată de splitare 1/50. Programul de temperatură: temperatura injectorului 250°C, temperatura sursei MSD 230°C, temperatura cvadrupolului MSD 150°C; temperatura în compartimentul coloanei a fost menținută constantă timp de 20 minute la 50°C, apoi temperatura a crescut cu 10°C/min până la 250°C unde a fost menținută constantă încă 5 minute. Modul de achiziție a spectrelor: SIM, domeniul de masă urmărit: 15–500 unități atomice de masă, interpretarea rezultatelor obținute: Agilent Technologies ChemStation software. După înregistrarea spectrelor de masă, metanolul a fost identificat prin compararea timpilor de retenție ai picurilor corespunzătoare din cromatogramele distilatelor, respectiv cromatograma unei probe etalon ce conține metanol. Metoda a fost validată conform normelor de validare, într-un studiu anterior (8, 9, 10).

REZULTATE ŞI DISCUȚII

Din cromatogramele obținute pentru probele analizate, după integrarea picurilor corespunzătoare metanolului, valorile ariilor au fost utilizate pentru calcularea concentrației în metanol (µg/ml sânge, respectiv g țesut), valori redate în tabelul I. S-a calculat, de asemenea, concentrația metanolului în g la 1000 ml sânge sau g țesut, cât şi valoarea medie a concentrației metanolice.

TABELUL I

Valorile concentrației metanolice din sânge şi organe determinate prin GC/MS

Nr.	Distilat	Concentrația metanolului		
		µg/mL sânge, sau g țesut	mg/mL sânge, sau g țesut	g/1000 mL sânge/g țesut
1	Sânge	1720,342	1,720	1,720
2	Ficat	795,506	0,795	0,795
3	Bilă	2661,306	2,661	2,661
4	Stomac	2287,104	2,287	2,287
5	Intestin	1639,867	1,639	1,639
6	Rinichi	1590,389	1,590	1,590
Valoare medie				1,78

Datele experimentale obținute pun în evidentă valorile crescute ale concentrațiilor metanolice atât în sânge, cât şi în organele cercetate. Astfel, în sânge, concentrația metanolului este de 1,720 g‰, în ficat de 0,795 g ‰, în bilă de 2,661 g ‰, în stomac 2,287 g ‰, în intestin de 1,639 g ‰, iar în rinichi de 1,590 g ‰.

Din figura 1 se constată că valoarea cea mai mare a concentrației metanolice s-a determinat în bilă 2,661 g‰, iar valoarea cea mai scăzută în ficat, respectiv 0,795 g‰. Probele din intestin şi rinichi au valori apropiate şi anume 1,639 g‰ în intestin şi 1,590 g‰ în rinichi. Valoarea medie a concentrației metanolice obținute a fost de 1,78 g‰, valoare apropiată cu cea obținută din sânge, intestin şi rinichi.

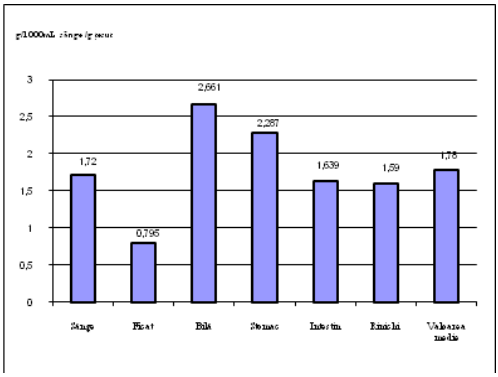

Fig. 1. Variaţia concentraţiei de metanol în sânge şi organe

Concentraţia metanolică în sânge, rinichi şi intestin reprezintă fiecare aproximativ 16% din concentraţia metanolică totală (valori apropiate de concentraţia medie). Ponderea cea mai mare se găseşte în bilă (24,88%), urmată de stomac (21,38%) (fig. 2).

DISCUŢII ŞI CONCLUZII

Faţă de datele din literatură, valorile concentraţiei metanolice obţinute în urma analizei probelor biologice se încadrează în intervalele valorice obţinute şi în alte cazuri de intoxicaţii letale cu metanol, respectiv intervalul 0,84–5,43g‰ metanol în sânge (11, 12).

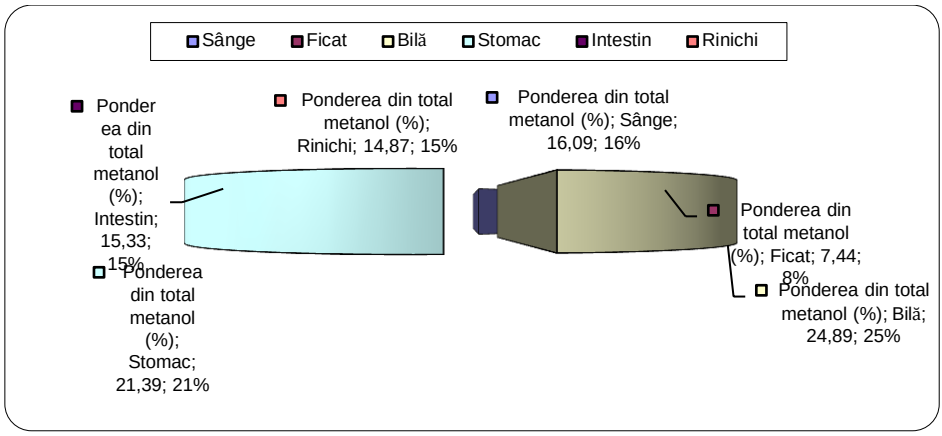

Fig. 2. Ponderea concentraţiei metanolice determinată în sânge şi organe

Datorită coeficientului lipo–hidrosolubilitatate, metanolul se distribuie în majoritatea organelor, cu precădere în organele cu concentraţii mari de apă (2, 3). În cazul de faţă, în bilă concentraţia metanolică este de trei ori mai mare faţă de concentraţia metanolică din ficat, unde s-a înregistrat cea mai scăzută valoare. Variaţia mare a valorilor concentraţiilor metanolice este explicată, în primul rând, de examenul anatomo-patologic. În cazul de faţă, steatoza hepatică cu degenerescenţă grasă diagnosticată la nivelul ficatului explică distribuţia neuniformă a toxicului. Valorile mari ale concentraţiei metanolice din stomac şi intestin pun în evidenţă faptul că metanolul a fost ingerat. Ştiind că rata metabolică a metanolului este de 25 mg/kg corp/h faţă de 175 mg/kg corp/h pentru alcool etilic, în cazul de faţă, decedata prezenta o alcoolemie de 1,90 g‰, rata de metabolizare a metanolului fiind extrem de scăzută, permiţând bioacumularea toxicului. Astfel, consumul excesiv de băuturi alcoolice coroborat cu tarele de sănătate (leziuni bacilare vechi, steatoză hepatică) şi, nu în ultimul rând, concen-

trația mult peste limită a metanolului din băuturile alcoolice consumate, au dus în cazul de față la deces. În urma studiilor întreprinse pe distilatele alcoolice naturale s-a constatat că 20% dintre acestea prezintă concentrații metanolice ce depășesc limitele admise (1g/100 ml etanol anhidru) (13), valoarea concentrației metanolice determinată în sânge și organe fiind fatală chiar și în cazul unui organism sănătos.

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PHARMACOKINETIC MODELING OF GLIMEPIRIDE PLASMA CONCENTRATION IN HEALTHY SUBJECTS

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PHARMACOKINETIC MODELING OF GLIMEPIRIDE PLASMA CONCENTRATION IN HEALTHY SUBJECTS (Abstract): **Aim**: To determine the pharmacokinetics of glimepiride, a sulfonylurea antidiabetic agent, after single dose administration in healthy subjects. **Material and methods** Pharmacokinetic data for modeling were extracted from a single-center, randomized, single-dose, fasting state, two–way crossover bioequivalence study on 4 mg glimepiride in 24 healthy subjects. **Results**: Plasma concentrations of glimepiride were measured using a validated LC/MS/MS method. The pharmacokinetic parameters were calculated using non-compartmental analysis. Different pharmacokinetic models were tested to evaluate pharmacokinetics of glimepiride. The optimal model was chosen based on Akaike's Information Criteria. **Conclusion**: Compartmental analysis demonstrated that oral glimepiride tablets obey one compartment open model with rapid absorption following a first order kinetics and a short half-life. **Key words:** GLIMEPIRIDE, AKAIKE CRITERIA, COMPARTMENT MODEL, PHARMACOKINETICS.

Glimepiride is the first representative of the third class of sulfonylurea antidiabetic agents. Glimepiride is frequently used in monotherapy or in combination with other glucose lowering agents in non–insulin dependent diabetes mellitus treatment. Its primary mechanism of action is stimulation of release of insulin from pancreatic beta cells (1). In addition, extrapancreatic effects (such as increasing sensitivity of peripheral tissues to insulin) play an important role in antidiabetic effect of glimepiride (2).

The present study is designed to carry out the pharmacokinetic modeling of glimepiride in plasma after the administration of a single oral dose to healthy subjects.

MATERIAL AND METHODS

Study Protocol

The study was approved by an Independent Ethics Committee and National Medicine Agency. The single–center, single–dose, randomized, open label, fasting state, 2-way cross–over study was performed in

accordance with Declaration of Helsinki (3) and Guideline of Good Clinical Practice (4). A signed informed consent was obtained from all participants before the study.

Selection of Subjects

Twenty-four healthy Caucasian adult male and female subjects were selected for the study according to general requirements for bioequivalence studies (5). Subjects with no history of chronic or acute disorders were selected for the study. Volunteers unable to provide consent, with abnormal vital sign measurement or laboratory testing for hepatitis B or C or HIV, with abnormal findings on electrocardiogram (ECG) or with history of significant use of alcohol and drugs were excluded.

Subjects were considered eligible for enrollment in the study based on medical and medication histories, demographic and clinical data, ECG, physical examination, clinical laboratory testing, serum pregnancy test and urinary drug of abuse screen. No medication was allowed two weeks before and through the study.

Study design

The study design was a 2-way cross over study. All subjects were fasted for at least 10 h prior to drug administration. At time zero, an intravenous cannula was inserted into forearm vein and blank blood samples were collected. After baseline blood sampling, one 4 mg glimepiride tablet of either generic or brand formulation was administered according to the randomization schedule. Standard meals were allowed 4 h, 8 h and 12 h after drug administration.

Blood samples for the determination of glimepiride concentrations were collected in EDTA tubes pre-dose and at 0.33; 0.66; 1.0; 1.33; 1.66; 2.0; 2.33; 2.66; 3.0; 3.5; 4.0; 6.0; 8.0; 10.0; 12.0; 16.0 and 24.0 h post-administration. Plasma was separated after centrifugation and frozen and stored at -25°C until analysis.

PK Sample Analysis

Glimepiride plasma concentrations were determined using a validated LC/MS/MS method. Gliclazide was used as internal standard (6).

Pharmacokinetic and statistical analysis

The primary non-compartmental pharmacokinetic parameters were maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under curve (AUC), mean residence time (MRT) and elimination rate constant (k_e). Both C_{max} and T_{max} were obtained from the plots of plasma concentration versus time. AUC was calculated using the trapezoidal method (log-linear). The k_e was obtained by linear regression of the terminal log linear phase of the concentration-time curve. The elimination half-life (t_{half}) was determined as $0.693/k_e$.

Both non-compartmental and compartmental pharmacokinetic data analyses were performed with the software program Kinetica® 5.0 (Thermo, Philadelphia) (7).

For compartmental analysis six mathematical compartmental models with first order absorption and elimination were used:

I. one-compartment, 0 order with lag time model
II. two-compartment, 0 order with lag time model
III. one-compartment, 1st order with lag time model
IV. one-compartment, 1st order without lag time model
V. two-compartment, 1st order with lag time model
VI. two-compartment, 1st order without lag time model.

The data were fit both to one compart-

ment and two compartment open models. The most appropriate model was chosen based on the Akaike information criteria (8).

The equations describing the compartments are:

$$C_p = C_1 e^{-k_d t} + C_2 e^{-ka t}$$

for one compartment model and

$$C_p = C_1 e^{-\alpha t} + C_2 e^{-\beta t} + C_3 e^{-ka t}$$

for two-compartment model, where C_p is the plasma concentration (ng/mL); k_d and β – disposition rate constants (h^{-1}); k_a is absorption rate constant (h^{-1}); α is distribution rate constant (h^{-1}); and C1, C2 and C3 are macrorate constants (9).

Discrimination between different pharmacokinetic models was performed for each study by minimizing the Akaike's information criterion test and Schwartz criteria and optimizing both the fitted versus the observed concentrations versus time and the weighted residuals versus the observed values relationship.

RESULTS

All data are presented as means ± standard deviation (SD) and CV%. In the study were enrolled 24 healthy Caucasian subjects with mean age 23.71 ± 3.96 years (range 19 - 32 years), mean height 1.75 ± 0.1 m (range 1.61 - 1.93 m), mean body weight 70.31 ± 12.33 kg (range 51 – 93 kg) and a mean BMI 22.56 ± 2.07 (range 19.00 – 25.0). All subjects completed the study and were included in analysis.

The mean plasma serum concentration vs. time curves after administration of the generic formulation of glimepiride is presented in fig. 1.

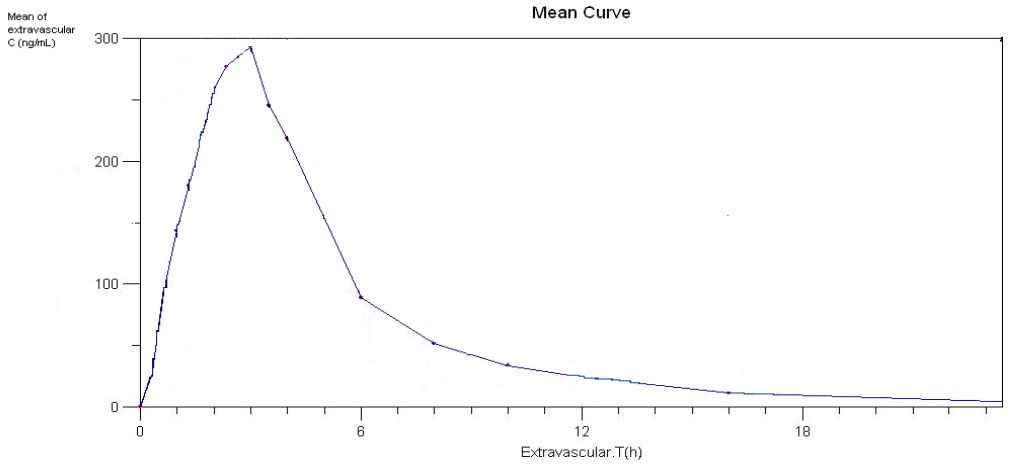

Fig. 1. Mean plasma concentration-time profile of glimepiride after a 4 mg single dose administration in healthy subjects (n = 24)

After single oral dose administration of 4 mg glimepiride, a C_{max} of 334.83 ng/ml was reached at 2.94 h. Elimination plasma half-life was 3.38 h. Pharmacokinetic parameters obtained by non-compartmental analysis are provided in table I.

The individual Akaike's values for the three analysed one-compartment models are presented in fig. 2. The main pharmacokinetic parameters calculated using this model are presented in table II.

TABLE I
Pharmacokinetic parameters of glimepiride by non-compartmental analysis

Pharmacokinetics parameter	Mean ± SD (CV%)
C_{max} (ng/ml)	334.83 ± 93.31 (27.87%)
T_{max} (hours)	2.94 ± 1.07 (36.34%)
AUC_{last} (ng*h/ml)	1449.69 ± 410.68 (28.33%)
T_{half} (hours)	3.38 ± 1.84 (54.46%)
MRT (hours)	5.56 ± 2.04 (36.75%)

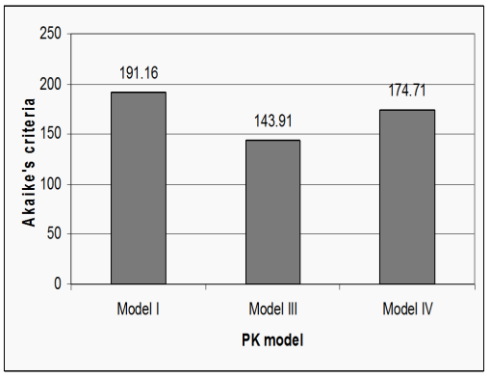

Fig. 2. Values of Akaike's index for one – compartment models

TABLE II
Pharmacokinetic parameters of glimepiride by compartmental analysis

Pharmacokinetic parameter	Mean ± SD (CV%)
$C_{maxcalc}$ (ng/ml)	290.99 ± 90.15 (30.98%)
$T_{maxcalc}$ (hours)	2.4 ± 0.59 (24.36%)
AUC (ng*h/ml)	1420.96 ± 401.96 (28.29%)
K_a (h^{-1})	0.74 ± 0.30 (41%)
Lag time	0.59 ± 0.42 (70.53%)
K_{el} (h^{-1})	0.48 ± 0.12 (25.25%)

DISCUSSION

For compartmental analysis, a comparison of Akaike's index criteria values demonstrated that a one-compartment was superior to a two-compartment model. In addition, the correlation matrix generated in the output report by Kinetica® accused a strong correlation between several parameters when a two-compartment model was attempted. This is an indication for over-parametrization and therefore, the one compartment model was chosen to explain the data. The discrimination between one-compartment models was based on the Akaike's index value. The model that fits better the data had to be characterized by a smaller Akaike's index. The analysis of the Akaike's index values revealed that the most appropriate model used to characterize pharmacokinetic of glimepiride was a one-compartment model with lag time.

Important data are available for characterization of glimepiride pharmacokinetics. Pre-authorization research and post–marketing experience of brand formulation Amaryl® (Aventis) reported information of glimepiride in various conditions: after single and multiple doses in healthy subjects, influence of age, co-medication, and disease state on plasma profile of glimepiride in both healthy individuals and patients (10). Some reports presented that glimepiride pharmacokinetics can be described by a two-compartmental model with a lag time (11). The investigators found a C_{max} of 191.5 ng/mL reached at approximately 4 h after administration of a single dose of 4 mg glimepiride in healthy subjects. After single dose administration of Amaryl® 4 mg tablets in 12 healthy subjects, C_{max} of glimepiride of 308 ± 69 ng/mL was reached in 2.1 ± 0.6 h. Almost similar results were found after single dose administration in patients with type 2 diabetes mellitus: C_{max} of 352 ± 222

ng/mL reached in 2.08 ± 0.51 h. In both healthy subjects and patients, glimepiride absorption was complete (100%) with significant absorption of glimepiride within 1 h after administration (11). The volume of distribution of glimepiride was 8.8 L (113 mL/kg) after intravenous administration. Elimination half-life in healthy subjects was 1.5 h. A significant increase of plasma elimination of glimepiride to 5.30 h was found in type 2 diabetes mellitus patients with altered renal function (12).

CONCLUSIONS

Both non-compartmental and compartmental pharmacokinetic analyses were performed for plasma concentration of a generic formulation of glimepiride after oral single-dose administration of 4 mg in healthy subjects.

In pharmacokinetic studies, it is necessary to select the correct model to allow an estimation of pharmacokinetic parameters with small bias and good precision to predict concentrations. This selection is based on analysis of Goodness of fits assessed with Akaike's Information Criteria, Schwartz Criteria, Residual Sum of Squares, standard deviation of the regression and determination coefficient. In the present study, Akaike's Information Criteria was the primary criteria for discrimination between pharmacokinetic models.

Overall, based on the detailed compartmental analysis, the results of this single dose study demonstrated that oral glimepiride 4 mg tablets obey one compartment model with lag time and with rapid absorption and a relatively short half-life. Further investigations will be performed on compartmental pharmacokinetic analysis to offer more flexible study application.

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VALIDAREA UNEI METODE HPLC PENTRU DETERMINAREA OCHRATOXINEI A

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VALIDATION OF A HPLC METHOD FOR OCHRATOXIN A DETERMINATION (Abstract): Ochratoxin A is a mycotoxin produced by various species of *Aspergillus* and *Penicillium*. Ochratoxin A has been detected in cereals and cereal products, coffee beans, beer, wine, spices, pig's kidney and cow's milk. For ochratoxin A, a HPLC method was developed and validated. Ochratoxin A was determined by RP-HPLC, using a liquid chromatograph type HP 1090 Series II, equiped with a fluorescence detector. The analysis was performed with a Phenomenex column, type Luna C18(2) 100A (150 x 4,6 mm; 5µm) with a mobile phase consisting of a mixture of acetonitrile/water/acid acetic (99/99/2), a flow of 0,7 mL/min. For detection, the wavelenght of excitation was 228 nm and wavelenght of emision was 423 nm. The calibration graph was linear in 6,25-50 ng/mL concentration range (r^2 = 0,9991). The detection limits was 1,6 ng/mL and the quantification limit was 4,9 ng/mL. The method precision (RSD = 2,4975 %) and the accuracy (recovery was 100,1 %) were studied. The HPLC method was applyed for ochratoxin A from food samples with good results. **Key words**: OCHRATOXIN A, VALIDATION, HPLC

Ochratoxina A este o micotoxină elaborată de specii de *Aspergillus* în zonele calde şi în cele tropicale şi de specii de *Penicillium* în zonele cu climă temperată (1). Ochratoxina A a fost detectată în cereale şi derivate, cafea, bere, vin, condimente şi în produse de origine animală - rinichi de porc şi lapte de vacă. Pentru determinarea ochratoxinei A a fost dezvoltată şi validată o metodă de analiză prin cromatografie de lichide de înaltă performanţă (HPLC), metodă utilizată la determinarea ochratoxinei A din probe de produse alimentare.

MATERIAL ŞI METODĂ

- Ochratoxina A from *Aspergillus ochraceus* (Sigma);
- Metanol Chromasolv ® min 99,9% (Sigma-Aldrich) ;
- Acetonitril R Chromasolv ® min 99,8% (Riedel-de Haën);
- Acid acetic glacial 100% (Sigma-Aldrich);
- Apă purificată (rezistivitate 18,2 MΩ);
- Balanţă analitică Adam;
- Filtre Millipore 45 µm;
- Cromatograf de lichide de înaltă performanţă de tipul HP 1090 Series II

prevăzut cu detector de fluorescenţă tip HP 1046 A;
- Coloană cromatografică Phenomenex Luna C18(2) 100A (150 x 4,6 mm, 5µm);
- Soluţie etalon stoc (40 µg/mL): 1 mg ochratoxină A se dizolvă în metanol şi se completează în balon cotat la 25 mL cu acelaşi solvent. Concentraţia soluţiei a fost verificată spectrofotometric (coeficientul molar de extincţie în metanol al ochratoxinei este de 6640 $cm^{-1}mol^{-1}$).
- Soluţie etalon de lucru: din soluţia stoc s-a preparat soluţia etalon de lucru de concentraţie 0,8 µg/mL în metanol. Din soluţia de lucru s-au preparat prin diluţie cu metanol soluţii cu concentraţii cuprinse între 6,25 ng/mL şi 800 ng/mL.

Mod de lucru

În literatura de specialitate există numeroase metode de determinare a ochratoxinei A prin metoda HPLC (2, 3, 4, 5). În cele ce urmează ne-am propus elaborarea unei metode simple şi rapide.

Determinările s-au realizat folosind ca faza staţionară coloana cromatografică Phenomenex Luna C18(2) (150 x 4,6 mm, 5µm), iar ca fază mobilă amestecul format din CH_3CN-H_2O-CH_3COOH (99:99:2, v/v/v), debitul fazei mobile fiind de 0,7 mL/min; temperatura în compartimentul coloanei a fost stabilită la 25°C. Detecţia s-a realizat în fluorescenţă cu λem = 228 nm şi λex = 423 nm. În vederea analizei, din soluţiile de lucru obţinute prin diluţie în metanol (6,25- 800 ng/mL) se injectează un volum de 20 µL. Timpul necesar unei analize este de aproximativ 16 min. ţinând cont că timpul de retenţie a ochratoxinei A este Tr = 13±5 % minute.

REZULTATE ŞI DISCUŢII

Pentru validarea metodei de analiză (6, 7, 8, 9) ne-am propus urmărirea următorilor parametri: selectivitatea, specificitatea metodei, determinarea liniarităţii, a limitei de detecţie, a limitei de cuantificare, a preciziei (precizia sistemului şi precizia metodei) şi a exactităţii.

Selectivitatea

Pentru determinarea selectivităţii metodei s-a procedat astfel: din analiza cromatogramelor soluţiilor de probă nu apar alte picuri la acelaşi timp retenţie, iar rezoluţia faţă de picurile adiacente este suficient de mare.

Specificitatea

Pentru determinarea specificităţii metodei a fost analizat solventul utilizat la prepararea soluţiilor standard şi a probelor. La timpul de retenţie corespunzător ochratoxinei A nu s-au obţinut picuri interferente.

Liniaritatea

Pentru determinarea liniarităţii au fost preparate soluţii de ochratoxină A în metanol în modul descris, pe intervalul 6,25 - 800 ng/mL. Pentru fiecare concentraţie s-au efectuat un număr de 3 determinări şi s-a calculat valoarea medie a ariei picului corespunzător ochratoxinei A. Datele experimentale obţinute sunt prezentate în tabelul I.

Valorile medii ale ariei picurilor au fost reprezentate grafic în funcţie de concentraţie şi s-a calculat ecuaţia dreptei de regresie (fig. 1).

Pe intervalul de concentraţie 6,25 - 800 ng/mL, variaţia ariei picului ochratoxinei A este liniară (r^2 = 0,9991, unde r^2 = coeficient de regăsire), dar, având în vedere faptul că intenţia este de a determina concentraţii cât mai mici de ochratoxină A din probe, am calculat ecuaţia curbei de calibrare pe intervalul 6,25 - 50 ng/ mL.

TABELUL I

Date experimentale pentru determinarea liniarității metodei de determinare a ochratoxinei A prin metoda HPLC

Conc (ng/mL)	Arie pic			
	I	II	III	Medie
6,25	10,10	10,40	10,30	10,27
12,50	14,00	14,90	15,92	14,94
20,00	19,22	19,10	19,66	19,32
25,00	23,30	22,80	22,90	23,00
30,00	26,38	25,88	25,26	25,84
40,00	32,26	31,30	32,41	31,99
50,00	38,90	37,70	38,23	38,28
100,00	68,70	68,90	66,97	68,19
150,00	100,50	96,90	96,10	97,83
200,00	135,23	136,74	131,03	134,33
400,00	256,24	259,21	258,90	258,12
600,00	386,14	385,03	380,50	383,89
800,00	504,40	497,30	500,80	500,83

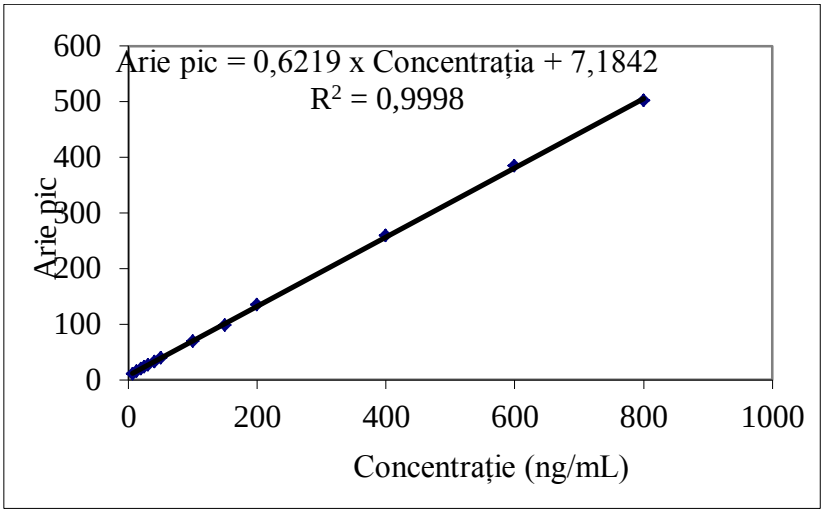

Fig. 1. Dreapta de regresie la determinarea ochratoxinei A prin metoda HPLC

Prin prelucrarea statistică se obține ecuația dreptei de calibrare Arie pic = 0,6339 x Concentrația + 6,7353 (r^2 = 0,9991), dar şi eroarea standard a dreptei de regresie (SE = 0,3123). Rezultatele obținute sunt prezentate în tabelul II.

TABELUL II

Evaluarea statistică a liniarității

Coeficient de regăsire (r^2)	0,9991
Eroare standard (SE)	0,3123
Intercept	6,7353
Panta	0,6339

Limita de detecţie şi limita de cuantificare

S-a calculat limita de detecţie (LD) şi limita de cuantificare(LQ) utilizând relaţiile:

$$LD = \frac{3,3 \times SE}{Panta} = \frac{3,3 \times 0,3123}{0,6339} = 1,6 ng/mL$$

$$LQ = \frac{10 \times SE}{Panta} = \frac{10 \times 0,3123}{0,6339} = 4,9 ng/mL$$

unde SE este eroarea standard a regresiei.

Precizia sistemului şi a metodei

În cadrul preciziei au fost studiate precizia sistemului pentru concentraţii de 25 ng/mL (pentru un număr de 10 determinări) şi precizia metodei pe un interval de 20 % faţă de concentraţia de interes (pentru un număr de 3 determinări la 3 nivele de concentraţie). Folosind ecuaţia dreptei de calibrare se calculează concentraţia pentru fiecare probă în parte şi procentul de regăsire. Datele experimentale obţinute sunt prezentate în tab. III şi tab. IV.

TABELUL III

Date experimentale referitoare la precizia sistemului

Nr. det.	Arie pic
1	22,71
2	23,22
3	22,90
4	22,84
5	22,83
6	22,73
7	22,74
8	23,19
9	23,30
10	22,84
Medie	**22,93**
SD	**0,2212**
RSD %	**0,9645**

După prelucrarea statistică a datelor obţinute se observă că deviaţia standard relativă (RSD) are valoarea 0,9645 %, faţă de maximul de 2 % prevăzut de normele europene, deci sistemul este precis (10).

TABELUL IV

Date experimentale referitoare la precizia metodei de determinare a ochratoxinei A prin metoda HPLC

Nr. det.	Concentraţie teoretică (ng / mL)	Arie	Concentraţie calculată (ng / mL)	Regăsire (%)
1	20	19,22	19,69	98,5
2		19,10	19,50	97,5
3		19,66	20,38	101,9
4	25	23,30	26,13	104,5
5		22,80	25,34	101,4
6		22,90	25,50	102,0
7	30	26,38	30,98	103,3
8		25,88	30,20	100,7
9		25,26	29,21	97,4
Date statistice			**Medie**	**100,8 %**
			SD	**2,5174**
			RSD	**2,4975 %**

Din datele experimentale obținute se observă că metoda este precisă, deviația standard relativă fiind RSD = 2,4975 %, față de maximul de 5 % prevăzut de normele europene (10).

Exactitatea metodei

Pentru determinarea exactității metodei pe un interval ± 20% față de concentrația de interes, au fost analizate 3 soluții de concentrații diferite, de 3 ori fiecare. Pentru fiecare analiză a fost determinată aria picului corespunzător ochratoxinei A şi s-a calculat concentrația folosind ecuația curbei de calibrare. S-a calculat regăsirea pentru fiecare probă în parte, valoarea medie şi intervalul de regăsire. Datele experimentale obținute sunt prezentate în tabelul V.

TABELUL V

Date experimentale referitoare la exactitatea metodei de determinare a ochratoxinei A prin metoda HPLC

Nr. det.	Concentrație teoretică (ng / mL)	Arie	Concentrație calculată (ng / mL)	Regăsire (%)
1	20	19,35	19,90	99,49
2		19,18	19,63	98,15
3		19,70	20,45	102,25
4	25	22,76	25,28	101,11
5		23,07	25,77	103,06
6		22,97	25,61	102,43
7	30	25,21	29,14	97,14
8		25,61	29,77	99,24
9		25,32	29,31	97,71
Date statistice			**Media**	**100,1 %**
			Min	**97,1 %**
			Max	**103,1 %**

Din datele experimentale obținute se observă că regăsirea medie este de 100,1 % pe intervalul 97,1-103,1 %, comparativ cu o limită de ± 10% prevăzută de normele europene, de unde rezultă că metoda este exactă (10).

CONCLUZII

A fost elaborată şi validată o metodă HPLC pentru detectarea ochratoxinei A. Astfel, determinările se efectuează pe o coloană Luna C18(2) 100A (150 x 4,6 mm, 5µm), cu o fază mobilă formată din CH_3CN-H_2O-CH_3COOH (99:99:2, v/v/v), debit 0,7 mL/min, cu detecție în fluorescență (λem = 228 nm, λex = 423 nm). Metoda este liniară pe domeniul de concentrații ales (6,25-50 ng/mL), ecuația dreptei de regresie fiind Arie pic = 0,6339 x Concentrația + 6,7353 (r^2 = 0,9991). Au fost calculate limitele de detecție (1,6 ng/mL) şi de cuantificare (4,9 ng/mL). S-a studiat precizia sistemului (RSD = 0,9645 %), precizia metodei (RSD = 2,4975 %) şi exactitatea (regăsirea medie = 100,1 %) pe intervalul 97,1 -103,1 %.

Metoda a fost aplicată cu rezultate bune pe probe de produse alimentare.

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CONSIDERAŢII BIOMECANICE CU PRIVIRE LA FOLOSIREA SUBSTITUIENŢILOR OSOŞI ÎN TRATAMENTUL CHIRURGICAL AL OSTEOMIELITELOR CRONICE

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BIOMECHANICAL CONSIDERATION REGARDING BONE SUBSTITUTES USE IN CHRONIC OSTHEOMIELITIS SURGICAL TREATMENT (Abstract): The main aim of this study is to present the use of bone substitutes in chronic ostheomielitis surgical treatment and its biomechanical influence. **Material and methods**: First step is three-dimensional reconstruction to one femur using CT sections; the second is the reconstruction of infected bone volume, synthesis of material properties, synthesis of loading forces, synthesis of boundary condition and, finally, finite element analysis. **Results**: The calculated Von Mises stress as a result of finite element analysis indicates that bone substitute implantation lead to a stress distribution in bone similar with a health femur. **Key words**: OSTHEOMIELITIS, BONE SUBSTITUTE, FINITE ELEMENT ANALYSIS

Osteomielita este inflamaţia osului şi a măduvei sale produsă prin infectarea pe calea sângelui (hematogenă) cu stafilococul auriu (o bacterie care populează şi suprafaţa tegumentară) sau alţi germeni (pneumococ, colibacil). Boala se poate prezenta sub o forma cronică sau acută.

Osteomielita cronică este o inflamaţie a osului care nu a fost precedată de fenomene de osteomielită acută. Clinic, semnul cel mai important îl constituie durerea, care este spontană şi intermitentă, calmându-se prin repaus. Aceasta are în general caracter nocturn.

În cazul osteomielitei cronice, tratamentul de elecţie va fi cel chirurgical, tratament ce vizează îndepartarea sechestrelor osoase ce întreţin infecţia.

Incizia se face la nivelul arătat de radiografie sau în locul unde există fistule ce drenează puroi din profunzime şi se îndepărtează sechestrul osos, care uneori poate fi de mărime apreciabilă, chiuretându-se cavitatea restantă.

Pentru o reintegrare socială rapidă a pacientului, este necesară umplerea cavităţii restante, rezultate în urma chiuretării, cu substitut osos care consolidează zona afectată, pe de o parte, iar pe de altă parte, în condiţiile în care substitutul osos este bioresorbabil, permite o regenerare facilă a ţesutului osos (1).

În această lucrare se va face o evaluare a stării de tensiuni care apar la nivelul unui femur afectat de osteomielită, a distribuţiei eforturilor unitare atunci când, în urma unei intervenţii chirurgicale, se îndepărtează ţesutului infectat şi în situaţia în care în cavitatea rezultată în urma chiuretării se introduce substitut osos.

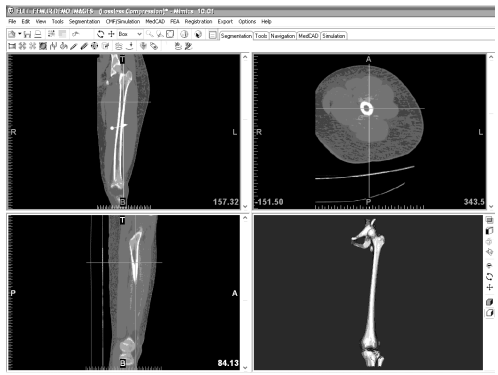

Fig. 1. Secţiunile CT şi rezultatul reconstrucţiei tridimensionale

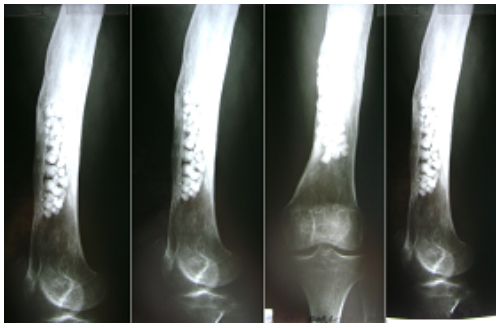

Fig. 3. Radiografii care stau la baza reconstrucţiei tridimensionale a zonei de os infecate

Comparaţia dintre cele două cazuri demonstrează un beneficiu biomecanic deosebit de important al substitutlui osos asupra osului ce se afla în proximitatea zonei infectate.

MATERIAL ŞI METODĂ

Modelarea virtuală ce va fi descrisă în cele ce urmează şi care este folosită frevent în evaluarea tensiunilor de la diferite nivele ale organismului uman, precum în: endoprotezare, radioterapie etc. (2,3), presupune parcurgerea următorilor paşi: sinteza obiectivelor analizei, sinteza geometrică a structurii, sinteza proprietăţilor de material, sinteza încărcărilor ce acţionează asupra structurii, sinteza reazemelor şi analiza propriuzisă (4,5,6).

a) ***Sinteza obiectivelor analizei.*** Analiza în element finit ce va fi făcută în continuare, urmăreşte evaluarea statică a tensiunilor, a deformaţiilor şi a deplasărilor apărute la nivelul femurului, în cele trei cazuri enunţate în introducere: femur normal, femur infectat şi femur tratat chirurgical cu cavitate restantă şi cavitate restantă umplută cu substitut osos.

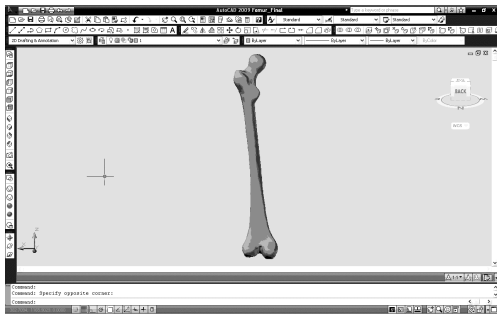

Fig. 2. Reprezentarea femurului în 3D în urma reconstrucţiei în AutoCAD 2009

b) ***Sinteza geometrică a structurii***. Pentru analiza curentă, care va fi făcută tridimensional (3D), se apelează la secţiunile CT ale coapsei, incluzând partea distală a pelvisului şi partea proximală a gambei (fig. 1). Secţiunile se inserează în soft-ul Mimics 10.01 care are posibilitatea de a reconstrui tridimensional, în urma aplicării unui set minim de comenzi, structurile osoase. Din modelul rezultat în Mimics, s-au extras secţiunile importante ale femurului, utilizând un alt soft dedicat reconstrucţiei 3D: AutoCAD 2009 (fig. 2).

În continuare se procedează la delimitarea zonei infectate din femur. În acest sens sunt utilizate un set de radiografii, care permit determinarea formei volumului infectat şi localizarea acestuia (fig. 3, 4).

Următorul pas constă în exportarea solidului din AutoCAD, reprezentat de femurul infectat, în soft-ul de analiză în elemnet finit Algor FEMPRO V. 23.1. În AutoCAD, se exportă şi se salvează fisierul cu extensia *.*sat. După deschiderea soft-ului Algor se deschide fisierul exportat anterior. Se dă comanda mesh, rezultatul fiind o discre-

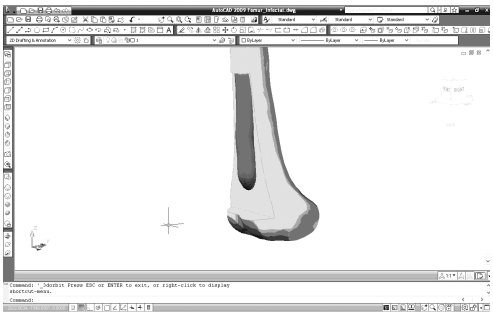

Fig. 4. Reprezentarea reconstrucţiei tridimensionale a zonei de os infectate

Fig. 5. Femurul infectat după operaţia de mesh-are

TABELUL I

Caracteristicile de material pentru fiecare componentă a structurii

Componentă	Modul de elasticitate [MPa]	Coeficientul lui Poisson	Densitate [kg/m^3]
Os	13.800	0,34	1100
Os infectat	150.000	0.4	1500
Substitut osos	55	0.45	1000

tizare a solidului reprezentat de femurul infectat. Rezultatul poate fi vizualizat în fig. 5.

c) Sinteza proprietăţilor de material

Pentru a se putea face analiza în element finit, trebuie definite, pe rând, pentru fiecare componentă a structurii : tipul de material, în care se vor defini, următoarele mărimi :

- densitatea materialului [kg/m^3] ;
- modulul de elasticitate [N/m^2] ;
- coeficientul lui Poisson ;
- modulul de elasticitate transversal [N/m^2] sau [Pa].

Se vor defini caracteristicile de material pentru os, ţesut osos infectat şi substitut osos (7,8,9) (tab. I).

d) Sinteza forţelor ce acţionează asupra structurii. Pe lângă muşchii care produc mişcările de flexie-extensie, abducţie--adducţie şi de rotaţie de la nivelul articulaţiei şoldului, mai sunt muşchii care au origine pe femur : biceps femural porţiunea scurtă şi cei trei muşchi vaşti, lateral, medial şi intermediar (fig. 1). În aplicarea forţelor asupra femurului s-a considerat forţa care acţionează asupra capului femural (care include forţele date de muşchii abductori, ischio-gambieri şi drept femural) şi forţele date de muşchii care au origine pe femur (fig. 6).

Valorile forţelor aplicate sunt (4) :

F_1 = 80N (muşchiul biceps femural porţiunea scurtă) ;

F_2 = 80N (muşchiul vast lateral) ;

F_3 = 70N (muşchiul vast intermediar) ;

F_4 = 90N (muşchiul vast medial) ;

F_5 = 2500N (forţa care acţionează asupra capului femural).

e) Sinteza reazemelor. Reazemele (locul de sprijin) se consideră a fi pe suprafaţa condililor femurali, şi reprezintă forţe de reacţiune ale forţelor musculare care acţionează pe femur (fig. 6).

REZULTATE

Odată parcurse toate etapele premergătoare amnalizei cu element finit, se dă comanda *analyse*.

Rezultatul distribuţiei de tensiuni este reprezentat şi poate fi vizualizat în fig. 7, unde sunt ilustrate stările de tensiuni pentru un femur normal (fig. 7.a), un femur infectat (fig. 7.b), femurul infectat după chiuretarea zonei infectate (fig. 7.c) şi situaţia în care cavitatea rezultată în urma chiuretării este plombată cu substitut osos (fig. 7.d). În fig. 7.e este reprezentat femurul mesh-at împreună cu volumul cavităţii chiuretate.

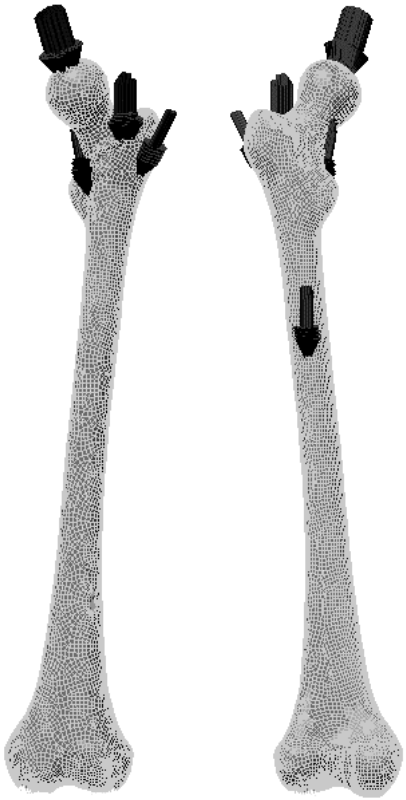

Fig. 6. Forţele date de muşchii vaşti, biceps femural (porţiunea scurtă) şi rezultanta de la nivelul capului femural

DISCUŢII

Se iau în discuţie cele patru cazuri analizate anterior.

În fig. 7, sunt ilustrate detaliile care cuprind zona în care se află infecţia osoasă şi mesh-ul femurului infectat, pentru a se putea face o corespondenţă corectă între distribuţia de tensiuni a fiecărui caz analizat şi poziţionarea volumului infectat (ulterior chiuretat sau a zonei cu substitut osos).

În comparaţie cu distribuţia de tensiuni de la nivelul femurului normal (fig. 7.a) se observă că pentru femurul infectat, în zona infecţiei, tensiunile au o valoare mai mică (fig. 7.b).

Femurul chiuretat prezintă o arie mare a tensiunii de valoare maximă (7kPa), în comparaţie cu cele ilustrate în figurile 7 a, b şi c.

Pentru femurul tratat cu substitut osos tensiunea are aceleaşi valori maxime ca şi pentru femurul normal (fig. 7.d), diferenţa fiind infomă.

Distribuţiile de tensiuni ilustrate în fig. 7, oferă informaţii asupra comportării biomecanice a osului. Pentru femurul cu infecţie osoasă, se observă că osul sănătos, adiacent infecţiei osoase, este mai puţin încărcat în comparaţie cu femurul normal.

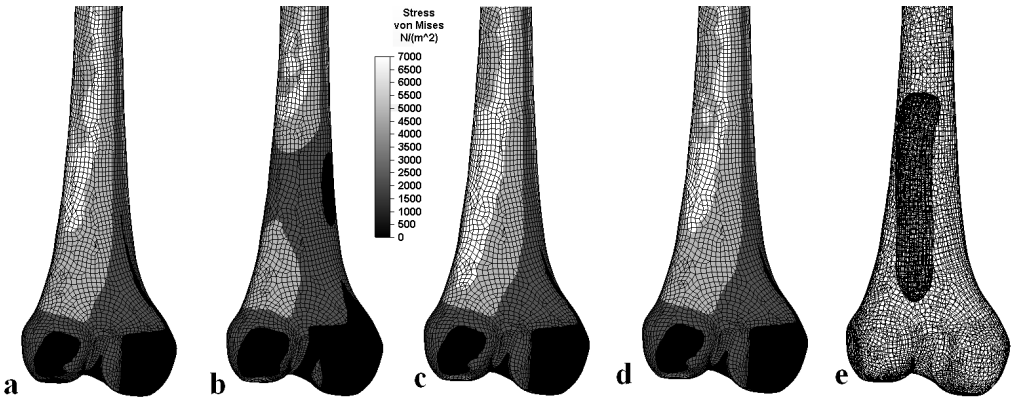

Fig. 7. a – distribuţia de tensiuni pentru femurul normal ; b – distribuţia de tensiuni pentru femurul cu infecţie osoasă ; c – distribuţia de tensiuni pentru femurul la care s-a îndepărtat infecţia osoasă prin chiuretaj ; d – distribuţia de tensiuni pentru femurul tratat cu substitut osos ; e – reprezentarea mesh-ată a femurului, cu evidenţierea zonei infectate.

Conform principiilor remodelării osoase şi ale mecanotransducţiei (10,11,12,13) într-un os mai puţin încărcat decât în condiţii fiziologice, normale, nu se mai realizează o reconstrucţie osoasă eficientă, ceea ce duce la o rarefiere osoasă, scăderea densităţii osului şi, implicit a rezistenţei la rupere (tensiunea de rupere a osului este direct proporţională cu densitatea osului la pătrat).

Acest lucru presupune posibilitatea apariţiei unei fracturi patologice, coroborată şi cu fragilitatea osului infectat (rigiditate mai mare, modul de elasticitate mai mare dar şi o elasticitate scăzută).

Astunci când, însă, distribuţia de tensiuni maxime se întinde pe o arie mai mare (fig. 7.c), osul este suprasolicitat, fiind posibilă o fractură înainte ca osul să se adapteze, în sensul densificării şi rigidizării.

În situaţia tratării zonei infectate cu substitut osos distribuţia de tensiuni ilustrază condiţiile unei regenerări ososase eficiente, fapt care, combinat cu proprietatea de resorbţie a substitutului osos, duce la refacerea ţesutului osos distrus prin infecţie (14,15).

Concluzia care se poate trage în urma analizei cu element finit este că, în cazul unei osteomielite cronice (semnul cel mai important il constituie durerea care este spontana si intermitenta, calmandu-se prin repaus), netratate, pot surveni complicaţii cu caracter biomecanic, una dintre ele fiind fractura.

CONCLUZII

În urma acestui studiu se pot trage următoarele concluzii :

1. Distribuţia de tensiuni pentru un femur cu infecţie osoasă :

a. Induce la nivelul osului adiacent infecţiei tensiuni mai mici decât în cazul osului sănătos ;

b. Tensiunea mai mică, presupune existenţa unor deformaţii mai mici ale osului adiacent infecţiei, o circulaţie ineficientă a fluidului prin canalicule şi, în consecinţă, o remodelare osoasă ineficientă, cu reducerea rezistenţei mecanice a osului ;

c. Încărcarea ineficientă a osului adiacent infecţiei osoase poate duce la fractura femurului în zona infectată, ştiut fiind faptul că solicitările complexe care apar la nivelul unui os lung duc la apariţia unor tensiuni mari, mai ales in zona diafizei osului ;

2. Starea de tensiuni pentru femurul chiuretat :

a. Induce la nivelul osului o tensiune întinsă ca arie depăşind, chiar proiecţia cavităţii pe suprafaţa osoasă ;

b. Tensiunea mare prezentă pe o arie întinsă, suprasolicită osul, fiind posibilă fracturarea.

3. Distribuţia de tensiuni pentru un femur tratat cu substitut osos :

a. Induce la nivelul osului adiacent substitutului tensiuni cu aceleaşi valori ca şi în cazul femurului normal, dar cu o arie de acţiune mai mare ;

b. Exitenţa unei tensiuni fiziologice, presupune o remodelare corectă a osului în zona considerată ;

c. Substitutul osos are în componenţă hidroxiapatită, ceea ce oferă o bază pentru reconstrucţie osoasă şi în regiunea care a fost infectată ;

4. Din cele enunţate anterior, se poate trage concluzia că tratamentul infecţiei osoase cu substitut osos este benefică, mai ales din punctul de vedere al comportării biomecanice a osului.

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NOUTĂŢI

Boala osoasă Şi hipercalciura la copil

În literatura de specialitate există puţine studii privind masele osoase şi hipercalciuria idiopatică al copil (IH). Când a fost efectuată, măsurarea densităţii minerale osoase (BMD) a relevat o scădere semnificativă a Z-scorului pentru copiii cu IH, la nivelul apofizei spinoase spinei lombare şi mai puţin important la nivelul colului femural. Câteva investigaţii au descris natura mecanismelor implicate în pierderea de masă osoasă la aceşti copii. Unele studii desemnează creşterea reabsorbţiei osoase ca mecanism al pierderii osoase. Nu sunt raportări privind evaluarea prin biomarkeri specifici pentru formarea osoasă la copii cu IH. Totuşi, cei mai mulţi dintre copiii cu IH din aceste raportări au demonstrat o creştere staturală normală, ceea ce pare puţin probabilă în condiţiile unei alterări în formarea osoasă. Cauza creşterii rezorbţiei osoase, de asemenea, nu este pe deplin stabilită dar, factori genetici, indisciplina alimentară şi alterarea producţiei de citokine au fost propuşi drept contributori la descreşterea BMD observată la copiii cu IH. O fixare minerală optimă în os pe parcursul copilăriei şi al adolescenţei este importantă în atingerea unui vârf al masei osoase şi poate servi la prevenirea apariţiei osteoporozei la adult. Aceasta justifică eforturile de a înţelege mecanismele de pierderea a masei osoase la copiii cu IH (Zerwekh JE. Bone disease and hypercalciuria in children. *Pediatr Nephrol* 2010 ; 25 (3) : 395-401).

Mihaela Munteanu

GRAPEFRUIT JUICE – DRUG INTERACTIONS: IMPORTANCE FOR PHARMACOTHERAPY

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GRAPEFRUIT JUICE-DRUG INTERACTIONS: IMPORTANCE FOR PHARMACOTHERAPY (Abstract): In spite of known health benefits of grapefruit juice, its consumption in combination with drugs requires caution. The drugs most susceptible to pharmacokinetic interactions with clinical significance are those with narrow therapeutic index and low bioavailability due to important first-pass metabolism. Most vulnerable populations are elderly, cirrhotics, subjects with genetic polymorphisms and individuals taking other CYP3A4 inhibitors. The major drug classes that have been reported to present interactions with grapefruit juice are antiallergics, antibiotics, antimalaria drugs, anxiolytics, calcium channel blockers, HIV protease inhibitors, HMG-CoA reductase inhibitors; the degree of pharmacokinetic interaction varies among the compounds of the same class. **Key words**: GRAPEFRUIT, PHARMACOKINETIC INTERACTION, BIOAVAILABILITY

Grapefruit juice (GJ) is widely preferred among consumers as it is a good source of vitamin C, beta carotene, lycopene, folate, bioflavonoids, furanocoumarins, potassium and fibers (1).

Epidemiological facts, clinical trials and animal studies provide strong evidence that GJ reduces risk of cardiovascular diseases (2) and some types of cancer (3,4,5,6,7).

In spite of all these health benefits, mixing GJ with drugs needs special caution. GJ is known to increase the oral bioavailability of many CYP3A4 substrates by inhibiting intestinal phase metabolism and by down regulating CYP3A4 in the small intestine (8).

However, recent data indicate that other mechanisms than inhibition of CYP3A4 are involved in GJ - induced pharmacokinetic interactions. GJ also inhibits some drug transporters like P-glycoprotein (P-gp) and Organic Anion Transporting Polypeptides (OATP) (9). This may help to explain the inconsistent data regarding GJ - drugs interactions and the large variability of the aria under curve (AUC) among patients.

The degree of the interaction with an enzyme inhibitor can be assessed based on midazolam effect. The pharmacokinetic interactions are classified as follows:

- weak - less than two fold increase of AUC of midazolam;
- moderate - two to five fold increase of AUC of midazolam;
- strong - more than five fold increase of AUC of midazolam (10).

REPORTS ON GRAPEFRUIT JUICE-DRUG INTERACTIONS

The GJ - **calcium channel blocker** interaction has been known since 1989 (11).

The co-administration of GJ with some **dihydropyridine** calcium channel antagonists like felodipine, nifedipine (12), nicardipine (13), nimodipine (14), nisoldipine (15) and nitrendipine (16) leads to increased AUC ranged from 1.5-fold to 4.0-fold. Especially among the elderly, the increased bioavailability is associated with enhanced reduction of both systolic and diastolic blood pressure and increased frequency of vasodilatation--related side events. The interaction between amlodipine and GJ doesn't present clinical significance because amlodipine has high (80%) oral bioavailability (17).

In the **phenylalkylamines** calcium channel antagonists group, verapamil exhibits a weak interaction with GF and a high inter-subject variability. In a study realized by Fuhr, prolongation of PR interval above 350 ms occurred in two of the 24 individuals in the group receiving GJ (18).

In the **benzothiazepine** calcium channel antagonists group, diltiazem has been found to have an increased bioavailability when co-administrated with GJ but these results need further investigation (19).

Carvedilol is a racemic mixture in which nonselective **β-adrenoreceptor blocking** activity is present in the S(-) enantiomer and **α1-adrenergic blocking** activity is present in both R(+) and S(-) enantiomers at equal potency. Carvedilol has an absolute bioavailability of approximately 25%-35% due to a significant stereoselective first-pass metabolism by CYP2D6, CYP2C9 and CYP3A4 enzymes. Following oral administration in healthy subjects, plasma levels of R(+) -enantiomer is 2 to 3 times higher than S(-) -enantiomer. Subjects with genetic polymorphism (poor metabolizers) present almost 3-fold higher plasma concentrations of R(+) -carvedilol and 1.25 - fold higher plasma levels of S(-)-carvedilol compared to extensive metabolizers. Drinking GJ while taking carvedilol may increase side effects of carvedilol and modify the therapeutic activity. In these circumstances, until new data is available, patients with heart failure receiving carvedilol should avoid GJ intake (8).

Losartan - **angiotensin II receptor (type AT1) antagonist** - is an orally active drug that undergoes substantial first - pass metabolism by CYP2C9 and CYP3A4 enzymes. It is converted, mainly to an active carboxylic acid metabolite (E3174) which is 10-40 times more potent than the parent drug. In healthy volunteers, GJ increases significantly the time to losartan appearance in serum and decreases significantly AUC of the E3174 metabolite suggesting loss of drug efficacy (20).

Amiodarone is an **antiarrhythmic** drug which can cause QTc interval prolongation and severe cardiac ventricular arrhythmia - torsade de pointes. Amiodarone is metabolized by CYP3A4 to N-desethylamiodarone - a metabolite with more potent anti-arrhythmic properties. GJ increases the amiodarone plasma concentrations and inhibits almost completely the production of the active metabolite leading to fewer side effects but also to diminished therapeutic action (21).

Even it has good oral bioavailability (which varies widely 45-100% between patients), the antiarrythmic quinidine should not be associated with GJ without cautious risk and benefit assessment. Careful monitoring of this drug is important due to its narrow therapeutic index and similarity in patient response to sub-therapeutic and toxic amounts of the drug.

HMG-CoA reductase inhibitors - a class of lipid-lowering agents - are associated with the increase the risk of myopathy and rhabdomyolysis, particularly in patients taking higher doses.

Lovastatin, simvastatin, and atorvastatin are highly prone to interactions due to their reduced oral bioavailability as a result of

presystemic metabolism by CYP3A4 (22). Two days of pretreatment with 200 ml double strength GJ three times a day (high dose of GJ) increases AUC for lovastatin and lovastatin acid 15- and respectively 5-fold, without influencing the half-life.

Similar results was obtained by Lilja et al. (23) who showed that GJ increased AUC of simvastatin 16-fold and the Cmax of simvastatin acid about 7-fold. When simvastatin is taken 24 hours after ingestion of high dose GJ, the effect on the AUC of simvastatin is only about 10% of the effect observed during concomitant intake of GJ and simvastatin.

In contrast, pretreatment with 200 ml double strength grapefruit juice three times a day for 2 days increased AUC of atorvastatin only 2.5-fold (22). The half-life of atorvastatin increased from 7.8 hours to 13.3 hours (25). Analogous study reported that GJ didn't change AUC of pravastatin.

As fluvastatin has a good oral bioavailability and is predominantly metabolized by CYP2C9, its use together with GJ is not a matter of concern. In patients who consume GJ together with HMG-CoA reductase inhibitors, potential alternative agents are pravastatin, fluvastatin, or rosuvastatin (8).

At therapeutic drug concentration, sildenafil selectively **inhibits phosphodiesterase PDE5** to increase intracellular cGMP concentration in *corpus cavernosa*. At higher drug concentration, the selectivity of sildenafil for PDE5 is lost and other forms of PDE are inhibited, resulting in a generalized increase in intracellular cGMP and systemic vasodilatation. The clearance of sildenafil is reduced in healthy elderly volunteers (over 65 years) and the free plasma concentrations are approximately 40% greater than in healthy younger volunteers (18-45 years). After GJ consumption, the magnitude of sildenafil AUC increase is variable ranging from 0.8 to 2.6. As AUC can increase even more depending on brands, batches and the volume of GJ intake it is recommended that the combination of sildenafil and GJ should be avoided (26). Also, interaction with tadalafil or vardenafil may cause serious systemic vasodilatation especially when combined with a nitrate (8).

Five of twelve drug withdrawals from the market from 1997 and 2002 were caused by pharmacokinetic interactions. **H1-receptor antagonists** astemizol and terfenadine and the gastrointestinal prokinetic agent, cisapride are CYP3A4 substrates; increased concentrations of these drugs due to pharmacokinetic interactions were associated with QT prolongation, torsades de pointes and other ventricular arrhythmia. Severe side--effects were also reported for calcium channel blockers mibefradil and HMG-CoA reductase inhibitor cerivastatin which are also CYP3A4 substrates (27). Ebastine and loratadine, other H1-receptor antagonists, which undergo important first-pass metabolism by CYP3A4 may also present higher concentrations levels after GJ administration. Although the co-administration of loratadine with CYP3A4 inhibitors such as erythromycin, ketoconazole, clarithromycin and cimetidine is associated with increases the loratadine's levels, the prolongation in the QTc interval doesn't occur. The arrhythmogenic potential of the ebastine is higher as it is chemically related to terfenadine.

Special care should be taken into consideration also for psychiatric patients who use drugs with narrow therapeutic index such as tricyclic **antidepressants** (clomipramine) or **anticonvulsivants** like carbamazepine (28). According to Lee et al. (29), GJ increases bioavailability of sertraline with 50%. Regarding **anxiolytics**, in healthy volunteers, midazolam AUC increased with 52% when given with GJ and triazolam AUC with 96% as compared to control group (30, 31, 32). The administration of GJ needs special caution in cirrhotic patients, elderly individuals or subjects taking other CYP3A4

inhibitors. In these patients GJ increased the AUC of midazolam by 106% and decreases the ratio of the AUCs alpha-hydroxymidazolam (metabolite) /midazolam by 85% (33) which may result in excessive sedation and other side-effects. Furthermore, GJ raise dramatically the AUC of non-benzodiazepine anxyolitic buspirone by 9.2 fold (34).

Interesting results are provided by an experimental study which shows that GJ significantly increases the blood concentration of **morphine** in morphine-tolerant rats (35). Also, a clinical trial showed that GJ augments significantly the bioavailability of dextromethorphan with up to three days persistence of the juice impact (36).

Even if GJ almost doubles the AUC for macrolides like clarithromycin the clinical significance of this pharmacokinetic interaction is not relevant (37, 38).

The inhibitory activity of GJ does not always have negative impact as it can also be used to maintain the drug effectiveness. Artemether is a **antimalarial drug** which undergoes high presystemic metabolism by CYP3A4 and presents a high relapse rate during monotherapy (due to autoinduction). GJ increases the oral bioavailability of artemether (39), achieves complete protection of the host from damage induced by schistosomal infection (40) but still cannot prevent the time-dependent reduction in bioavailability.

Halofantrine, another antimalarial drug, presents an oral bioavailability of 10% and is metabolized by CYP3A4 to the less cardiotoxic metabolite, N-debutylhalofantrine. Charbit et al. showed that GJ administration increased halofantrine AUC by 2.8-fold and decreased N-debutyl-halofantrine AUC by 2.4-fold. As GJ prolonged QTc with approximately 82%, this beverage should be contraindicated during administration of halofantrine (41).

Ten years ago a report drew attention to potential fatal interaction between GJ and other drug with narrow therapeutic index – colchicine (an agent used for **gout** treatment) (42). In addition, in Caco-2 cells GJ reduced ratio of basal-to-apical transport to apical-to-basal transport suggesting that, *in vivo,* the bioavailability of this drug might be increased by GJ consumption (43).

Another class of drugs which presents clinically relevant pharmacokinetic interactions with GJ is the **inhibitors of HIV protease** - ritonavir, indinavir, saquinavir, nelfinavir (44). The bioavailability of saquinavir increases twice after GJ consumption (45).

The co-administation of **immunosuppressive** agent cyclosporine (frequently used in the prophylaxis of organ rejection) with GJ increases AUC by 186% as compared with water (46, 47, 48). Also concentration of tacrolimus should be closely monitored when combined with GJ (49).

On the contrary, GJ decreased AUC of the **antineoplasic** agent etoposide with approximately one quarter when administered concomitantly (50). Studies realized on Caco-2 cells indicate that the ratio of basal-to-apical transport to apical-to-basal transport of [3H] vinblastine was reduced by extracts of GJ (51, 52). Further research is needed to underline the clinical significance of this interaction.

Interaction between **hormones** like ethinyl--estradiol (53) or methylprednisolone and GJ can be considered weak and seem to be unlikely to be clinically relevant. It has to be mentioned that a decrease in morning cortisol plasma concentrations has been observed after administration of methylprednisolone with GJ (54,55). Recent data show that regular intake of GJ presents an important impact on the bioavailability of budesonide nearly doubling it (56).

Our data show that, in healthy volunteers, the association of non-steroidal anti-inflammatory drugs like celecoxib or diclofenac with GJ presents a large inter-subject variability. In one subject taking celecoxib, Cmax increased by 3.17 fold and AUCtotal by

1.83 fold. Also, in one case, GJ administration increased Cmax of diclofenac by 3.27 fold and decreased elimination half-life by 2.81 fold.

CONCLUSION

When there is a concern for drug toxicity related to increased plasma drug concentration it is recommended that GJ consumption should best be avoided entirely during pharmacotherapy.

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NOUTĂŢI

COMPUŞI CU ACŢIUNE ANTI-OSTEOPOROTICĂ DIN PLANTE MEDICINALE DIN INDIA

Acest studiu a avut drept scop evaluarea *in vitro* a efectelor osteogenice ale unor plante utilizate în medicina tradiţională indiană în tratamentul fracturilor osoase : *Allophylus serratus* (Roxb) Kurtz (*Sapindaceae*), *Cissus quadrangularis* (*Vitaceae*), *Vitex negundo* (Linn.) (*Verbenaceae*). Din aceste plante au fost izolaţi 14 compuşi. Posibilele efecte osteogenice ale acestor compuşi au fost evaluate *in vitro* pe culturi primare de osteoblaste. 5 dintre compuşii testaţi (rutozida, 6'-O-trans-cinamoil catalpolul, agnuzida, negundozida, luteolina) au determinat o creştere a ratei de diferenţiere a osteoclastelor şi a mineralizării. Rezultatele obţinute justifică utilizarea acestor specii în medicina tradiţională indiană în tratamentul fracturilor osoase (Kumar M, Rawat P, Dixit P et al. Anti-osteoporotic constituents from Indian medicinal plants. *Phytomedicine* 2010 ; 17 : 993-999).

Anca Miron

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *SALVIA OFFICINALIS* L. FROM ROMANIA

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CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *SALVIA OFFICINALIS* L. FROM ROMANIA (Abstract): The quantitative and qualitative analysis of essential oils obtained from *Salvia officinalis* L. (*Lamiaceae*) cultivated in Romania is reported in this paper. **Material and methods**: The essential oils were obtained from fresh and dried material harvested from plants cultivated in Cluj (Romania) and from 2 commercial samples, by hydrodistillation. The essential oils were analysed by GS-MS. **Results**: 12 compounds were found in each oil, 9 of them being common in all samples. The α-thujone was the major compound in all analysed oils (31.23-52.86%), followed by camphor and viridiflorol. Chemically, the oxygenated monoterpenes are present in highest quantity (49.78-79.2%). **Conclusions**: The monoterpenoid ketones' presence (á-thujone, â-thujone, camphor) in high amounts in the essential oils of Romanian sage may develop toxicity for these products and may limit their medicinal use. **Key words**: ESSENTIAL OIL, *SALVIA OFFICINALIS* L., OXYGENATED MONOTERPENES

PANTOTHENIC ACID – APPLICATIONS, SYNTHESIS AND BIOSYNTHESIS

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PANTOTHENIC ACID – APPLICATIONS, SYNTHESIS AND BIOSYNTHESIS (Abstract): Pantothenic acid, also known as vitamin B5 or anti-stress vitamin, is a water-soluble vitamin with various applications in pharmaceutical industry (ointments, solutions for treating lesions and irritation of the skin or mucous membranes, multivitamin supplements - tablets, capsules, effervescent tablets, chewable tablets, syrups), food industry (dietetic foods, baby products, cereals, juices), cosmetics (component of shampoos, hair masks, balms, creams and sunscreen lotions, baby products), and widely used as feed additive. Currently, pantothenic acid is produced by chemical synthesis using pantolactone and the calcium salt of β - alanine, but it can also be obtained by fermentation processes which involve different bacterial species, as well as a number of yeasts. Lately, the demand for pantothenic acid for cosmetic and dermatocosmetic industry registered a significant increase. In this context, obtaining pantothenic acid by fermentation has become a promising method because it does not have a negative environmental impact, it uses renewable resources and cheap raw materials, and global consumer demand for organic products is increasingly higher. **Keywords:** PANTOTHENIC ACID, APPLICATIONS, PRODUCTION.

Pantothenic acid is an organic acid having the formula $C_9H_{17}O_5N$ (fig. 1). From the chemical point of view, pantothenic acid is the amide of pantoic acid with β-alanine.

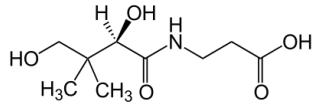

Fig. 1. The chemical structure of pantothenic acid

Pantothenic acid was discovered in 1931 by RJ Williams. In 1933, vitamin B5 was isolated from yeast, and a few years later, RJ Williams successfully establish the chemical structure and extract the compound from liver (1). The main role of pantothenic acid in cells is the synthesis of coenzyme A (CoA). Coenzyme A acts as an acyl group carrier to form acetyl-CoA and other related compounds. CoA is very rich in energy and enzymatically reacts on two distinct paths: an anabolic pathway, where acetyl-CoA is the starting point in many biosynthetic processes (in the biosynthesis of fatty acids, cholesterol, and acetylcholine) and a cata-

bolic pathway that continues with the tricarboxylic acid cycle (Krebs cycle) and ends with the respiratory chain. Pantothenic acid in the form of CoA is also involved in signal transduction and enzyme activation and deactivation (2, 3) (fig. 1).

PANTOTHENIC ACID APPLICATIONS

Regarding the human body, this compound is involved in the health of the digestive, nervous, circulatory, and skeletal systems, skin and hair, as well as in the synthesis of hormones (insulin, adrenaline) (4). Combs G.F. Jr. (5) consider that it also plays an important role in increasing the immunity of human body. Panthenol, another form of vitamin B5, is used in hair care products, considering that it interferes in maintaining a healthy aspect of it, acting as a humectant to facilitate the increase of water content and elasticity. At the same time, it protects the skin from harmful effect of radiation, moisturizes the skin, mucous membranes and cornea. In addition, the administration of high doses of pantethine (500-1200 mg/day) to subjects suffering from dyslipidemia (blood lipid pathological change) and diabetes, it was found that it is effective in reducing the level of cholesterol and triglycerides in the blood, treating fatty liver and related diseases. Some studies suggest that vitamin B5 supplements may speed the healing of superficial wounds, especially after surgery. This quality is important in case of burns, when a significant proportion of micronutrients are lost, phenomenon that increases the risk of infection and slows healing (4).

Pantothenic acid is found in all living cells (pantos = everywhere) and is widely found in foods, so deficiency of this substance is generally rare encountered. The richest sources of vitamin B5 are: yeast, royal jelly, cereals, vegetables, egg yolk, liver and kidney, beef, soy, nuts/peanuts, walnuts, mushrooms (4, 6) (tab. I).

TABLE I

Sources of vitamin B5 and content

Sources	Content, mg/100g	Sources	Content, mg/100g
Meat		**Vegetables**	
beef	0.3-2	avocado	1.1
pork	0.4-3.1	broccoli	1.2
young cattle kidney	3.9	cabbage	0.1-1.4
chicken liver	9.7	carrots	0.27
young cattle liver	8	cauliflower	1
pork liver	7	lentil	1.4
Cereals		potatoes	0.3
wheat flour	0.21	soybeans	1.7
wheat bran	2.1	tomatoes	0.3
whole wheat	1.2	**Nuts**	
soy flour	1.4	Cashew	1.3
oatmeal	1.1	Peanuts	2.8
whole rice	1.1	walnuts	0.8
Fruits		**Others**	
apples	0.1	eggs	2.9
strawberry	0.3	milk	0.2
oranges	0.25	mushrooms	2.1
blackcurrants	0.4	yeast	5.3-11

Recommended daily doses of vitamin B5 (tab. II) depend on, besides age and sex, on daily caloric consumption and physiological state of the body (7). Vitamin B5 deficiency symptoms include: fatigue, insomnia, depression, irritability, listlessness, nausea and vomiting, abdominal cramps, muscle weakness, burning sensation in the extremities. In hypovitaminosis, at molecular level, changes occur in the biosynthesis of corticosteroids and cholesterol, inhibition of the oxidative decarboxylation pathway of pyruvate and metabolism of fatty acids, as well as in the antibody biosynthesis process.

TABLE II

Recommended daily doses of pantothenic acid

Pediatrics	Recommended daily intake, mg	Adults	Recommended daily intake, mg
0 - 6 months	1.7	19 years and older	5
6 months - 1 year	1.8	pregnant women	6
1 - 3 years	2	breastfeeding women	7
4 - 8 years	3		
9 - 13 years	4		
14 - 18 years	5		

THE CHEMICAL SYNTHESIS OF PANTOTHENIC ACID

The production of calcium pantothenate, pantothenic acid commercial form, is performed chemically, by heating pantolactone with β-alanine (calcium or sodium salt) in methanol or ethanol (fig. 2).

Racemic pantolactone was obtained in a yield of about 90% from isobutyraldehyde, formaldehyde and hydrogen cyanide, without the need for separation of the intermediate products. Saponification of the second intermediate with a strong acid causes the formation of pantolactone in the reaction media, which can be separated by extraction and/or distillation (6).

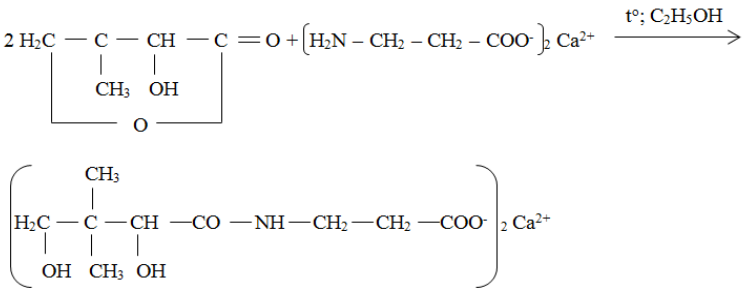

Fig. 2. Calcium pantothenate production by chemical synthesis

For obtaining biologically active enantiomer, R (D)-pantolactone, several methods were developed (6):

1. fractional crystallization of diastereoisomeric salts or amides of pantothenic acid with alkaloids or synthetic chiral amines;

2. the cleavage of the racemic mixture and recycling of S-enantiomer - can be made either by heating with an aqueous solution of NaOH at 150°C for a few hours, until the obtaining of sodium pantoat, or by treatment with very alkaline amines or alkoxides;

3. stereo selective hydrogenation of 2-oxolactone (obtained either by oxidation of 2-aminolactone or pantolactone, or by acid treatment with dimethylpiruvic formaldehyde).

The industrial synthesis of β-alanine can be performed by two methods (6):

a) the addition of ammonia to acrylonitrile, followed by basic hydrolysis of the nitrile with formation of the sodium salt of β-alanine; after sulfuric acid treatment, evaporation and extraction of the residue with methanol, crystalline β-alanine is obtained;

b) ammonia addition to acrylic acid, in which case the sodium salt formation is avoids.

An important problem associated with industrial obtain of calcium pantothenate, apart from the use of toxic chemical reagents - hydrocyanic acid, is the laborious and expensive step of optical resolution of racemic pantolactone. Thus, numerous studies have concentrated on the development of more efficient methods for the preparation of D-pantolactone, the biologically active isomer (8, 9).

Therefore, a more economical alternative to conventional resolution, that uses a quite expensive synthetic alkaloid series, is the biotransformation of racemic pantolactone using a fungal enzyme, lactonohydrolaze, as biocatalyst. The stereospecific hydrolysis of D-lactone is catalysed by this enzyme. Because D-pantolactone is one of the preferred substrates, lactonohydrolaze rapidly converts it in D-pantoic acid, while the L-enantiomer of the racemic mixture remains unchanged and will be racemized and recycled for a new cycle of optical resolution (8, 10).

Some strains of fungi belonging to the genera *Fusarium*, *Gibberella* and *Cylindrocarpon* have a high activity of this enzyme (8, 9, 11). For example, by incubating the *Fusarium oxysporum* cells for 24 hours at 30°C and a pH value of 7, D-pantolactone from the racemic mixture (700 g/l) is 90% hydrolyzed to D-pantoic acid, while the L-isomer remains unchanged (8, 9).

A simplified procedure ensures biocatalytic production of D-pantolactone, with an enantiomeric excess, from 3-hydroxy-2,2-dimethyl-propionaldehyde and hydrogen cyanide, followed by acid hydrolysis. As biocatalyst, the hydroxyl-nitrilase isozyme from *Prunus amygdalus* is used, the reaction being performed at a low pH value to avoid spontaneous formation of the racemic nitrile. A suitable biocatalyst for this process, which is stable and has a high activity to acidic pH, may be obtained from hydroxyl-nitrilase isoenzyme V (10, 12).

BIOSYNTHESIS OF PANTOTHENIC ACID

The development of genetic engineering techniques and the progress of DNA manipulation help researchers, who by modifying chromosomal genes or introducing additional extra chromosomal genetic material, managed the development new mutant strains with the ability to synthesize much larger amount of pantothenic acid as compared with the wild strains.

Gram positive bacteria *Coryne bacterium glutanicum*, used for large-scale production of amino acids (L-glutamic acid, L-lysine) was studied for the biosynthesis of vitamin B5. Thus, if the wild strain produces about 9 mg/l pantothenic acid, by chromosomal deleting the *ilvA* gene coding threonine-dehydratase and over expression combined *ilvBNCD* and *panBC* genes, scientists were able to accumulate more than 1 g/l vitamin in the supernatant (13, 14).

Saccharomyces cerevisiae has the capacity to produce endogenous β-alanine and, therefore, to biosynthesize de novo pantothenic acid, as long as *FMS1* gene, encoding amine oxidase that ensures the transformation of spermine in the β-

alanine, is over expressed (15). Over expression of *FMS1* caused excess pantothenic acid to be excreted into the medium, whereas deletion mutants required β-alanine or pantothenic acid for growth. White et al. concluded that yeast is naturally capable of pantothenic acid biosynthesis, and that β-alanine is derived from methionine via a pathway involving spermine.

For *Escherichia coli*, the responsible genes for encoding enzymes needed for the biosynthesis of vitamin B5 are: *panB*, *panC*, *panD* and *panE*. By overexpressing these genes, a new strain with superior pantothenic acid productivity has been developed (10, 11). Laudert and Hohmann used glucose as a substrate and β-alanine as a precursor and after 72 hours of fermentation was obtained a concentration of 66 g/l pantothenic acid. The main disadvantage of this process, beside the fact that β-alanine is added as a co-substrate, is that over 40 % of the amount of useful product does not come from carbohydrate substrate, but from the synthetized β-alanine.

A promising method for obtaining vitamin B5 is by fermentation processes using the *Bacillus subtilis* gram-positive bacteria. Normally, *Bacillus subtilis* synthesizes only small amounts of pantothenic acid required to refill their needs. By genetic engineering the over expression of the genes *panBCDE*, *ilvBNC* and *ilvD* of bacteria can be achieved, thereby obtaining a higher productivity - 86 g/l in only 48 hours, compared to the fermentation of mutant strain of *E. coli*. In addition, in the case of *Bacillus subtilis*, it is not necessary to add exogenous β-alanine. Therefore, the biotechnologies methods are a feasible alternative and economically solution in the production of vitamin B5 *vs.* chemical synthesis (10, 16).

The pantothenic acid obtained by these methods has to be further extracted from natural extracts or biosynthesis media. The separation of pantothenic acid can be carried out by crystallization, ion exchange, chromatography, and physical extraction (17-20). Further studies focused on the separation of pantothenic acid by reactive extraction, method that has been used for the recovery of biosynthesis products (organic acids, antibiotics, vitamins, amino acids etc.) from aqueous solution using an organic phase in which different extractants are added (21).

Although biosynthesis is an environmentally friendly and fairly cheap alternative to obtain vitamin B5, it is not currently applied to industrial level due to much lower productivity compared with chemical methods. Further efforts are needed to develop new strains whose metabolic flux to be directed mainly towards the synthesis of pantothenic acid, so as to ensure a higher yield (10).

CONCLUSIONS

Industrial production of pantothenic acid is based on chemical synthesis, but an attractive alternative for obtaining this compound, in terms of required steps and consumption of raw materials and energy, is biosynthesis by microorganisms.

With the use of chemical and genetic engineering, scientists were able to improve the properties of the biosynthetic product and also to achieve much higher production yields for pantothenic acid. By modifying chromosomal genes, *Coryne bacterium glutanicum* can produce more than 1 g/l pantothenic acid, compared with about 9 mg/l produced by the wild strain. Introducing additional extra chromosomal genetic material and over expressing some genes led to the development of new strains with superior pantothenic acid production. Thus, *Escherichia coli* can produce in 72 hours of fermentation 66 g/l pantothenic acid, using glucose as a substrate and β-alanine as a precursor, and *Bacillus subtilis* was able to produce 86 g/l vitamin B5 in

only 48 hours.

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VISIBLE SPECTROPHOTOMETRIC METHOD FOR AMIODARONE

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VISIBLE SPECTROPHOTOMETRIC METHOD FOR AMIODARONE (Abstract): Amiodarone is an antiarrhythmic agent used for various types of tachyarrhythmia, both ventricular and supraventricular (atrial) arrhythmia. A spectrophotometric method for the assay of amiodarone was established. Based on the reduction of potassium ferricyanide in hydrochloric acid medium to potassium ferrocyanide forming a blue colored complex ferric ferrocyanide with Fe (III) ions. The compound was most stable in a mixture of ethylic alcohol and water (2:1, v/v) and it had an absorption maximum at 725 nm. The data were according to the Lambert-Beer Law in the concentration range of 0.5-5.0 µg / sample: correlation and coefficient R = 0.99977, R^2 = 0.999541, slope of the line 0.12775, intercept 0.042077. The detection limit (DL) was 0.1032 µg / sample and the quantification limit (QL) 0.344µg/ sample. **Key words:** AMIODARONE; SPECTROPHOTOMETRY, VALIDATION.

Amiodarona, a class III of antiarytmic reagent has been clinically used as one of the most powerful antiarrhythmic drugs for the treatment of a wide variety of arrhythmias. Its main effect is to delay repolarization and to prolong the action potential duration of atrial and ventricular muscle without altering the resting membrane potential. Amiodarone thus lengthens the refractory period of atrial and ventricular myocardium, the atrioventricular node, and of the accessory pathways which mediate Wolff-Parkinson-White (WPW) syndrome.

This paper presents a new spectrophotometric quantitative method of amiodarone using as reagent potassium ferricyanide and ferric chloride in hydrochloric acid medium forming ferric ferrocyanide.

The proposed method is based on the reaction of amiodarone with ferric ferrocyanide in hydrochloric acid medium is released and it reduces the proposed reagent in a blue compound with a maximum of absorption of 725 nm.

The following protocol was set. 2 ml ferric ferricyanide solution was considered ideal for the reduction and the necessary acidity level of the reaction was obtained by adding 1 ml 2N hydrochloric acid solution. It was established that the absorbance could be read after at least 20 minutes after addition of all reagents, and the reaction product was stable for at least 30 minutes.

This paper presents the validation of the VIS spectrophotometric determination method for amiodarone.

MATERIAL AND METHODS

- Stock solution of amiodarone 0.1 mg/ml: 0.010 g amiodarone is dissolved in

100 ml ethylic alcohol. Standard solution with concentrations ranging between 0.5-5.0 μg/ml are prepared from this solution;

- $FeCl_3$ - 0.04% (m/v) aqueous solution;
- $K_3[Fe(CN)_6]$ - 0.02% (m/v) aqueous solution;
- main reagent: 1:1 mixture of $FeCl_3$ - 0.04% (m/v) aqueous solution and $K_3[Fe(CN)_6]$ - 0.02% (m/v) aqueous solution;
- HCl 2N solution;
- Spectrophotometer UV-VIS Hewlett-Packard 8453.

The proposed method for determining of amiodarone is based on the reduction of potassium ferricyanide in hydrochloric acid medium which forms ferric ferrocyanide when reacting to ferric chloride. The blue colored compound is most stable in a mixture of ethylic alcohol and water (2:1 v/v) and has an absorption maximum at 725 nm against a witness whose absorbance is negligible.

The reactions that take place:

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \qquad E_{o\ ox.\ red} = 0.356V;$$

$$Fe(CN)_6^{4-} + 4Fe^{3+} \rightarrow Fe_4[Fe(CN)_6]^3;$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \qquad E_{o\ ox.\ red} = 0.771V.$$

1 ml sample containing 0.5-5.0 μg/sample amiodarone is measured in a series of 5 ml volumetric flasks. 1 ml HCl 2N, 2 ml ferric ferrocyanide freshly prepared reagent is added to the sample and then it is brought to 5 ml with ethylic alcohol. The mixture is homogenized and it is left to rest for 20 minutes, then the absorbance of the blue compound is measured at 725 nm against a witness.

RESULTS AND DISCUSSION

In order to validate the VIS spectrophotometric method for the assay of amiodarone, the following parameters have been studied: the detection wavelength, the linearity, the detection and the quantification limit, the precision of the system, the precision of the method and the accuracy.

Establishing the optimum detection wavelength [4-7]

From the absorption spectrum of the colored compounds, we noticed that the formed complex shows a 725 nm a maximum of absorbance at a wavelength of when compared to the reagent and amiodarone alone as it is shown in Figure 1.

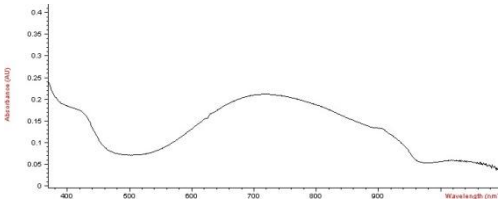

Fig. 1. Absorption VIS spectrum for the colored compounds

Linearity [4-7]

For the study the linearity of the method, we prepared amiodarone solutions by diluting the stock solution with distilled water in the 0.5–5.0 μg/sample concentration range. These solutions were processed according to the method. After obtaining the final product, we read the absorbance of each sample against a witness prepared in the same conditions at 725 nm. After processing the data through mathematical regression, a calibration curve could be obtained showed in the second Figure. The obtained data and the statistical evaluation are registered in table I and II.

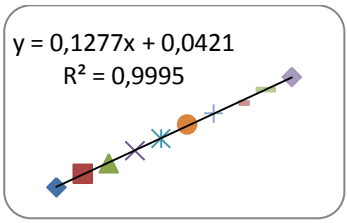

Fig. 2. Calibration curve

The equation of the calibration curve calculated through mathematical regression has the following profile:

Absorbance = 0.12775 concentration + 0.043077

TABLE I

The study of the linearity of the method

No	Conc. µg/ml	Absorbance				
		I	II	III	IV	Average
1.	0.5	0.10541	0.10563	0.10428	0.09888	0.10355
2.	1.0	0.17155	0.17461	0.17848	0.17408	0.17468
3.	1.5	0.22765	0.22521	0.23532	0.23042	0.22965
4.	2.0	0.30590	0.30255	0.26677	0.30866	0.29597
5.	2.5	0.35890	0.36288	0.36101	0.35985	0.36066
6.	3.0	0.43285	0.43601	0.43775	0.42559	0.43305
7.	3.5	0.48851	0.49473	0.49116	0.49220	0.49165
8.	4.0	0.54602	0.54824	0.53924	0.55502	0.54713
9.	4.5	0.61588	0.62043	0.61476	0.61021	0.61532
10.	5.0	0.66819	0.71276	0.67625	0.67168	0.68222

TABLE II

Linearity – statistical evaluation

Correlation coefficient (r)	0.999541
Regression coefficient (r^2)	0.99977
Standard error (SE)	0.0043946
Intercept	0.042077
Slope	0.12775

After the statistical evaluation of the linearity, the detection limit was determined that represents the minimum concentration of the compound in the sample that can be detected but not necessarily quantified using the formula (SE is the regression standard error):

LD = 3 x SE / slope = 0.1032 µg/sample

We have determined the quantification limit (LQ) that represents the minimum concentration of the amiodarone that can be determined with an acceptable precision and accuracy, in the described experimental conditions, it was calculated using the formula:

LQ = 10 x SE/ slope = 0.344 µg/sample.

Precision characterizes the consistency degree of the analytical results, when the analysis method is repeatedly applied to the same sample to be analyzed. It represents the performance degree of the methods and the instruments reflected on the results obtained under normal working conditions.

The performed studies for determining the precision include the precision of the system and the precision of the amiodarone determination method.

In order to evaluate the precision of the system, we have done 6 determinations for

the same sample (2 μg/sample), under the same experimental conditions and then we have calculated the standard deviation and the relative standard deviation (table III).

TABLE III
Precision of the system

No	Absorbance	No.	Absorbance	Statistical Data
1.	0.41667	6.	0.43294	Mean = 0.42452 SD = 0.006238 RSD = 1.47 %
2.	0.43193	7.	0.42417	
3.	0.42403	8.	0.42249	
4.	0.42361	9.	0.41765	
5.	0.41855	10.	0.43316	

After the statistical processing of the obtained data, we have found that the value of the relative standard deviation (RSD) is 1.4694 %, which shows that the system is precise.

Precision of the method

We have evaluated the precision of the method for a concentration range of ± 25 % compared to the target value (2 μg/sample). In order to validate the method (repeatability assessments) we have analyzed two sets of samples, each in different day, with at least 9 determinations covering the specific concentration area (tables IV and V).

The calculated confidence range for each individual value using equation (3) (for 8 degrees of freedom and a precision of 95%, t = 2.31) is 97.15 – 106.01 % because $x - (t\ SD) < \mu < x + (t\ SD)$ (3).

Calculating the confidence range for each individual value, for 8 degrees of freedom and a precision of 95% (t = 2.31), according to the equation (3), we have obtained: 98.48 – 104.95 %.

For the first set of determinations, we have obtained RSD = 1.89 %, for the second set RSD = 1.40 % and for both sets RSD = 1.65 %, values smaller than 2%, but closer to the precision of the system (RSD = 1.47 %). All these values confirm that the proposed method is precise.

TABLE IV
Precision of the method - first set

No	Theoretical concentration (μg/sample)	Absorbance	Calculated concentration (μg/sample)	%	Statistical Data
1	1.5	0.30393	1.5300	102.00	Mean = 101.58 SD = 1.9185 RSD = 1.89 %
2		0.30766	1.5600	104.00	
3		0.29896	1.4900	99.34	
4	2.0	0.36862	2.0500	102.50	
5		0.37359	2.0900	104.50	
6		0.36116	1.9900	99.50	
7	2.5	0.43082	2.5500	102.00	
8		0.42709	2.5200	100.80	
9		0.42336	2.4900	99.60	

TABLE V
Precision of the method – second set

No	Theoretical concentration (µg/sample)	Absorbance	Calculated concentration (µg/sample)	%	Statistical Data
1	1.5	0.30393	1.5300	102.00	Mean = 101.71% SD = 1.40 RSD = 1.38 %
2		0.30268	1.5199	101.33	
3		0.29895	1.4900	99.33	
4	2.0	0.36625	2.0309	101.55	
5		0.37036	2.0640	103.20	
6		0.36196	1.9965	99.82	
7	2.5	0.43456	2.5801	103.20	
8		0.43393	2.5750	103.00	
9		0.43082	2.5500	102.00	

The accuracy of the method is expressed by its recovery its expressed by a percentage of analyzed substance and it depends on the quality of the results due to the analysis method, the equipments, the reagents and the quality of the operations necessary for obtaining these results.

In order to determine accuracy of the amiodarone determination method, we used the addition method. Using the regression equation and the values of the corresponding absorbance, was calculated the concentration in µg/sample for each sample (table VI).

TABLE VI
The accuracy of the method

No	Theoretical concentration (µg/sample)	Absorbance	Calculated concentration (µg/sample)	Recovery %	Statistical Data
1	1.5	0.29897	1.4901	99.34	Mean = 100.14% Min = 98.00 % Max =102.35 %
2		0.29902	1.4905	99.37	
3		0.30297	1.5223	101.48	
4	2.0	036825	2.0470	102.35	
5		0.36285	2.0036	100.18	
6		0.36196	1.9965	99.82	
7	2.5	0.41838	2.4500	98.00	
8		0.42592	2.5106	100.42	
9		0.42558	2.5079	100.32	

The statistical data shown in Table VI shows that in the concentration range of 0.5 – 5.0 µg amiodarone per sample, the mean recovery is 100.14% (not less then 98.00 % and not more than 102.35 %). These values proved that the method was accurate.

CONCLUSIONS

Following the study carried out on the reaction of the ferric ferrocyanide with amiodarone a new spectrophotometric method for the assay of amiodarone is proposed.

The proposed method is simple, fast and it has the advantage of an increased sensitivity; the detection limit is 0.1032μg/sample and the quantification limit is 0.344μg/sample with amiodarone clohridate.

The analysis method was validated the setting optimum wavelength of detection at 725 nm. We also studied the linearity of the method in the range of 0.5– 5.0 μg amiodarone /sample, the correlation coefficient was r^2 = 0.99977, the accuracy (mean recovery =100.14%).the precision of the system (RSD = 1.47 %) and the precision of the method (RSD = 1.65 %).

In conclusion, the proposed method is linear, precise, accurate, simple and fast and it can be applied with good results for the assay with amiodarone in different pharmaceutical dosage forms.

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INTERACŢIUNI ÎNTRE ANTICOAGULANTELE ORALE ŞI ALTE MEDICAMENTE ÎN UNITĂŢILE DE PRIMIRE URGENŢE – O ABORDARE FARMACOEPIDEMIOLOGICĂ

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INTERACTIONS BETWEEN ORAL ANTICOAGULANTS AND OTHER MEDICATION IN EMERGENCY DEPARTMENT-A PHARMACOEPIDEMIOLOGIC APPROACH (Abstract): Oral anticoagulants (OAC) are commonly used as a life-long therapy in the prevention of systemic embolism in patients with atrial fibrillation, valvular heart disease, prosthetic heart valves, and in the primary and secondary prevention of venous thromboembolism, in patients with acute myocardial infarction and angina pectoris. Drugs, herbs, and multivitamin supplements can alter the absorption, pharmacokinetics or pharmacodynamics of OAC. **Aim**: To investigate the possible interactions between oral anticoagulants and different drugs administered in patients in emergency department. Methods: This 1- year exploratory investigation on 93 patients on OAC (aged 30 – 85 years), admitted at two emergency departments of the Iasi „Sf. Spiridon" Hospital and Piatra Neamţ Hospital, consisted in their medical records analysis: diagnosis, recommended treatement. Data were scored and statistically analyzed by t-test (Windows Excel). The experimental protocol was in agreement with the recommendations of the Gr.T. Popa University Committee for Research and Ethical Issues. **Results and conclusions**: Analysis and statistical processing of data shows that during the study period 56 patients which received oral anticoagulant therapy associated with different other medication belonged to the 60 and 80 years age group. This finding suggests that the pathology is represented especially by atrial fibrillation and myocardial infarction. The correlation between the therapy consisting in OAC and sartan or nonsteroidal anti-inflammatory drugs and International Normalized Ratio (INR), revealed a high level of anticoagulation and an increase in hemorragic complications. **Key words**: ORAL ANTICOAGULANTS, DRUG INTERACTIONS, SARTANS

Anticoagulantele orale (ACO) sunt folosite în terapia pe termen lung, pentru prevenţia trombemboliilor sistemice la pacienţii cu fibrilaţie atrială cronică, cu boli cardiace valvulare sau cu proteze valvulare şi în tromembolismul pulmonar (1,2,3). Numeroase studii prezintă informaţii referitoare la interacţiuni ce apar între anticoagulantele orale şi diverse medicamente utilizate concomitent (4,5,6).

Controlul efectului anticoagulant se realizează prin măsurarea timpului de protrombină (timpul Quick), care evaluează activitatea complexului protrombinic, fiind sensibil, mai ales, în lipsa factorilor II, VII şi X ai coagulării (7). Datorită posibilelor

erori se recomandă folosirea ca reactiv de referinţă a unei tromboplastine sensibil standardizate (preparată din creier uman), exprimarea rezultatelor făcându-se prin raportul INR (International Normalized Ratio) şi prin determinarea timpului tromboplastinei parţial activate (aPTT) (8,9).

Lucrarea îşi propune să realizeze unele investigaţii ale interacţiunilor între anticoagulantul oral cumarinic (ACOC), acenocumarol şi alte medicamente recomandate pacienţilor aflaţi în situaţii de urgenţă.

MATERIAL ŞI METODĂ

Studiul s-a desfăşurat pe o perioadă de un an pe un număr de 93 de pacienţi (cu vârste cuprinse între 30 ani şi 85 ani), care au primit tratament cu anticoagulante orale. Cerecetările efectuate au cuantificat nivelul de anticoagulare al pacienţilor care au fost asistate în Unitatea de Primire Urgenţe (UPU) a Spitalului de Urgenţe „Sf. Spiridon" Iaşi şi Spitalul Judeţean de Urgenţe Neamţ (SJN), pentru diverse afecţiuni care au necesitat terapie cu ACOC pe termen lung şi care au primit şi alte medicamente, care au putut interacţiona cu acestea. S-au stabilit corelaţii clare între eficacitatea terapeutică a ACOC şi diverse medicamente utilizate simultan.

Criteriile de includere au fost: persoane cu domiciliul stabil în localitate, vârsta între 18-85 ani, prezentarea la UPU pentru efectuarea analizelor lunare sau de urgenţă, iar criteriile de exclu:dere au cuprins: vârsta sub 18 ani sau peste 85 ani, refuzul subiecţilor, date incomplete, persoane cu domiciliul în alte localităţi.

Culegerea datelor preliminare s-a efectuat cu ajutorul registrelor de consultaţii din UPU, a anchetei privind utilizarea anticoagulantelor orale, prin măsurători clinice şi determinări de laborator şi au luat în consideraţie sănătatea pacientului şi condiţiile de viaţă (vârstă, sex, ocupaţie); rezultate recente privind: timpul Quick (TQ), INR, aPTT efectuate în UPU, date referitoare la tratament, respectiv ACO, sartani sau antiinflamatoare nesteroidiene (AINS). Într-o etapă următoare, au fost definite cazurile şi expunerea, studiul urmărind şi măsurarea asocierii dintre comorbiditate ca factor de risc cu implicaţie în relaţia de cauzalitate. Studiul s-a efectuat cu acordul Comisiei Etice a UMF ''Gr.T. Popa'', Iaşi. Datele au fost centralizate şi prelucrate statistic cu ajutorul testului *t-Student* din Microsoft Office Excel.

REZULTATE ŞI DISCUŢII

1. Distribuţia pe sex. La SJ Neamţ, în perioada studiată, s-au prezentat un număr de 35 de pacienţi care au fost trataţi simultan cu ACOC şi sartani sau AINS, din care 22 au fost de sex masculin.În perioada studiată, la UPU a Spitalul „Sf. Spiridon" Iaşi s-au prezentat un număr de 58 de pacienţi, care au fost trataţi simultan cu ACOC şi sartani sau AINS, din care 30 au fost de sex masculin (tab. I).

TABELUL I

Distribuţia pe sex a pacienţilor incluşi în studiu.

Spitalul Judeţean PiatraNeamţ			**Spitalul „Sf. Spiridon" Iaşi**		
Sex	Nr.	%	Sex	Nr.	%
Masculin	22	62,9	Masculin	30	51,7
Feminin	13	37,1	Feminin	28	48,3
Total	35	100,0	Total	58	100,0

2. Distribuția pe grupuri de vârstă. În studiu au fost incluşi pacienţi cu vârste între 30 şi 85 de ani, care s-au prezentat la Unităţile de Primire Urgenţe. Cei mai mulţi dintre pacienţi aparţin grupei de vârstă cuprinsă între 70 şi 80 de ani, media de vârstă fiind de 64,5 ani la Spitalul Judeţean Neamţ (41,7%) şi 70,4 ani la Spitalul „Sf. Spiridon" Iaşi (36,2%).

TABELUL II

Distribuţia pe grupuri de vârstă a pacienţilor din cele două grupuri de studiu.

	Grupul de vârstă (ani)	Nr.	% *
Pacienţi	30 – 40	1	1,7
Spitalul „Sf. Spiridon" Iaşi	40 – 50	2	3,4
	50 – 60	8	13,8
	60 – 70	12	20,7
	70 – 80	21	36,2
	80	14	24,1
	Total	58	100,0

	Grupul de vârstă (ani)	Nr.	% *
Pacienţi	30 - 40	3	8,6
Spitalul Județean Piatra Neamţ	40 - 50	1	2,9
	50 - 60	4	11,4
	60 - 70	10	28,6
	70 - 80	13	37,1
	80	4	11,4
	total	35	100,0

*pentru valorile mai mici de 10, proporțiile sunt orientative.

3. Distribuția pacienților în relație cu diagnosticul. Centralizarea datelor a arătat că la UPU a SJ Neamţ, diagnosticul principal al pacienţilor a fost FiA (42,9%), urmat de accidente după administrarea de ACOC (17,1%) şi de IMA (14,3%) (tab. III).

Studiul efectuat a evidenţiat faptul că la UPU a Spitalului „Sf. Spiridon" Iaşi, cea mai mare parte din pacienţi prezentau FiA (58,6%) şi, în ordine descrescătoare a incidenţei, IMA (25,9%) şi accidente după administrarea de anticoagulante orale ACOC (6,9%)(tab. IV).

TABELUL III

Distribuţia în relaţie cu diagnosticul pacienţilor de la UPU a SJ Neamţ

Pacienți	Afecțiune	Nr.	% *
Spitalul Județean, Piatra Neamţ	fibrilaţie atrială (FiA)	15	42,9
	infarct miocardic acut (IMA)	5	14,3
	tromboză venoasă profundă (TVP)	1	2,9
	tromboembolism pulmonar (TEP)	2	5,7
	accident vascular cerebral (AVC)	2	5,7
	accidente după ACOC	6	17,1
	proteză valvulară	2	5,7
	altele	2	5,7
	Total	35	100,0

*pentru valorile mai mici de 10, proporțiile sunt orientative.

TABELUL IV

Distribuţia în relaţie cu diagnosticul pacienţilor de la UPU Spitalul „Sf. Spiridon" Iaşi

	Afecţiunea	Nr.	% *
Pacienţi **Spitalul „Sf. Spiridon" Iaşi**	FiA	34	58,6
	sindroame coronariene acute (SCA)	15	25,9
	TVP	2	3,4
	AVC	2	3,4
	accidente după ACOC	4	6,9
	proteză valvulară	1	1,7
	Total	58	100,0

*pentru valorile mai mici de 10, proporţiile sunt orientative.

4. Distribuţia pacienţilor în relaţie cu medicaţia primit. Dintre cei 35 de pacienţi incluşi în studiu care s-au prezentat la UPU Piatra Neamţ cu tulburări de coagulare, 12 au fost trataţi cu acenocumarol (Sintrom(R) sau Trombostop(R)), 12 cu ACOC şi AINS, iar 11 au primit tratament cu ACOC şi sartani (tab. V).

TABELUL V

Distribuţia în funcţie de tratament pacienţilor de la UPU din cele două spitale

Pacienţi	Tratament	Nr.	%
Spitalul Judeţean Piatra Neamţ	ACOC	12	34,3
	ACOC+AINS	12	34,3
	ACOC+sartani	11	31,4
	total	35	100,0
Spitalul Clinic de Urgenţă „Sf. Spiridon" Iaşi	ACOC+sartani	27	46,6
	ACOC+AINS	24	41,4
	ACOC	7	21,1
	total	58	100,0

Dintre cei 58 de pacienţi incluşi în studiu care s-au prezentat la UPU „Sf. Spiridon " Iaşi cu tulburări de coagulare, 27 au primit tratament cu ACOC şi sartani, 24 cu ACOC şi AINS, şi 7 doar cu acenocumarol (Trombostop(R)).

5. Corelaţii între diagnostic, sex, tratament şi TQ, INR, aPTT INR este parametrul cel mai utilizat în evaluarea eficacităţii terapiei cu ACOC. Riscul complicaţiilor hemoragice sau tromboembolice creşte dacă INR este în afara intervalului terapeutic recomandat.

Centralizarea datelor studiului de la UPU Piatra Neamţ privind tipul de tratament primit de pacienţi, evidenţiază faptul

că există o corelaţie pozitivă (p<0,01) între tipul de tratament şi nivelul INR şi negativă pentru aPTT, la persoanele care au primit concomitent ACOC şi AINS, dar şi la cei care au utilizat ACOC şi sartani. Astfel, se observă că nivelul INR este crescut, ca urmare a interacţiunilor medicamentoase apărute, atât la cei care sunt trataţi simultan cu ACOC şi sartani, cât şi la cei care au primit ACOC şi AINS (tab. VI).

TABELUL VI

Corelaţii – UPU a SJ Neamţ

Elemente pentru corelaţii	Indicatori statistic-matematici	Diagnostic	Distribuţia pe sex	Tratament	TQ	INR	aPTT
Diagnostic	Corelaţie Pearson	1	,218	-,198	,006	,122	,035
	Sig. (2-tailed)		,215	,364	,972	,492	,844
Distribuţia pe sex	Corelaţie Pearson	,218	1	,285	,117	,176	-,165
	Sig. (2-tailed)	,215		,177	,504	,313	,344
Tratament	Corelaţie Pearson	-,198	,285	1	,298	,198	,045
	Sig. (2-tailed)	,364	,177		,158	,353	,836
TQ	Corelaţie Pearson	,006	,117	,298	1	-,207	,192
	Sig. (2-tailed)	,972	,504	,158		,232	,269
INR	Corelaţie Pearson	,122	,176	,198	-,207	1	**-,653****
	Sig. (2-tailed)	,492	,313	,353	,232		,000
aPTT	Corelaţie Pearson	,035	-,165	,045	,192	**-,653****	1
	Sig. (2-tailed)	,844	,344	,836	,269	,000	
	N	34	35	24	35	35	35
** Corelaţia este semnificativa la nivelulul 0,01							

Studiul efectuat la UPU „Sf. Spiridon” Iaşi evidenţiază faptul că există o corelaţie pozitivă (p< 0,05) între tipul de tratament şi nivelul INR, TQ şi negativă pentru aPTT, la persoanele care au primit concomitent ACOC şi AINS, dar şi la cei care au utilizat ACOC şi sartani. De asemenea, există o corelaţie semnificativă statistic între nivelul INR, TQ şi diagnosticul principal al pacienţilor (p< 0,01). Astfel, se constată că nivelul INR este crescut, ca urmare a interacţiunilor medicamentoase apărute, la pacienţii trataţi simultan cu ACOC şi sartani, cât şi la cei care au primit ACOC şi AINS (tab. VII).

Într-un studiu se arată existenţa de interacţiuni medicamentoase între ACOC şi AINS din 112 pacienţi 46% au prezentat creşterea INR peste nivelul terapeutic după introducerea AINS (13). Fukuda T. et al (12) au stabilit influenţa metabolismului candesartanului asupra ACOC într-un studiu in vitro realizat la Şcoala de Ştiinţe Farmaceutice de la Universitatea Osaka.

TABELUL VII.

Corelaţii – UPU a Spitalului „Sf. Spiridon” Iaşi

Elemente pentru corelaţii	Indicatori statistic-matematici	Distribuţia pe sexe	Grupuri de vârstă	Diagnostic	Tratament	TQ	INR	aPTT
Distribuţia pe sexe	Corelaţie Pearson	1	,168	,009	,122	,210	,245	,305
	Sig. (2-tailed)		,208	,946	,361	,113	,064	,251
Grupuri de vârstă	Corelaţie Pearson	,168	1	-,125	**,282***	-,034	-,025	-,044
	Sig. (2-tailed)	,208		,351	,032	,798	,851	,872
Diagnostic	Corelaţie Pearson	,009	-,125	1	,184	**,313***	**,347****	,305
	Sig. (2-tailed)	,946	,351		,167	,017	,008	,251
Tratament	Corelaţie Pearson	,122	**,282***	,184	1	-,114	-,047	-,418
	Sig. (2-tailed)	,361	,032	,167		,395	,725	,108
TQ	Corelaţie Pearson	,210	-,034	**,313***	-,114	1	**,890****	**,743****
	Sig. (2-tailed)	,113	,798	,017	,395		,000	,001
INR	Corelaţie Pearson	,245	-,025	**,347****	-,047	**,890****	1	**,501***
	Sig. (2-tailed)	,064	,851	,008	,725	,000		,048
aPTT	Corelaţie Pearson	,305	-,044	,305	-,418	**,743****	**,501***	1
	Sig. (2-tailed)	,251	,872	,251	,108	,001	,048	
	N	16	16	16	16	16	16	16
* Corelaţia este semnificativă la nivelului 0,05 Sig. (2-tailed)								
**Corelaţia este semnificativă la nivelului 0,01 Sig. (2-tailed)								

CONCLUZII

Utilizarea anticoagulantelor orale concomitent cu alte tratamente, cu AINS sau cu sartani, fără modificarea dozelor iniţiale, a determinat creşterea nivelului de anticoagulare la aceşti pacienţi, obiectivat prin creşterea nivelurilor INR, TQ şi prelungirea aPTT. Din studiul efectuat la cele două UPU a două spitale judeţene din Moldova se concluzionează următoarele:

- se observă un număr mai mare de pacienţi de sex masculin, la ambele UPU, cu un plus la Piatra Neamţ, respectiv, 62,7% bărbaţi, faţă de Iaşi, 51,7% bărbaţi;

- grupa de vârstă a pacienţilor este asemănătoare la cele două spitale, dar cu o medie de vârstă mai mare pentru pacienţii de la Iaşi, respectiv 70,4 ani, faţă de 64,5 ani, la Neamţ;

- în ceea ce priveşte diagnosticul prin-

cipal pentru care s-au utilizat ACOC, rezultatele arată că FiA este cea mai des întâlnită la pacienţii prezentaţi la ambele spitale judeţene, urmată de IMA în ambele UPU;

- nivelul mediu al INR la pacienţii sosiţi la cele două UPU este asemănător (4,51 la Neamţ, faţă de 4,58 la Iaşi);

- corelaţiile semnificative statistic sunt asemănătoare la cele două spitale judeţene, între nivelul INR, TQ, şi tipul de tratament primit de pacienţi.

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OPTIMIZATION OF TOPICAL GELS WITH BETAMETHASONE DIPROPIONATE: SELECTION OF GEL FORMING AND OPTIMAL COSOLVENT SYSTEM

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OPTIMIZATION OF TOPICAL GELS WITH BETAMETHASONE DIPROPIONATE: SELECTION OF GEL FORMING AND OPTIMAL COSOLVENT SYSTEM (Abstract): The purpose of these studies was to develop a 0.05 % betamethasone gel characterized by physical-chemical stability and good release properties. The preliminary studies were designed to select the gel-forming agents and the excipients compatible with betamethasone dipropionate. In order to formulate a clear gel without particles of drug substances in suspension, a solvent system for the drug substance was selected. The content of drug substance released, the rheological and in vitro release tests were the tools used for the optimal formulation selection. A stable carbomer gel was obtained by solubilization of betamethasone dipropionate in a vehicle composed by 40 % PEG 400, 10 % ethanol and 5 % Transcutol

The use of topical corticosteroids started the revolutionary management of skin diseases. In topical formulations, betamethasone dipropionate (BDP), a synthetic adrenocorticosteroid, is indicated for relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses.

For topical formulation, the vehicles have a significant influence in stability and efficiency. The excipients could act by increasing and optimizing the thermodynamic activity of drug in the vehicle and promote the release from vehicle to the skin (1, 2, 3). The incompatibilities between drug substance and excipients were the primary cause of degradation reactions.

The goal of these studies was to develop a 0.05 % betamethasone (B) gel by selecting the excipients capable to assure, in the same time, the solubilization of BDP, chemical stability and good release properties of drug from vehicles.

MATERIAL AND METHODS

Betamethasone dipropionate (Farmabios, Italy); Benzyl alcohol (Brenntag Tesenderlo Chemie NV, Germany); Carbomer *Carbopol® Ultrez 10* (Lubrizol Corporation, USA); Caprylocaproyl Macrogolglycerides *Labrasol®* (Gattefosse, Canada); Diethylene Glycol Monoethyl Ether *Transcutol®* (Gattefossé, France); Dimethyl sulfoxide *DMSO* (Gaylord Chemical, Germany); Glycerol (Evayap International, Turkey); Hexylene Glycol - *HG* (Adiego Hnos. S.A., Spain); Hydroxypro-

pyl methylcellulose (HPMC) - Metholose 60-SH 4000, Shin-Etsu, Japan; Isopropyl myristate - IPM (Cognis, Germany), Macrogol 400-*PEG 400* (Sasol, Slovakia); N-methyl pyrrolidone (NMP) *Pharmasolve*® (ISP, Switzerland); Poloxamer 407 *Lutrol*® F 127 (BASF, Germany), Propylene glycol *PG* (SC Oltchim, Romania) and Sodium alginate *Manucol LKX* (ISP, Germany) were used. All chemicals were either analytical or pharmaceutical grade and they were used as received.

Preparation of gels

For the preparation of 8 gels formulation (G-G8), 0.05 % BDP was suspended in four types of gel forming agents, at two different concentrations each: carbomer, poloxamer, metholose and sodium alginate (Na alginate).

For the formulations coded G9 to G32, the BDP was first suspended/dissolved in different mixtures of aqueous excipients with humectants, solvents or permeation enhancers and then incorporated in the gel mass (4).

BDP suspensions in different aqueous excipients

Suspensions of 0.05 % B (w/v) were prepared using binary mixtures (1:1, w/w) of water and different excipients: DMSO, Glycerol, HG, IPM, Labrasol, NMP, PEG 400, PG and Transcutol® (Table II). The suspensions were stored at temperatures of 40±2°C and 75±5 % RH (relative humidity) for 3 months.

In Vitro Drug Release Studies

The *in vitro* release studies were carried out in static type diffusion cells having 15-mm orifice (1.76 cm^2 diffusion area) and 7 mL capacity. The studies were performed using synthetic nylon membranes (25 mm diameter, 0.45 μm) and hydroalcoholic receptor medium (phosphate buffer pH 7.4 : ethanol 1:1, w/w), thermostated at constant temperature (35±0.5°C). Samples were collected every hour, for six hours and the BDP content was analyzed spectrophotometrically at 254 nm. The total amount of BDP was determined in each cell. The micrograms per cubic centimeter released at each time interval were plotted against square root of time. We determined the slope of the line (R, release rate), using linear regression.

Determination of Viscosity

A rotational viscometer Brookfield RVDV II + Pro and adapter (Helipath Stand with TC spindle), rotated at a speed of 7 rpm, was used to measure the apparent viscosity of the preparations.

Related substances content

For evaluating the B related substance content (any impurity - AI and total impurities - TI), a validated HPLC analytical method was used. The analytical equipment consisted of a liquid chromatograph equipped with UV detector and recorder. The chromatographic conditions were: chromatographic column: Lichrosorb RP 18, 250×4,6 mm, 5 μm; mobile phase – acetonitrile:water 40:60 (v/v); flow rate: 1.0 mL/min.; detector: UV at 254 nm; temperature: 30°C. European Pharmacopoeia 6.0 specifies the following limits for related substances in the monograph "Betamethasone dipropionate": any impurity - max. 1.50 %; total impurities - max. 2.50 % (5,6, 7).

RESULTS AND DISCUSSION

In the first stage of studies, one series of 8 gels was prepared and evaluated after 24 hours and after 1 year from preparation (maintained at 25±2°C) (Table I).

The poloxamer (22 %), metholose and sodium alginate do not allow drug extrac-

tion from gel structure in the sample analytical preparation before HPLC analysis. In G4 formulation, the high level of related substances reflects the incompatibilities between poloxamer and BDP. The sodium alginate forms unaesthetic gels with slight brown color and with filaments in their structure. The optimum gel forming agent for BDP gels was carbomer.

With the purpose of dissolving BDP and obtaining a gel without particles in suspension, the compatibility of BDP with a series of liquid excipients with humectants, solvents or permeation enhancers was tested (8, 9, 10, 11). Composition of suspensions is presented in table II.

TABLE I
Composition and properties of BDP gels from stage I

Gels name and gel-forming	Apparent viscosity, cP	R $\mu g/cm^2/s^{1/2}$	Related substances, %			
			T_0		$T_{12\ months}$	
			AI	TI	AI	TI
G1. Carbomer 1 %	46875	2.27	0.422	0.422	0.736	0.786
G2. Carbomer 0.75 %	39714	3.21	0.448	0.448	0.781	0.840
G3. Poloxamer 22 %	105143	4.58	-	-	-	-
G4. Poloxamer 16.5 %	70429	4.59	0.849	0.968	2.697	4.047
G5. Metholose 4 %	40142	4.23	-	-	-	-
G6. Metholose 3 %	10857	3.72	-	-	-	-
G7. Na alginate 6 %	43150	4.21	-	-	-	-
G8. Na alginate 4 %	11000	3.03	-	-	-	-

TABLE II
Compatibility studies of BDP with liquid excipients

Composition of suspensions	Related substances, %			
	T_0		$T_{3months}$	
	AI	TI	AI	TI
0.05 % B in Water	0.498	0.674	3.097	3.704
0.05 % B in Water/DMSO	0.480	0.650	3.191	4.366
0.05 % B in Water/Glycerol	0.215	0.611	3.131	4.788
0.05 % B in Water/HG	0.517	0.823	2.264	5.986
0.05 % B in Water/IPM	0.857	0.997	1.837	4.435
0.05 % BDP in Water/Labrasol	0.523	0.696	2.354	5.193
0.05 % BDP in Water/NMP	0.593	0.767	2.341	4.360
0.05 % B in Water/PG	0.518	0.657	3.194	4.404
0.05 % B in Water/PEG 400	0.486	0.622	1.593	2.486
0.05 % B in Water/Transcutol	0.467	0.615	0.851	2.379

The measure of compatibility was the level of related substances evaluated initially and after 3 months, when keeping the suspensions at 40±2°C and 75±5 % RH (Table II). The lower values of B related substances were recorded when using PEG 400 and Transcutol. These excipients were selected for the solubilization of BDP in gel formula. In stage two of the studies, a second series of gels was prepared with

different amounts of above-mentioned excipients. PEG 400 used as unique solvent, in concentrations between 5 and 60 %, was not able to maintain the drug substance dissolved.

Adding Transcutol to the formula determined the formation of a clear gel, without particles in suspension (G23, G25, G26). The gels without drug crystals have the disadvantage of high levels of PEG 400 (over 50 %), excipient with irritating properties in topical applications. Consequently, to reduce the PEG 400 content, the formula G19 was selected for optimization. To this formula, different amounts of ethanol were added (5 to 15 %).

TABLE III

Optimization of gels formula by system solvent selection

Solvent	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
PEG 400, %	10	10	10	20	20	20	30	30
Transcutol, %	0	5	10	0	5	10	0	5
BDP crystal presence	+	+	+	+	+	+	+	+
Solvent	**G17**	**G18**	**G19**	**G20**	**G21**	**G22**	**G23**	**G25**
PEG 400, %	30	40	40	40	50	50	50	60
Transcutol, %	10	0	5	10	0	5	10	0
BDP crystal presence	+	+	+	+	+	+	-	+
Solvent	**G25**	**G26**	**G27**	**G28**	**G29**	**G30**	**G31**	**G32**
PEG 400, %	60	60	40	40	40	40	40	40
Transcutol, %	5	10	5	5	5	10	10	10
Ethanol, %	0	0	5	10	15	5	10	15
BDP crystal presence	-	-	+	+	+	+	-	-

+ Crystal present; - Crystal absent

Formula G31, containing 1 % carbomer, 40 % PEG 400, 5 % Transcutol and 10 % ethanol, is considered the final formula (Table III). This formula was examined throughout accelerated stability studies (40±2 °C; 75±5 % RH) for 6 months (Table IV).

TABLE IV

Physical and chemical properties of final formula

Analysis time	**Apparent viscosity, cP**	**R, $\mu g/cm^2/s^{1/2}$**	**BDP crystal**	**Related substances, %**	
				AI	**TI**
T_0	49020	3.35	-	0.489	0.511
$T_{6months}$	47545	3.29	-	1.211	2.271

CONCLUSIONS

The carbomer was the gel-forming agent compatible with BDP and which allows the extraction of drug and proper use of analytical methods. The effect of low release properties of carbomers is neutralized by PEG 400 and ethanol, excipients with good solvent properties for BDP. Supplementary to the molecular dissolution of drug substance, the use of Transcutol, excipient which is

also a permeation enhancer, confers the advantage of therapeutic efficiency (3, 9). The 0.05 % B gel formula characterized by solubilization of drug substance in a vehicle composed by 1 % carbomer, 40 % PEG 400, 10 % ethanol and 5 % Transcutol represents the most stable formulation obtained during this research. After 6 months of accelerated stability studies, the content of related substances was below the maxim admissibility level specified in the European Pharmacopoeia. By extrapolation, the formula may be considered stable for 2 years. The formula also presents optimum physical properties (appearance and viscosity) for topical application.

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CARACTERISTICILE ŞI PROPRIETĂŢILE DE SUPRAFAŢĂ ALE POLI(ETER)URETANULUI PU-PTHF/MDI/HPC CU POTENŢIALE APLICAŢII ÎN PROTEZAREA CARDIOVASCULARĂ

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SURFACE PROPERTIES AND CHARACTERISTICS OF PU-PTHF/MDI/HPC POLY (ETHER)URETHANE FOR CARDIOVASCULAR PROSTHESIS (Abstract): The polyurethanes are very used materials for medical devices. Their segmented block-copolymeric structures endows them a wide range of versatility in terms of tailoring their physical properties, and tissue compatibility. **Material and method**: A new polyether-urethane based material (PU-PTHF/MDI/HPC) was studied on bio-integration capacity in cardiovascular applications. In this respect, surface and bulk structure analysis, albumin and fibrinogen adsorption (in noncompetitive and competitive condition) and thrombogenicity have been evaluated. It was found that PU-PTHF/MDI/HPC surfaces had different roughness and bulk interconnected, well developed porous structure. As protein adsorption properties, preferential adsorption for albumin of material was observed. **Results**: Thus, 0.280 ± 0.04 mg/cm^2 and 0.260 ± 0.01 mg/cm^2 of albumin adsorb on PU-PTHF/MDI/HPC in noncompetitive and competitive conditions, while fibrinogen adsorption was 0.044 ± 0.02 mg/cm^2 and 0.040 ± 0.01 mg/cm^2. The clot amount test experiment have shown that clot weight of control blood was 24.8 ± 2.03 mg, the clot weight formed by polyurethane was 28.4 ± 2.93 mg and the clot weight formed by thrombogenic positive control (collagen membrane) was 42.5 ± 3.50 mg. **Conclusions**: From our study we could conclude: 1 - PU-PTHF/MDI/HPC polyurethane membranes have two-interface structure that could be advantageous for cavitary or tubular medical devices; 2 – the protein adsorption results and clot formation test revealed a thromboresistent behavior of PU-PTHF/MDI/HPC, appropriate for cardiovascular applications. **Key words:** POLYURETHANE, PROTEIN ADSORPTION, THROMBORESISTANCE, CARDIOVASCULAR APPLICATIONS

Polimerii poliuretanici sunt o clasa importantă de materiale sintetice, utilizate pe larg în domeniile biomedicale. Numeroase produse comerciale pe bază de polimeri poliuretanici cu aplicaţii cardiovasculare precum grefe vasculare, valve cardiace, componente ale inimii artificiale, baloane pentru angioplastii, catetere sau altele sunt în prezent disponibile (1,2,3,4). Principalele avantaje ale acestui tip de polimer sunt datorate structurii bloc-segmentate, în care alternează segmentele rigide şi cele flexibile. Astfel, se pot obţine numeroase structuri cu proprietăţi mecanice, fizico-chimice şi de suprafată diferite, în funcţie de componentele de pornire a sintezei, a modului de conducere a reacţiei de polimerizare, precum şi de metodele de obtinere a suporturilor (filme, bureţi, spume etc.). Diversitatea poliuretanilor, explicată prin varietatea segmentelor rigide şi

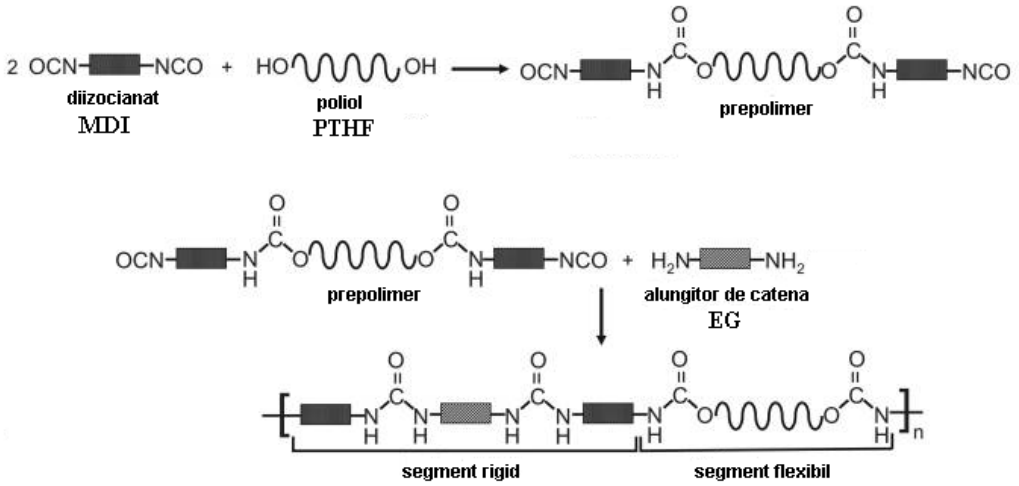

Fig. 1. Reprezentarea schematică a principiului de obţinere PU-PTHF/MDI/HPC

a celor flexibile utilizate la sinteza diblоc--polimerilor, este imbogăţită continuu datorită tehnologiilor noi de obţinere a structurilor mai complexe, de tipul tribloc sau a celor de hidrogeluri (5,6).

În calitate de segmente flexibile, la sinteza poliuretanilor sunt utilizaţi macrodiolii de tip: polietilenglicol (PEG), polietilenoxid (PEO), polipropilenoxid (PPO), policaprolactonă (PCL) şi multe altele. Pentru segmenele rigide sunt frecvent utilizaţi diizocianaţii de tip: diizocianatohexan (HDI), diizocianatobutan (BDI), difenilmetandiizocianat (MDI) şi altele. Prin interacţia celor două componente se obţine un prepolimer, care este extins mai apoi cu ajutorul unui alungitor de catenă de tipul etilenglicolului (EG) a butandiolului (BD) şi a altor molecule cu proprietăţi de legare asemanatoare.

Deşi poliuretanii sunt o clasă de materiale cu o capacitate structurală nelimitată, întrunirea concomitentă a cerinţelor mecanice, precum şi a celor de biocompatibilitate (in particular a celor de hemocompatibilitate) este greu de realizat. Numeroase studii din ultimii ani au fost dedicate găsirii unor formule ideale de structură pentru susţinerea unor funcţii biologice concrete (2,7, 8,9), capitol din care face parte şi această cercetare. Astfel, în aceasta lucrare un nou poliuretan di-bloc segmentat, modificat prin amestec fizic cu derivatul celulozic – hidroxipropilceluloza (HPC) a fost studiat pentru caracteristicile şi proprietăţile sale de integrare functională la interfaţa cu sângele. Materialul a fost selectat în cadrul unor studii anteriaore, ca fiind unul potrivit din punct de vedere mechanic si fizico-chimic pentru acest tip de aplicatii (10,11,12,13,14).

MATERIAL ŞI METODĂ

Materialul poliuretanic luat în studiu a fost obţinut prin reacţia de polimerizare folosind N,N-dimetilformamida (DMF, Fluka) ca solvent. Pentru sinteza poliuretanului, în calitate de macrodiol a fost utilizat polytetrahidroxifuranul (PTHF, Mn = 2,000 g/mol), iar ca diizocianat - 4',4'- difenilmetandiizocianatul (MDI, Merck). Derularea reacţiei de sinteză si a proprietăţilor fiyico-chimice obţinute a fost obictul studiilor anterioare (12,13). Etapelor de sinteză a materialului sunt reprezentate schematic a figura 1. Polimerul sintetizat, notat prin PU-MDI/PTHF/HPC a fost precipitat în apă caldă iar apoi uscat în condiţii de vacuum timp de câteva

zile, pentru obţinerea membranelor cu grosimea de aproximativ 1 mm.

Morfologia de suprafată şi structura internă a PU-MDI/PTHF/HPC a fost studiată cu ajutorul metodei de microscopia electronic de baleaj (SEM), utilizând procedura standard de pregătire a probei, descriesă în literatură (15). Analiza SEM a fost efectuată cu ajutorul microscopului electronic de baleaj VEGA TESCAN, in sistem de vid înaltă (high vacuum – HV) la o tensiune de acceleraţie a electronilor de 30 kV.

Sarcina electrică de suprafaţă a materialului a fost măsurată prin determinarea potenţialului zeta, cu ajutorul analizatorului electrocinetic SurPASS, Anton Paar GmbH. Principiul metodei se bazează pe masurarea potenţialul de interfaţă dintre stratul imobil, purtător de sarcină electrică (polimerul) şi cel difuz, cunoscut ca strat dublu electric creat la interfaţa polimer - lichid (16). Astfel, doua filme poliuretanice cu dimensiuni de 1,5 x 1,0 cm au fost montate pe cele 2 placi de teflon ale aparatului. Pentru fiecare determinare placile erau aduse faţă-în-faţă, astfel încât între cele două suprafeţe polimerice se crea un microcanal prin care se forta trecerea unui electrolit cu debit al fluxului de 80 ml/min la o presiune de 300 mbar În calitate de electrolit s-a folosit o solutie 0,1M de NaCl. Valoarea potenţialului zeta se obţinea ca urmare a trecerii prin sistem a soluţiei de electrolit la interfaţa probei de analizat în direcţii alternative de patru ori (de două ori în fiecare direcţie). Valoarea potenţialului zeta s-a calculat utilizând ecuaţia Fairbrother–Mastin (ecuaţiile 1 şi 2), luându-se în considerare şi efectul conductivităţii suprafeţei.

$$\zeta = \frac{dU}{dp} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times K \quad (1)$$

unde: ζ - potenţialul zeta, dU/dp - panta potenţialului de electrofiltrare şi presiune, η - vâscozitatea electrolitului, ε_0 - permitivitatea vidului, ε - constanta dielectrică a electrolitului,

$$K = \frac{L}{A \times R} \quad (2)$$

unde: L - lungimea canalului de electrofiltrare, A - secţiunea canalului de electrofiltrare, R - rezistenţa internă a celulei de măsurare.

Pentru adsorbţia de proteine, albumina serică bovină (BSA, Sigma-Aldrich) şi fibrinogenul (fibrinogen liofilizat, > 95% coagulabil, Sigma-Aldrich) au fost utilizate.

Adsorbţia de albumina a fost determinată prin metoda indirectă, dozând concentraţia albuminei în soluţie fiziologică (4,5 mg/ml BSA în 0,9% NaCl), înainte şi după incubarea cu polimerul. Materialul cu o suprafaţă totală de 1 cm^2 era incubat într-un volum de 0,5 ml soluţie BSA, timp de 30 min, în condiţii de temperatură constantă, de $+37^0$C şi agitare orbitală de 150 rot/min. Înainte de incubare, probele de polimer erau hidratate în soluţie ser fiziologic steril. Dozarea albuminei s-a efectuat folosind metoda imunoturbidimetrică, cu anticorpi monospecifici pentru albumină, furnizati de kitul DiaLab (Austria). Pentru metodologia de dozare s-a respectat protocolul anexat kitului. Măsurătorile spectrofotometrice au fost efectuate utilizând spectrofotometrul UV-VIS Piccos 05. Formula de calcul pentru determinarea albuminei adsorbite a fost următoarea:

$$Proteina\ adsorbită\ (mg/cm^2) = (Ci - Cp)\ x\ V\ /\ S \quad (3)$$

unde, Ci şi Cp sunt concentraţiile iniţială şi cea de postincubare a soluţiei de proteină cu materialul poliuretanic, V – volumul de incubare a probei, iar S – suprafaţa probei incubate.

Ca şi în cazul albuminei, adsorbţia fibrinogenului a fost determinată prin metoda indirectă. Concentraţia soluţiei de fibrinogen a fost una specifică intervalului de valori normale în sânge, respectiv 3 mg/ml. Pentru dozarea fibrinogenului a fost utilizată metoda turbidimetrică Vincent, cu sulfat de sodium anhidru (17). Masuratorile spectro-

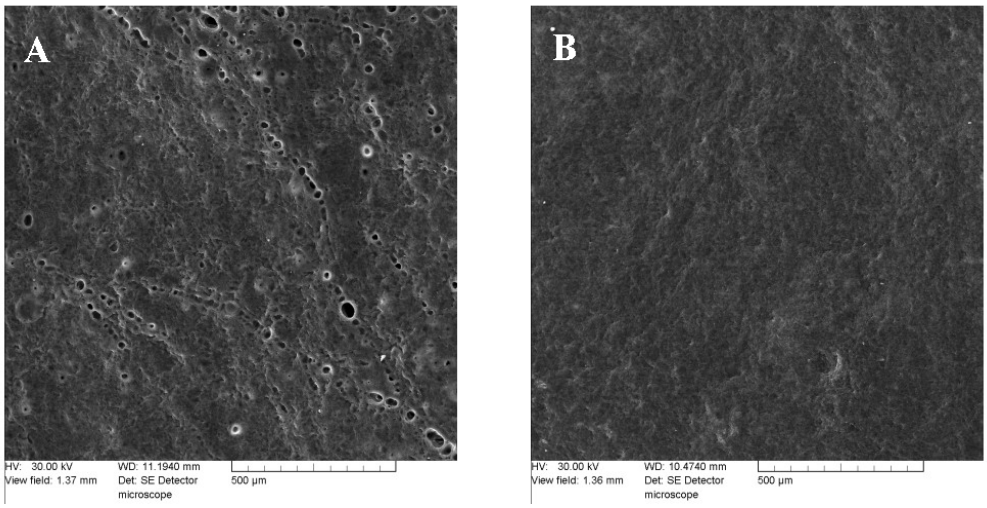

Fig. 2. Morphologia de suprafaţă a poliuretanului PU-PTHF/MDI/HPC
(*A* – faţa inferioara ; *B* – faţa superioară)

fotometrice au fost efectuate cu ajutorul spectrofotometrul UV-VIS Piccos 05. Formula de calcul pentru dozarea fibrinogenului a fost asemanatoare cu cea pentru calculul albuminei (ecuatia 3).

Activitatea trombogenă a materialului fost estimată cu ajutorul testului de formare a cheagului din sângele integral, *in vitro*, folosind metoda Nose (18) modificată în laborator şi descrisă în cercetarile noastre anterioare (11). Metoda reflectă capacitatea totală de coagulare a sângeli şi constă în incubarea unei suprafeţe de material (1 cm^2) cu 0,5 ml sânge integral (recoltat pe citrat de sodiu 3,8%), în condiţii de 37^0C timp de 30 min. La expirarea timpului de incubare, sângele era recalcifiat cu 0,05ml soluţie 0,1M $CaCl_2$, iar cheagul obţinut după 4 min de recalcifiere era cântărit cu ajutorul unei balanţe analitice. Rezultatul era comparat cu cel obţinut pentru sângele martor, incubat în aceleaşi condiţii, dar fără polimer.

REZULATE ŞI DISCUŢII

Din observaşiile privind caracteristicile morfologice de suprafaţă ale poliuretanului PU-PTHF/MDI/HPC (fig. 2) se poate observa că filmul poliuretanic exprimă două feţe cu porozitate şi rugozitate diferită. Structura bifacială este consecinta procesului de precipitare în apă.

Structura intrenă a materialului este reprezentată în figura 3. În imagine se pot observa pori de dimensiuni şi forme diferite. Se pot distinge : 1 - pori mari, cu o formă alungită, cu dimensiuni longitudinale cuprinse între 50 şi 500 μm şi cu dimensiuni transversale cuprinse între 50 şi 150 μm, formaţi ca urmare a pătrunderii, avansării şi fuziunii picăturilor de apă în polimer în timpul procesului de precipitare (fig. 3 A şi B) ; 2 - pori medii, de formă sferică, cu o distribuţie uniformă in întreaga grosime a materialului, cu dimensiuni situate în jurul valorii de 50 μm (fig. 3 C) ; 3 - pori foarte mici, cu diametrul de 1-4 μm (fig. 3 D). Din figura 3 se poate observa, de asemenea, că structura poarasă are forma unei reţele tridimensionale interconectate, permiţând din punct de vedere arhitectural şi dimensional invazia celulară, transferul de mase sau neovascularizarea. Mai mult decât atât, ca urmare a unei orientări bifaciale (asimetrice în raport cu cele doua feţe) a porozităţii, acest material poate

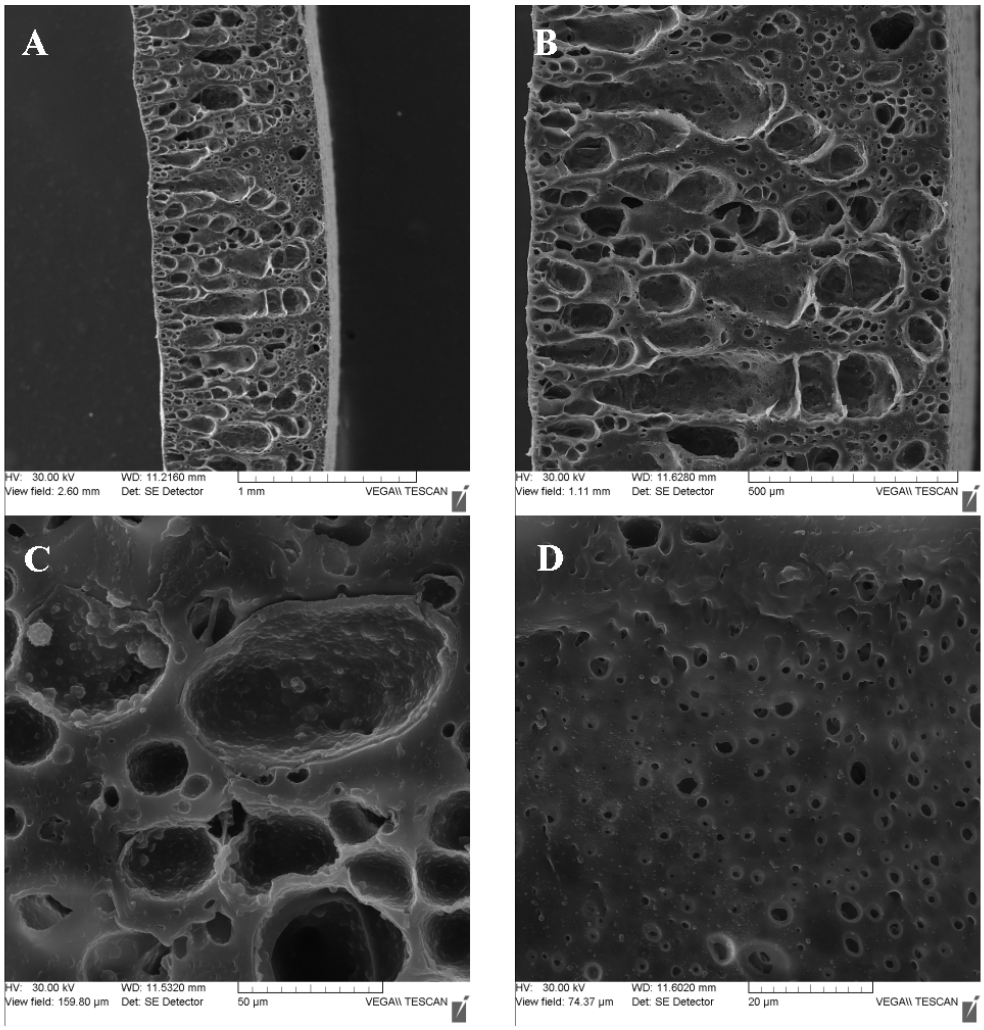

Fig. 3. Secţiune prin filmul poliuretanic PU-PTHF/MDI/HPC. Imagini de microscopie electronică de baleaj (SEM) (*A, B, C şi D* – putere de mărire diferită (scara alăturată fiecărei imagini)

fi util în proiectarea unor dispositive implantabile cu dublă interfaţă (de formă tubulară sau cavitară) pentru care este importantă porozitatea şi rugozitatea redusă orientată spre partea luminală sau cavitară şi porozitatea crescută orientată spre spaţiile interstiţiale. Acest tip de structură, dar şi proprietăţile elastice ale acestui material (9), pot fi considerate caracteristici favorabile pentru o integrare structurală corespunzătoare a materialului în cadrul unui tesut biologic.

Studiile privind capacitatea de integrare funcţională în cadrul potenţialelor aplicaţii cardiovasculare s-au axat pe determinarea unor proprietăţi de suprafaţă importante pentru biocompatibilitate, dar în special pentru hemocompatibilitate. Sarcina de suprafaţă, se ştie, determină iniţierea şi evoluţia fenomenelor nespecifice şi imediate, care au loc la interfaţa unui material sintetic cu ţesutu-

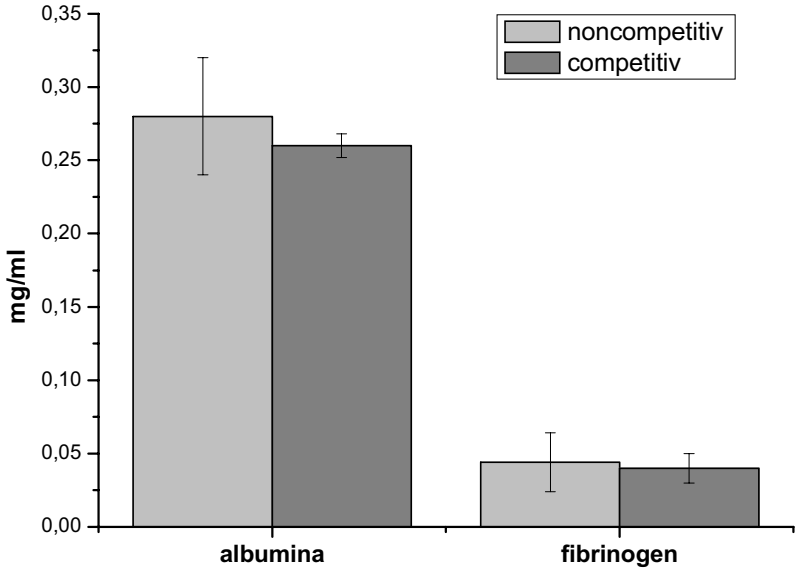

Fig. 4. Adsorpţia de albumină şi fibrinogen în condiţii noncompetitive şi competitive

rile vii. Aceasta intervine în adsorbţia proteinelor din fluidele biologice sau lichidele interstiţiale, ceea ce duce la activarea suprafaţei materialului. Astfel, afinitatea materialului pentru un anumit tip de proteine il poate vulnerabiliza, expunându-l atacului celular sau fenomenelor trombogene sau, din potrivă, il poate functionaliza în sensul biocompatibilizării - prin stimularea migrării, adeziunii şi populării cu celule funcţionale specifice. Este considerat, în general, că o suprafaţă încărcată negativ este un criteriu de biocompatibilitate, în timp ce sarcina de suprafaţă pozitivă favorizează denaturarea conformaţională a proteinelor adsorbite, expunând domeniile funcţionale din miezul hidrofob pe suprafaţa moleculei, astfel ducând la activarea reactiilor de corp străin sau a celor de coagulare la interactiunea cu sângele (19).

În studiul nostru, sarcina de suprafaţă exprimată de materialului PU-PTHF/MDI/HPC la un pH fiziologic de 7,3-7,4 a fost de +0,78 mV. Având în vedere că valoarea potenţialului zeta obţinută de noi a fost aproape de zero şi luând în consideraţie valorile descrise în literatură pentru alte tipuri de materiale (20), putem spune că suprafată PU-PTHF/MDI/HPC este neutră din punct de vedere electric.

Efectul unui potenţial aproape neutru asupra adsorbţiei de proteine a fost în continuare studiat pentru două proteine sanguine importante – albumina şi fibrinogenul. Rezultatele de adsorbţie obţinute în condiţii noncompetitive (soluţii fiziologice simulate simple) şi competitive (soluţie fiziologică simulată mixtă) sunt ilustrate in figura 4.

Din figura 4 se poate observa că PU-PTHF/MDI/HPC adsoarbe o cantitate semnificativ mai mare de albumină decât de fibrinogen. Putem spune, că proprietăţile de suprafaţă ale materialului studiat sunt selective pentru adsorpţia de albumină, atât în condiţii noncompetitive, cât şi în condiţii competitive. Nu s-au observat diferenţe semnificative ale adsorpţiei celor doua proteine în funcţie de prezenţa sau absenşa proteinei concurente, deşi există o uşoară tendinţă de reducere a cantităţii de proteină adsorbită în condiţii

TABELUL I
Cheagul format prin recalcifierea sângelui integral în prezenţa PU-PTHF/MDI/HPC

Suprafaţa incubată	Greutate cheag (mg)	% de cheag peste cel produs de martorul negativ
Martor negativ (fără polimer)	24,8±2,03	0
PU-PTHF/MDI/HPC	28,4±2,93	14,52
Martor pozitiv (colagen)	42,5±3,50	71,37

competitive, în special pentruu albumină. Aceasta poate fi cauzată de dislocarea albuminei de fibrinogen prin fenomenul Vroman, descris în literatura ca proces de înlocuire a primelor proteine adsorbite, de obicei de dimensiuni mai mici şi aflate în concentraţii mai mari, cu proteine de dimensiuni mai mari, care se gasesc în concentraţii mai mici, dar care pot avea o specificitate mai mare de interacţie cu suprafaţa (21). Având în vedere că rezultatele noastre nu au arătat diferenţe semnificative între albumina adsorbită în soluţie pură şi cea adsorbită în prezenţa fibrinogenului, putem spune că fenomenul Vroman, în cazul celor doua proteine, este slab exprimat, adsorbindu-se preferenţial albumina. Aceste date sunt corelate cu studiile noastre anterioare, care arată ca materialul PU-PTHF/MDI/HPC are o hidrofilie crescută, iar conform mai multor studii bibliografice, această proprietate favorizează adsorbţia albuminei (21,22,23). Astfel, rezultatele noastre au evidenţiat proprietăţi de suprafaţă favorabile unui comportamentul tromborezistent al materialul PU-PTHF/MDI/HPC. Pentru a demonsta acesta afirmaţie, însă, am efectuat testul *in vitro* de formare a cheagului din sângele integral. Rezultatele au fost comparate cu cele obtinute pentru un compus cunoscut pentru proprietăţile sale trombogene – un film de aceleaşi dimensiuni, obtinut din colagen bovin. Rezultatele sunt prezentate in tabelul I.

Din tabelul I se poate observa o diferenţă foarte mică între greutatea cheagului format în contact cu PU-PTHF/MDI/HPC şi cel al martorului negativ, obţinut prin recalcifierea sângelui în absenţa materialului. Având în vedere varianţa standard ale rezultatelor obţinute, diferenţa este la limita semnificaţiei statistice. Comparând rezultatul obţinut cu cele ale martorului pozitiv, se poate observa o diferenţă semnificativă între cele două situaţii, ceea ce ne permite să afirmăm că materialul PU-PTHF/MDI/HPC nu modifică proprietatea de coagulare a sângelui.

CONCLUZII

Membranele poliuretanice obtinute prin metoda de precipitare în apă din PU-PTHF/MDI/HPC sunt membrane bifaciale, având o porozitate şi rugozitate diferită a suprafeţelor. Structura internă a membranei este una poroasa, prezintă o reţea de pori interconectaţi, care din punct de vedere al dimensiunilor, poate permite neovascularizarea sau popularea cu celulele la interactiunea cu tesuturile biologice. Sarcina electrică de suprafaţă a membranelor PU-PTHF/MDI/HPC este una usor pozitivă, aproape neutră, ceea ce descurajează adsorpţia de fibrinogen la concentraţia lui fiziologică atât în condiţii idealizate, noncompetitive, cât şi în condiţii de competiţie cu alte proteine. Datorită proprietăţilor sale hidrofile şi a sarcinii superficiale neutre, membranele PU-PTHF/MDI/HPC favorizează adsorpţia de albumină atât în soluţia fiziologică simplă de albumină cât şi în prezenţa fibrinogenului. În final, studiul a demonstrat că membranele poliuretanice de tip PU-PTHF/MDI/HPC sunt tromborezistente, iar proprietăţile coroborate indică o capacitate crescută pentru biointegrare la condiţiile structurale şi funcţionale ale sistemului cardiovascular.

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MEASUREMENT OF TOTAL ANTIOXIDANT ACTIVITY WITH CHLORPROMAZINE RADICAL CATION

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MEASUREMENT OF TOTAL ANTIOXIDANT ACTIVITY WITH CHLORPROMAZINE RADICAL CATION (Abstract): In the present study there is described a simple and sensitive method for the evaluation of antioxidant activity in chlorpromazine-Fe (III) system using ascorbic acid as standard. **Material and method**: Chlorpromazine [2-chloro-N-(3-dimethyl-aminopropyl)-phenothiazine] is oxidized in acidic media by Fe (III) with the formation of a stable radical cation characterized by an intense visible absorbtion band (λ = 540 nm). The optimum parameters for the stability of the radical cation have been studied: molar ratio chlorpromazine-Fe (III), pH, solvents. Spectrophotometric methods have been used in these studies. **Results**: The chlorpromazine radical cation is stable in the acidic media (pH = 3) at a molar ratio chlorpromazine : Fe (III) of 2 : 1. Ascorbic acid reduces these radicals and induces a decrease of absorbance. Percent inhibition was calculated and plotted as a function of the concentration of standard antioxidant solutions. The results show that percent inhibition varies in a linear manner with the ascorbic acid concentration. Percent inhibition is higher when the antioxidant solution is added after generation of radical cation. **Conclusions**: It has been developed a method for evaluating antioxidant activity in the chlorpromazine : Fe (III) system using ascorbic acid as a standard. The method is fast, simple and sensitive; it can be applied for the detection and evaluation of the antioxidant activity of simple or complex systems. **Key words:** CHLORPROMAZINE RADICAL CATION, ANTIOXIDANT ACTIVITY, SPECTROPHOTOMETRIC METHODS

Oxidative stress created by in excess production of free radicals is involved in many degenerative diseases: coronary heart disease, stroke, diabetes mellitus, cancer, rheumatism, aging.

Free radicals derived from oxygen (reactive oxygen species – ROS) or nitrogen (reactive nitrogen species – RNS) are unstable, highly reactive and energized molecules having unpaired electrons. They play an important role in cellular injuries and also initiate the peroxidation of polyunsaturated fatty acids in biological membranes (1,2,3,4).

Aerobic organisms are protected from the radicals effects by a natural antioxidant defence system involving enzymatic and non-enzymatic mechanisms.

At the same time, the exogenous antioxidants from fruits and vegetables have protective effects against cell oxidation (5,6,7,8,9).

Different methods for the evaluation of antioxidant activity have been reported. Such methods often use stable free radicals for studying radical scavenging abilities (10,11, 12,13,14).

This study describes a simple and sensitive decolorization assay based on the generation of chromogenic chlorpromazine radical cation and on its scavenging by ascorbic acid.

MATERIAL AND METHOD

The following reagents were used :

- L (+)-ascorbic acid (Merck) as standard antioxidant ; 0.05 M stock solution was prepared in twice-distilled water ; the solution was diluted just before use ;
- iron (III)-chloride hexahydrate puriss. p.a. (Fluka Chemie Buchs), 0.05 M solution ;
- 37% hydrochloric acid (Farmitalia), 0.1 M solution ;
- chlorpromazine maleate, 0.01 M solution ; the solution was prepared in acidulated water, at light heating.

Experiments were performed using a Hewlett Packard 8453 UV–VIS spectrophotometer.

Preparation of chlorpromazine radical cation solution

Chlorpromazine solution (20 mL, 0.01 M) was added to 10 mL of 1.0 M HCl. Chlorpromazine acidic solution was then treated with 5 mL of 0.005 M $FeCl_3$. Radical cation solution had a maximum absorbance at λ = 540 nm and became stable after 10 min.

In order to study antioxidant activity, different volumes of ascorbic acid solution were added ; the final concentration of ascorbic acid in reaction mixture ranged between 0.125 μmol and 0.750 μmol. The absorbance was determined after 10 min.

Percent inhibition was calculated as : % inhibition = $(1-A_s/A_0)$ x 100, where A_0 is the absorbance of the unscavenged radical cation solution and A_s is the absorbance of the sample after the addition of antioxidant solution.

Percent inhibition was plotted as a function of the concentration of standard antioxidant.

RESULTS AND DISCUSSIONS

The most important property of phenothiazine and its derivatives is their susceptibility to oxidation. They are easily oxidized by different chemical, electrochemical, photochemical and enzymatic agents with the formation of a coloured oxidation product-intermediate radical cation (15).

Chlorpromazine reacts with $FeCl_3$ in acidic media yielding a red coloured radical cation as a result of univalent oxidation of phenothiazine ring.

In the present study, this chromogenic reaction was used in order to develop a decolorization assay for the estimation of antioxidant activity of some natural antioxidants. For this purpose, the optimum parameters for the stability of the radical have been studied.

The stability of oxidation products depends on acidity, concentration of oxidizing agents, time, temperature and the presence of some salts.

The stability of the cation radical colour depends mainly on the oxidizing agent. In the case of strong oxidants, the colour of radical disappears quickly due to the second step of reaction which leads to the formation of a colourless sulphoxid.

The effect of different concentrations of an oxidizing agent on the colour intensity and stability of the radical cation was studied (fig. 1). At a molar ratio chlorpromazine : Fe (III) of 2 : 1 the radical is stable for almost 1 h.

We preferred a molar ratio chlorpromazine : Fe (III) of 8 : 1 in order to avoid an excess of Fe (III) which might interfere with the reaction between antioxidant and radi-

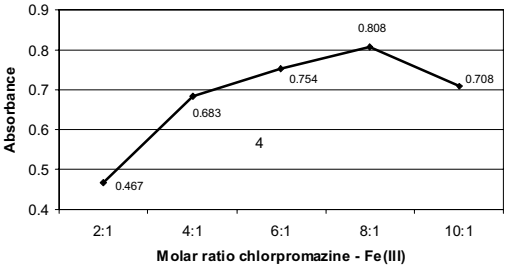

Fig. 1. The influence of molar ratio chlorpromazine : Fe (III) on the stability of the coloured radical cation

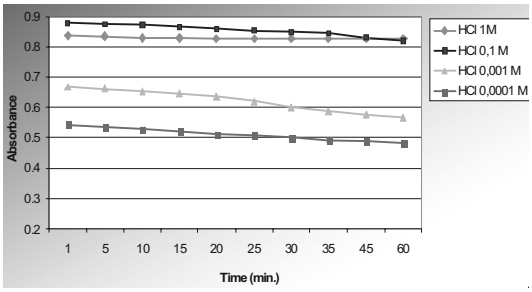

Fig. 2. Time course of chlorpromazine cation formation at different acid strengths (aqueous solutions of 0.001 M–1.0 M HCl)

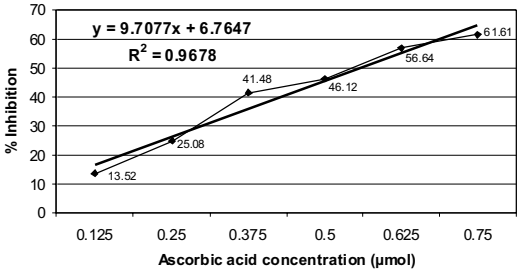

Fig. 3. Effects of ascorbic acid concentration on the inhibition of chlorpromazine radical cation (ascorbic acid was added after generation of chlorpromazine radical cation)

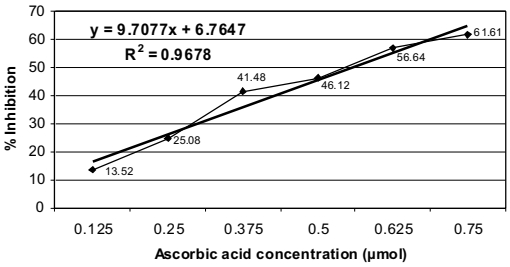

Fig. 4. Effects of ascorbic acid concentration on the inhibition of chlorpromazine radical cation (ascorbic acid was added before generation of chlorpromazine radical cation)

cal cation leading to erroneous results.

The effect of acid strength on the stability of chlorpromazine radical cation was also monitored (fig. 2).

The stability of the radical cation increases in an acid medium.

The inhibition or scavenging of the radical cation by ascorbic acid as standard antioxidant has been studied.

The reaction of ascorbic acid with chlorpromazine radical cation has been found to be fast, sensitive and reproducible.

Percent inhibition was calculated and plotted as a function of the concentration of standard antioxidant solutions in two series of determinations :

- ascorbic acid has been added to reaction mixture after generation of chlorpromazine radical cation (fig. 3) ;
- ascorbic acid has been added to reaction mixture before generation of chlorpromazine radical cation (fig. 4).

The results show that percent inhibition varies in a linear manner in both cases but % inhibition is higher when the antioxidant solution has been added after generation of radical cation.

CONCLUSIONS

The proposed assay for the evaluation of antioxidant activity in the chlorpromazine--Fe (III) system using the ascorbic acid as standard is fast, simple and sensitive ; it can be applied for the detection and evaluation of antioxidant activity of simple or complex systems.

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NOUTĂŢI

KERATINA 17 PROMOVEAZĂ PROLIFERAREA EPITELIULUI ŞI CREŞTEREA TUMORALĂ PRIN POLARIZAREA RĂSPUNSULUI IMUN ÎN PIELE

Tumorile cutanate bazocelulare, incluzând carcinomul bazocelular (BCC) şi hamartomul folicular bazocelular, sunt asociate cu un semnal aberant al genelor Hedgehog (Hh) şi, în cazul BCC, cu un set de multiple variante genetice ale keratinei 5 (codificată de gena KRT5) componenta a filamentului de keratina. Autorii au demonstrat că absenţa /lipsa în tumorile cutanate bazocelulare din cauze genetice a keratinei 17 şi deficitul de co-polimerizare cu keratina 5 întârzie in vivo iniţierea şi creşterea hamartoamelor foliculare bazocelulare la şoarecii care exprimă Hh în epiderm. Aceasta întârziere este precedată de reducerea inflamaţiei şi de polarizarea citokinelor inflamatorii de la Th1 si Th17 cu profil dominant spre un profil dominant Th2. Absenţa keratinei 17 atenuează de asemenea hiperplazia şi inflamaţia la modelele cu dermatită acută. Re-expresia keratinei 17 în *Gli2*^{tg} ; keratinocitele *Krt17*$^{-/-}$ induc selecţia Th1 cu rol deja stabilit în BCC. Se pare că keratina 17 are un rol modulator în expresia Hh în tumorile cutanate bazocelulare ceea ce ar putea avea importanţă în înţelegerea evoluţiei tumorilor cutanate, a psoriazisului şi a reparării leziunilor cutanate (DePianto D, Kerns ML, Dlugosz AA, Coulombe PA. Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin. *Nat Gen* 2010 ; 42 : 910–914).

Elena Braha

CERCETAREA STABILITĂŢII CEFIXIMEI, MATERIE PRIMA PENTRU OBŢINEREA DE FORME FARMACEUTICE

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CEFIXIME STABILITY ASSESSMENT, RAW MATERIAL USED FOR PHARMACEUTICAL FORMS (Abstract): In this paper we studied the stability of cefixime, a raw material used for pharmaceutical forms. **Material and method**: Quantitative analysis was made by using a UV spectrometric method. **Results**: We established the cefixime content after exposing on different conditions of temperature and environment. The obtained results demonstrated that cefixime is stable at environmental temperature and degrades fast and strong in aggressive environments. **Key words**: CEFIXIME, UV SPECTROMETRIC METHODS, STABILITY TESTS

IgE- AND NON-IgE MEDIATED FOOD ALLERGY IN CHILDREN

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IgE- AND NON-IgE MEDIATED FOOD ALLERGY IN CHILDREN (Abstract**). Aim**: To evaluate a group of children diagnosed with food allergies mediated by IgE or non-IgE mechanisms**. Material and Methods:** This retrospective study was conducted on 132 patients diagnosed with food-associated hives admitted over a 2-year period to the Pediatric Clinic II of the Iasi "St. Maria" Children's Hospital. **Results:** Serum levels of specific IgE were positive in 85 (64.39%) cases for one or more food allergens, while positive values of IgG4 were recorded in 21 (15.9%) cases. The most commonly involved allergens were cow milk, egg white, peanuts, wheat flour and fish, in agreement with data in the literature. Percentage frequency of risk factors for allergic sensitization was: artificial or mixed feeding (68.18%), family history of atopy (53.78%), food diversification before 6 months of age (52.27%), natural feeding for less than 3 months (37.87%), and prolonged antibiotic therapy (23.48%). Polysensitization to food and airborne allergens correlated with respiratory allergic manifestations (asthma and allergic rhinitis), occurring predominantly in the age group over 4 years, while atopic dermatitis was associated with food allergy in children up to 4 years of age. **Conclusions:** This study shows that IgE-mediated food allergies are more common, with a higher frequency in children below 4 years due to the interaction of genetic and environmental factors and immaturity of the immune system in the first years of life.
Keywords: FOOD ALLERGENS, IMMUNE MECHANISMS, IgG4 ANTIBODIES.

The prevalence of allergic diseases has increased in recent decades due to changes in eating behavior, involvement of environmental agents and infectious factors.

The most common cause of sensitization is food-related, the cutaneous, digestive and respiratory allergic manifestations developing concurrently or alternately.

Food allergy is defined as "a phenomenon in which adverse reactions are caused through antigen-specific immunological mechanisms after exposure to given food" (1).

The etiopathogenic factors involved in food allergy and therapeutic possibilities are important research goals.

MATERIAL AND METHODS

This retrospective study was conducted on 132 patients diagnosed with food-associated hives admitted over a 2-year period to the Pediatric Clinic II of the Iasi "St. Maria" Children's Hospital.

Total IgE was measured by ELISA, and the determination of serum specific IgE specific to 20 of the most common food and airborne allergens by CLA System *Quanti Scan* (Innogenetics, Heiden, Germany).

An enzyme immunoassay for the quantitative determination of IgG4 antibodies against 20 food allergens was performed using Fin Test Kit TM IgG4 Screen ELISA 20. The tested allergens were: egg white, cow milk, fish, wheat flour, rye flour, barley flour, orange, banana, kiwi, strawberry, celery, soy, carrot, tomato, hazelnuts, peanuts, curry, pepper, sesame, and pork.

Cutaneous testing was done with skin prick test for 7 food allergens (milk, egg, wheat, hazelnuts, nuts, fish, soy).

Statistical analysis was performed using statistical software SPSS10.0, and data were analyzed using descriptive and inferential statistics (Student t test).

RESULTS

Age group distribution showed that predominantly affected were the children aged under 4 years, 93 (70.45%) cases. The mean age at admission was 30 months.

Percent frequency of risk factors for allergic sensitization was as follows: artificial or mixed feeding (68.18%), family history of atopy (53.78%), food diversification before 6 months of age (52.27%), natural feeding for less than 3 months (37.87%), and prolonged antibiotic therapy (23.48%) (fig.1).

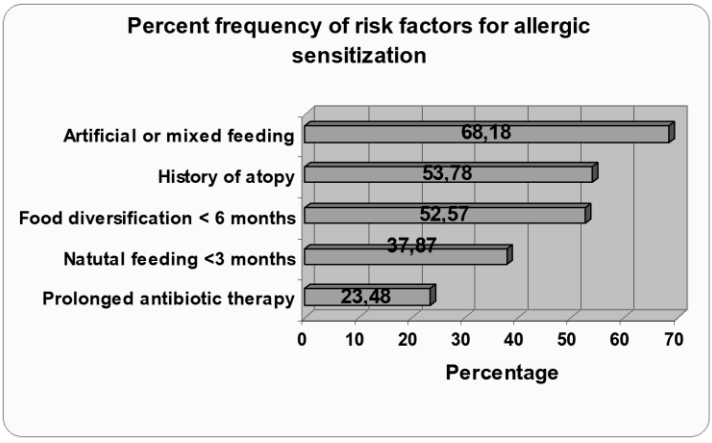

Fig. 1. Hierarchy of risk factors for allergic sensitization

All patients (132) presented hives-like skin manifestations, while associated digestive and respiratory manifestations were present in 18 (13.63%) and 16 (11.36%) cases, respectively.

The associated allergic manifestations were: atopic dermatitis in 22 (16.66%) cases, asthma 11 (8.33%) cases, angioedema 36 (27.27%) and allergic rhinitis in 5 (3.78%) cases.

In 85 (64.39%) cases specific IgE were positive for one or more food allergens. The allergens most commonly involved were: cow milk 28 cases (21.21%, p =

0.000), egg white 19 cases (14.39%, p = 0.000), peanuts 10 cases (7.57%, p = 0,000), fish 8 cases (6%, p = 0.000), soy 6 cases (4.54%, p = 0.000), nuts 6 cases (4.54%, p = 0.000), wheat flour 5 cases (3.78%, p = 0.003), strawberries 2 cases (1.51%, p = 0.011) and rye flour 1 case (0.75%, p = 0.150) .

All allergens were statistically significant except for rye flour (p = 0.150) (fig. 2).

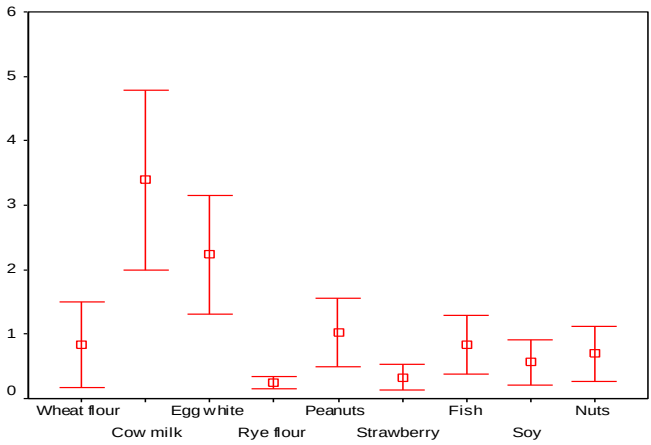

Fig. 2. The most common specific IgE allergens

In patients with low specific IgE levels but in which clinical manifestations and history were suggestive of allergy (47 cases - 35.60%), IgG4 antibodies against 20 food allergens were determined. Of these, 21 (15.9%) cases showed positive IgG4 levels against the following allergens: wheat flour (5 cases, p = 0.049), cow milk (3 cases, p = 0.072), egg white (2 cases, p = 0.061), peanuts (3 cases, p = 0.064), rye flour (2 cases, p = 0.040), strawberries (2 cases, p = 0.108), nuts (2 cases, p = 0.027), fish (1 case, p = 0.006) and soy (1 case, p = 0.087) (fig. 3). One patient presented mixed allergy to peanuts and nuts.

Of these allergens only wheat flour only (p = 0.049), rye flour (p = 0.040) and fish (p = 0.006) were statistically significant.

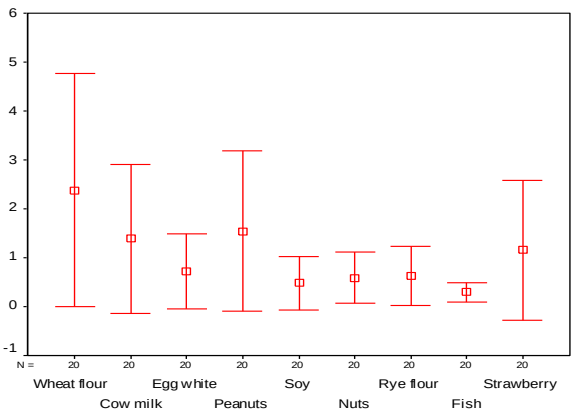

Fig. 3. The most common specific IgG4 allergens

In patients with positive IgG4 levels to different food allergens, 89% presented associated digestive and cutaneous symptoms, suggesting that the level of specific IgG4 antibodies is high in patients with gastrointestinal allergy, but has no predictive value.

Polysensitization to food and airborne allergens correlated with respiratory allergic manifestations (asthma and allergic rhinitis), occurring predominantly in the age group over 4 years, while atopic dermatitis was associated with food allergy in children under 4 years.

The reference values for specific IgG4 antibodies are listed in table I.

TABLE I
Interpretation of results for specific IgG4 antibodies

CLASS	REFERENCE VALUE	INTERPRETATION
0	<0.35 U/mL	**negative-** no IgG4 antibodies detected
1	0.35-0.7 U/mL	**borderline-** low level of IgG4 antibodies
2	0.7-3.5 U/mL	**positive-** moderate level of IgG4 antibodies
3	3.5-17.5 U/mL	**distinctly positive-** high level of antibodies
4	>17.5 U/mL	**Highly positive-** very high levels of IgG4 antibodies

DISCUSSION

Food allergy is defined as an immune response to food proteins affecting approximately 8% of children and 2% of adults (2), but it's the prevalence, as well as that of all allergic diseases is increasing.

In food allergy the mechanisms involved may be IgE or non-IgE mediated immune reactions. The immune mechanisms involved in non-IgE mediated reactions may be based on the participation of subtype 4 IgG antibodies. These types of IgG4 antibody mediated reactions are characterized by less severe and less specific symptoms, and have a late onset after the intake of the incriminated food (up to 72 hours).

There are several arguments supporting the involvement of IgG4 antibodies in non-IgE mediated food allergies: allergic reactions may occur independently of IgE specific antigen; subsequent decline of IgG antibody level when the incriminated food is removed from diet; specific IgG antibodies were identified in celiac disease, atopic eczema and in diseases associated with increased intestinal permeability (chronic inflammatory bowel disease) (3).

Besides hives and anaphylaxis triggered by IgE-mediated immune mechanisms, there are several cell-mediated diseases, such as food protein-induced eosinophilic esophagitis or enterocolitis. Non-IgE mediated food allergies that manifest with food protein-induced entercolitis syndrome or eosinophilic esophagitis are rare and have a favorable course on elimination diet (4).

Immune-mediated food allergies have to be distinguished from food intolerances caused by non-immune mechanism which are non-specific and their symptoms and those of irritable bowel syndrome often overlap (5). Allergic diseases by food sensitization are more common in the early years of life, their frequency decreasing with age, with the acquisition of tolerance

to food. Allergic manifestations with onset in the early years of life are the result of the interaction between genetic factors, environmental factors (artificial feeding, incorrect diversification, prolonged antibiotic therapy) and immaturity of the immune system in early childhood (6).

In our study group the diagnosis of food allergy was based on the criteria in international guidelines, suggestive clinical manifestations, thorough history, and positive levels of serum specific IgE and IgG4 against food allergens, positive skin tests, elimination diet, and oral loading test. The latter must be done under close medical supervision as it can cause such severe adverse reactions as anaphylaxis.

The analysis of specific IgE and IgG4 shows that 64.39% of the cases were IgE-mediated allergies and 15.9% IgG4-mediated allergies. The allergens most frequently involved were cow milk ($p = 0.000$), egg white ($p = 0.000$), peanuts ($p = 0.000$), wheat flour ($p = 0.003$) and fish ($p = 0.000$), in agreement with the data in literature (4, 7, 8). Correlation of skin test with specific IgE level showed a concordance between results. Skin tests have a sensitivity of 95% and a specificity of 50% (9). A negative skin test result has a high predictive value in children older than 1 year, while in children less than 1 year its negative predictive value is lower due to the particularities of cutaneous mast cells (small number and low reactivity). A positive skin test has a positive predictive value of only 50%. If history is not strongly suggestive of an allergy, the diagnosis should be confirmed by a challenge test (10).

With regard to egg allergy, recent studies estimate that it affects approximately 0.5-2.5% of children (11, 12), but there are studies reporting a higher percentage; thus, an Australian study suggests that 8.9% of children have allergic reactions to egg (13). In our study egg allergy is the second most common food allergy. Egg allergy is associated with a high risk of allergy to peanuts or other food allergens, atopic dermatitis, occurrence of respiratory allergy and asthma (14, 15, 16). The rate of resolution of egg allergy is slow; most studies show that tolerance to egg develops at age 3 (17), but there are studies suggesting that about half of children develop tolerance at age 12 (18). The latter study demonstrated persistent egg allergy in 42% of children in their teens, suggesting that the number of adults with egg allergy can increase with time, although current estimate of egg allergy among adults is 0.2% (19).

Factors predictive of the resolution of egg allergy are: initial reaction characteristics (isolated hives/angioedema versus other events), specific IgE level, intensity of skin prick test reaction, severity of atopic dermatitis, IgG4 level, response of interleukin 4 (IL-4). Of these, the first two are the most important predictors for resolution (11).

CONCLUSIONS

This study shows that IgE-mediated food allergies are more common, the highest percentage being recorded in children less than 4 years of age due to the interaction of genetic and environmental factors and immaturity of immune system in the early years.

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EVALUAREA COMPARATIVĂ A MODULUI DE ÎNCORPORARE AL UNUI SUPERDEZAGREGANT ANIONIC ASUPRA CALITĂȚII COMPRIMATELOR CU RANITIDINĂ

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COMPARATIVE EVALUATION OF THE ANIONIC SUPERDISINTEGRANT INCORPORATION MODE ON THE QUALITY OF RANITIDINE TABLETS (Abstract): The present study was based on the impact of the superdisintegrants incorporation mechanism on the immediate realese of the tablets final performances. The aim was the selection of the working method to obtain Ranitidine 150 mg tablets with the desiderate quality and in reproducible conditions. **Material and methods:** The effect of the incorporation mode of sodium starch glycolate on the aspect, granules size distribution and flowing properties of the lubricated product, and also the weight uniformity, hardness, disintegration, friability, and dissolution of the Ranitidine 150 mg tablets prepared by dry granulation was studied. The addition mode of the disintegrant was realized in three ways: intragranular, extragranular, and distributed equally between the two phases. The distribution range for the tablets weight was established. Relative standard distribution was calculed for the weight and hardness of the uncoated tablets. **Results:** The powder flow and, implicit, the weight uniformity of the uncoated tablets was positive influenced by the extragranular incorporation of the superdisintegrant. The disintegration time was identical for all the three disintegrant addition modes, and the hardness and the friability were not significantly influenced by working method, the obtained values were similar. For the developed formulations, the percent of the ranitidine dissolution was high, but higher in the extragranular incorporation. **Conclusions:** For the product quality the extragranular addition mode seemed the best method to incorporate the superdisintegrant. **Key words:** DRY GRANULATION, SUPERDISINTEGRANTS, INTRAGRANULAR INCORPORATION, EXTRAGRANULAR INCORPORATION, DISSOLUTION.

Eficacitatea terapeutică a unui medicament este dependentă de capacitatea sa de a elibera principiul activ, astfel încât acesta să poată fi absorbit de către membranele biologice. Prin urmare, în formularea comprimatelor cu eliberare imediată, se urmăreşte realizarea unui compromis între obținerea unor produse cu dezagregare şi dizolvare rapidă şi procesul de fabricație al unui produs cu rezistență mecanică la manipulare. Facilitarea dizolvării se realizează prin adăugarea superdezagreganților. Aceştia influențează atât viteza şi timpul de dizolvare, cât şi caracteristicile fizice ale comprimatelor, cum ar fi rezistența mecanică şi friabilitatea (1, 2, 3, 4).

MATERIAL ŞI METODE

Pentru realizarea studiilor s-au utilizat trei serii de comprimate filmate cu 150 mg ranitidină, obţinute prin granulare uscată, conform aceleiaşi formule de fabricaţie, dar cu mod diferit de încorporare al superdezagregantului (tab. I).

Celuloza microcristalină tip 102 a avut rol de diluant al substanţei active şi liant. Ca dezagregant s-a lucrat cu amidon glicolatul de sodiu, iar drept glisant s-a utilizat dioxidul de coloidal. Lubrifierea a fost realizată cu stearat de magneziu, iar acoperirea comprimatelor s-a efectuat prin filmarea cu un produs pe bază de alcool polivinilic - Opadry yellow sub formă de soluţie apoasă 16,7 %.

Amestecurile omogene, rezultate prin diluarea principiului activ cu / fără amidon glicolat de sodiu şi celuloză microcristalină 102 pe un omogenizator în V (15 minute la 7 rpm), s-au granulat pe un compactor Alexanderwerk WP 50N/75 (presiunea de compactare de 25 bari, sita de 1,25 mm), în condiţii similare de funcţionare a echipamentului. Lubrifierea, realizată pe omogenizatorul în V, s-a efectuat prin omogenizarea granulatului cu / fără amidon glicolat de sodiu, dioxid de siliciu coloidal (5 minute la 7 rpm) şi stearat de magneziu (3 minute la 7 rpm). Comprimatele biconvexe cu diametrul de 9 mm şi masa de 300 mg s-au obţinut pe un echipament Kilian S 250 (forţa de comprimare 15 kN, viteza de 25 rpm), filmarea lor efectuându-se într-un echipament Driacoater.

TABELUL I

Compoziţia calitativă a comprimatelor filmate

Materia primă	RA1 %(m/m)	RA2 %(m/m)	RA3 %(m/m)
Clorhidrat de ranitidină	53,85	53,85	53,85
Celuloză microcristalină 102	39,42	39,42	39,42
Amidon glicolat de sodiu intragranular	0,96	-	0,48
Amidon glicolat de sodiu extragranular	-	0,96	0,48
Dioxid de siliciu coloidal	0,96	0,96	0,96
Stearat de magneziu	0,96	0,96	0,96
Opadry yellow	3,85	3,85	3,85

Deoarece curgerea pulberilor omogene este importantă pentru uniformitatea masei comprimatelor, înainte de începerea etapei de comprimare a fost studiat comportamentul la curgere al produselor lubrifiate. S-au determinat: densitatea iniţială şi tasată, indicele Haussner şi Carr, aspectul şi distribuţia granulometrică. Comprimatele neacoperite au fost studiate comparativ din punct de vedere al uniformităţii masei, rezistenţei mecanice, friabilităţii, timpului de dezagregare. Pentru comprimatele filmate s-a determinat procentul de ranitidină dizolvată în 45 minute.

Aparatele utilizate au fost: microscop AxioScope A_1 cu o magnitudine de 50x - *aspectul granulatelor lubrifiate*, sortator Eweka cu şase tipuri de site (1000 μm, 500μm, 250 μm, 180 μm, 125 μm, 90 μm, 63 μm, 45 μm) - *granulometria*, aparat de măsură a densităţii tasate Electrolab ETD 1020 (cilindrul de 100 ml, înălţimea de tasare de 14 mm ± 2 mm, frecvenţa tasărilor de 300/minut, testele finalizându-se la

1250 tasări) - *densitatea şi indicii de curgere Haussner I_H şi Carr I_C,* balanţă analitică Mettler Toledo ABS - *masa medie şi uniformitatea masei comprimatelor,* tester 6D Dr. Schleuniger PHARMATRON - *rezistenţa mecanică,* tester Hanson Reserch QC 21 - *timpul de dezagregare,* friabilator tip Vankel AG 104 – *friabilitatea comprimatelor,* spectrofotometru Carry 50 – *procentul de ranitidină dizolvată.*

S-a calculat masa medie a procesului de comprimare (media maselor medii ale eşantioanelor), deviaţia standard SD a procesului (media deviaţiilor standard ale eşantioanelor) şi deviaţia standard relativă RSD a procesului (media deviaţiilor standard relative ale eşantioanelor). Pentru toate eşantioanele s-a calculat distribuţia procentuală a valorilor maselor individuale faţă de masa medie a eşantionului (încadrarea valorilor individuale).

Parametrii rezistenţă mecanică, dezagregare, friabilitate şi dizolvare s-au stabilit respectând prevederile *Farmacopeii Europene* 6.0 (5).

Determinarea procentului de ranitidină dizolvată după 45 minute s-a realizat prin metoda spectrofotometrică folosind ca mediu de dizolvare apa distilată la o temperatură de 37°± 0,5°C, la o viteză de rotaţie a paletelor de 50 rpm. S-a determinat absorbanţa soluţiei de referinţă şi probă la lungimea de undă de 314 nm în cuve de un cm grosime folosind apa distilată ca lichid de compensaţie. Testul s-a efectuat pe şase comprimate şi s-a calculat media procentelor de ranitidină dizolvată.

REZULTATE

Analiza distribuţiei granulometrice nu a arătat diferenţe semnificative între cele trei serii de produse lubrifiate, dimensiunea medie a particulelor având valori apropiate (fig.1).

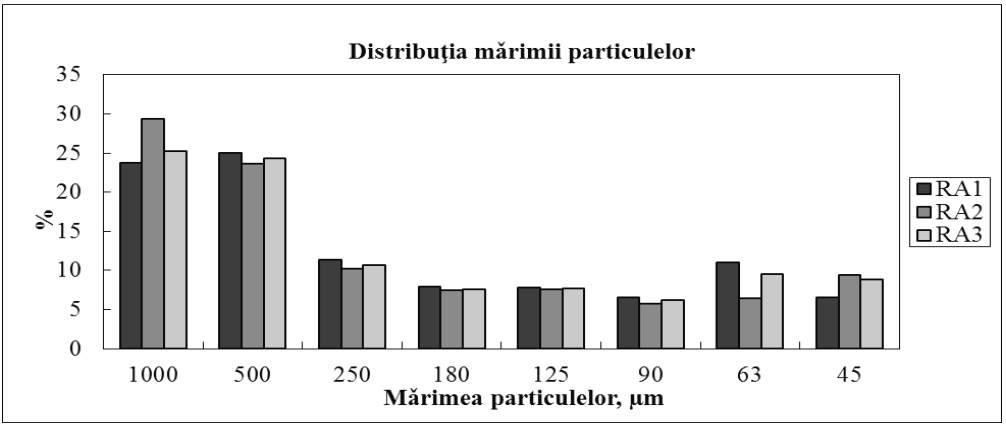


Fig. 1. Reprezentarea grafică a distribuţiei mărimii particulelor

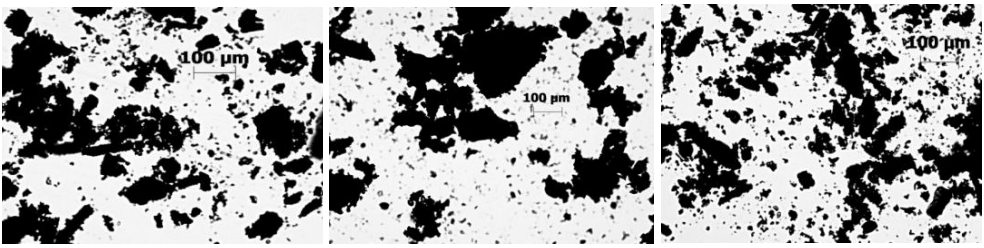
Valorile pentru densitatea produselor tasate au variat în succesiunea RA1> RA3>RA2, adăugarea superdezagregantului în etapa de lubrifiere determinând o tasare superioară a produsului. Corelaţia indice Haussner – indice Carr a arătat o curgere bună pentru RA2: $I_H < 1,25$ respectiv 1,239 şi $I_C < 20\%$, respectiv 19,277 (6). Seriile RA1 şi RA3 au prezentat o curgere mai slabă, dar suficientă pentru încadrarea masei comprimatelor în limitele de admisibilitate stabilite (tab. II).

TABELUL II

Determinarea factorilor de curgere pentru produsele lubrifiate

Parametrii analizați	RA1	RA2	RA3
Densitatea produsului netasat do (g/ml)	0,574	0,602	0,588
Densitatea produsului tasat df (g/ml)	0,781	0,746	0,763
Indicele Haussner I_H	1,359	1,239	1,297
Indicele Carr I_C (%)	26,437	19,277	22,935

Vizualizarea aspectului particulelor nu a evidențiat diferențe mari între cele trei serii de produs. S-a sesizat procentul mai mare de pulbere fină în cazul RA3 (fig.2).



(a) (b) (c)

Fig. 2. Aspectul particulelor lubrifiate de RA1 (a), RA2 (b), RA3 (c)

TABELUL III

Caracteristicile fizico chimice ale comprimatelor preparate

Parametri analizați	RA1	RA2	RA3
Masă medie comprimate (g)	0,30147	0,30042	0,30011
SD proces de comprimare (g)	0,0047	0,0006	0,0030
RSD proces comprimare (%)	1,55	0,19	1,02
distribuție masă comprimate (%)	-3,54 / +3,29	-0,41 / +0,59	-1,94 / +2,66
RSD rezistență la rupere (%)	1,82	1,02	1,56
timp dezagregare (min)	10	10	10
friabilitate (%)	0,29	0,13	0,20
ranitidină dizolvată (%)	94,86	99,80	98,10

Masa medie şi valorile maselor individuale ale comprimatelor neacoperite s-au plasat în intervalul 300 mg ± 3,5%. Rezistența mecanică (168 – 180 N) şi friabilitatea (0,13 – 0,29%) au fost satisfăcătoare, asigurând filmarea, ambalarea şi utilizarea comprimatelor. Dizolvarea ranitidinei s-a realizat în procente ridicate de peste 90% (tab. III).

DISCUȚII

Dintre caracteristicile produselor lubrifiate corespunzătoare celor trei formulări, modul de încorporare al superdezagregantului a influențat uşor numai comportamentul la curgere. Asocierea amidon glicolat de sodiu, dioxid de siliciu coloidal şi stearat de magneziu din etapa de lubrifiere a produsului RA2 a fost benefică proprietăților de curgere ale acestuia.

Uniformitatea masei comprimatelor neacoperite a fost favorizată de adiția extragranulară a superdezagregantului, dispersia datelor fiind mult mai redusă pentru RA2. Valoarea RSD foarte mică pentru RA2 s-ar explica prin curgerea superioară a produsului, lucru ce a permis reglarea mult mai bună a înălțimii camerei de comprimare.

Rezistența mecanică şi friabilitatea comprimatelor nu au fost influențate în mod semnificativ de modul de încorporare al dezagregantului. Timpul de dezagregare realizat a fost identic şi mare pentru toate cele trei tipuri de comprimate, fapt care s-ar putea datora solubilității ridicate în apă a clorhidratului de ranitidină. Fiind componenta majoritară din formularea comprimatelor, solubilitatea sa a influențat atât viteza cât şi mecanismul de dezagregare al comprimatelor. Rezultatele sunt în concordanță cu datele din literatură (2).

Adiția extragarnulară a asigurat de asemenea cea mai buna friabilitate şi dizolvare a comprimatelor. Variabilitatea existentă între cele trei formulări s-a putut explica prin interacțiunea ionică existentă între superdezagregantul anionic şi clorhidratul de ranitidină care este un cation. Date similare referitoare la întârzierea eliberării substanței active în cazul interacțiunii ionice apar şi în literatură (2, 3).

CONCLUZII

În formulările studiate, modul de încorporare al superdezagregantului nu a generat diferențe semnificative între caracteristicile comprimatelor. În toate cazurile s-au obținut comprimate cu rezistență mecanică bună, friabilitate scăzută şi o dizolvare rapidă a substanței active. Totuşi încorporarea extra-granulară a amidon glicolatului de sodiu a condus la comprimate cu o foarte bună uniformitate a masei.

Rezultatele studiului prezintă importanță pentru stabilirea modului de încorporare a superdezagregantului în formularea medicamentelor generice cu ranitidină, când există obligativitatea realizării unui medicament bioechivalent, cu un profil de dizolvare comparativ cu al medicamentului de referință.

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EVALUAREA SEMICANTITATIVĂ A METADONEI PRIN METODA CROMATOGRAFIEI ÎN STRAT SUBȚIRE (CSS)

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SEMI-QUANTITATIVE DETERMINATION OF METHADONE BY TLC (Abstract): Methadone is the main therapeutic option in heroin addiction treatment, but also an abuse substance. Given the analytical focus on the diagnosis of drug abuse and the usefulness of toxicological analysis methods in both overdose and monitoring substitution therapy, this study was aimed at the semi-quantitative determination of methadone by using Thin Layer Chromatography (TLC). By having the advantage of simplicity and rapidity, TLC finds its rightful place among the analysis methods when other relatively costly methods that involve instrumental performance are not available. **Materials and methods**: TLC plates (silicagel GF 254 Merck), developing system methanol:strong ammonia 100: 1,5, photometric quantification at 254 nm, using TLC Scanner 3 (Camag). **Results**: The results show that methadone can be determined semi-quantitatively in the chromatographic conditions mentioned in the two domains tested (5 – 40 μg and 10 – 80 μg), with the best results obtained in the 5 - 40 μg domain. **Conclusions**: Semi-quantitative TLC evaluation proposed by us has as main advantages the rapidity, simplicity and relatively low cost compared to other useful methods.
Key words: METHADONE, TLC, SEMI-QUANTITATIVE DETERMINATION

Metadona reprezintă principala opţiune terapeutică în cazul dependenţei de heroină. Ea se regaseşte însă şi în categoria substanţelor de abuz (1, 2).

În cursul tratamentului de substituţie cu metadonă, unii heroinomani pot avea recăderi. Deoarece menţinerea în programele de substituţie pe metadonă este condiţionată de abstinenţa de heroină sau alte opiacee, este esenţial să se testeze extraconsumul de opiacee.

Metodele cromatografice au o buna aplicabilitate în studiul analitico-toxicologic al substanţelor de abuz, îndeosebi în analiza pe probe biologice.

Deşi este o metodă mai puţin precisă decât cele trei metode recomandate de grupul de experti OMS (imunoanaliza, HPLC, GC-MS), metoda cromatografiei în strat subţire (CSS) îşi găseşte locul binemeritat printre metodele de analiză, atunci când nu se dispune de aparatura relativ costisitoare pe care o implică metodele instrumentale performante (3, 4). Dată fiind variabilitatea datelor şi a metodelor prezentate în literatură, ne-am propus aplicarea CSS pentru determinarea semicantitativă a metadonei la cantităţi de ordinul microgramelor-zecilor de micrograme (5, 6).

MATERIAL ŞI METODĂ

- Clorhidrat de metadonă s.r., Alcool metilic, Amoniac, Cloroform;
- Plăci de sticlă CSS Merck, gata preparate (silicagel 60 GF 254);
- Cuve de developare;
- Sistem Camag TLC Scanner 3 de achiziţie şi prelucrare a cromatoplăcilor, prevăzut cu cameră obscură, lămpi UV şi cuplat la calculator (soft WinCATS);
- Sistem semiautomat Camag Linomat 5 pentru spotarea probelor pe cromatoplacă;
- Aparat foto pentru captarea imaginilor;
- Balanţă Sartorius.

S-a preparat o soluţie stoc de clorhidrat de metadonă în cloroform de concentraţie 0,2% (2 mg/ml). Din această soluţie, am spotat cu sistemul Camag Linomat 5, cantităti diferite astfel încât să putem obţine o curbă de etalonare. Am testat două domenii de cantităţi: 5 – 40 µg şi 10 – 80 µg.

Developarea s-a realizat în sistemul metanol: amoniac 100:1.5, iar după migrare plăcile au fost examinate la lampa UV (254 nm). Cromatoplăcile au fost prelucrate cu ajutorul sistemului Camag TLC Scanner 3 (densitometru), prevăzut cu cameră obscură, lămpi UV şi cuplat la calculator.

REZULTATE ŞI DISCUŢII

După developarea în sistemul de solvenţi metanol: amoniac 100:1.5, examinarea cromatoplăcilor la lampa UV evidenţiază spoturile corespunzătoare metadonei, având fluorescenţă violetă. În sistemul de solvenţi menţionat, metadona este caracterizată de o valoare R_f de 0,5 (fig. 1 şi fig. 2).

Cu ajutorul sistemului TLC Scanner 3 au fost înregistrate densitogramele metadonei pe cele două domenii testate: 5 - 40 µg (fig. 3) şi respectiv, 10 - 80 µg (fig. 4). Datele obţinute prin cuantificarea fotometrică la 254 nm, au fost utilizate pentru trasarea dreptelor reprezentând variaţia ariei de sub curbă în funcţie de cantitatea de clorhidrat de metadonă (fig. 5, fig. 6).

Evaluarea semicantitativă a metadonei prin CSS se poate realiza utilizând următoarele condiţii experimentale: plăci TLC (silicagel GF 254 Merck), sistem de developare metanol: amoniac 100: 1,5 (R_f = 0,5), cuantificare fotometrică la 254 nm, prin scanarea plăcilor cromatografice cu un sistem Camag TLC Scanner 3 şi prelucrarea datelor cu ajutorul softului WinCATS şi a programului Microsoft Office Excel 2003.

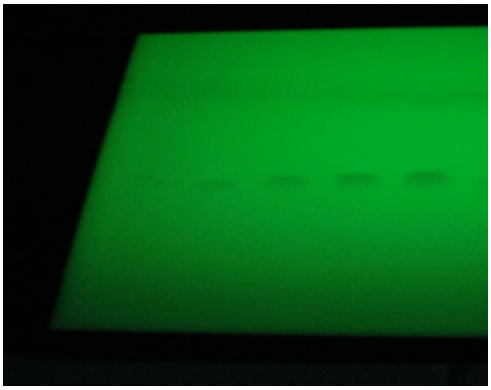

Fig. 1. Analiza CSS metadonei în sistemul de solvenţi metanol: amoniac 100:1.5, domeniul 5 – 40 µg

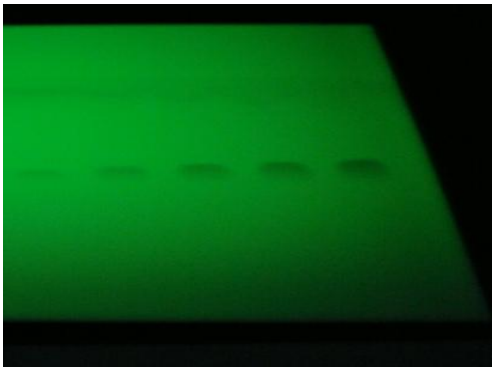

Fig. 2. Analiza CSS în sistemul de solvenţi metanol: amoniac 100:1.5, domeniul 10 – 80 µg

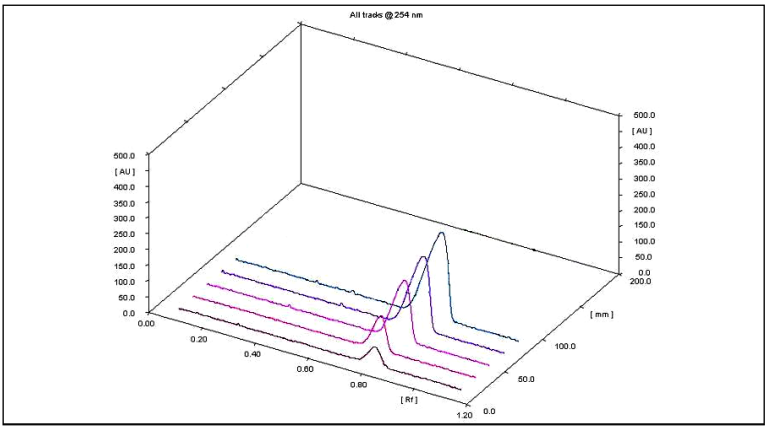

Fig. 3. Densitograma obţinută la evaluarea semicantitativă a metadonei în domeniul de concentraţii 5 – 40 µg/ml

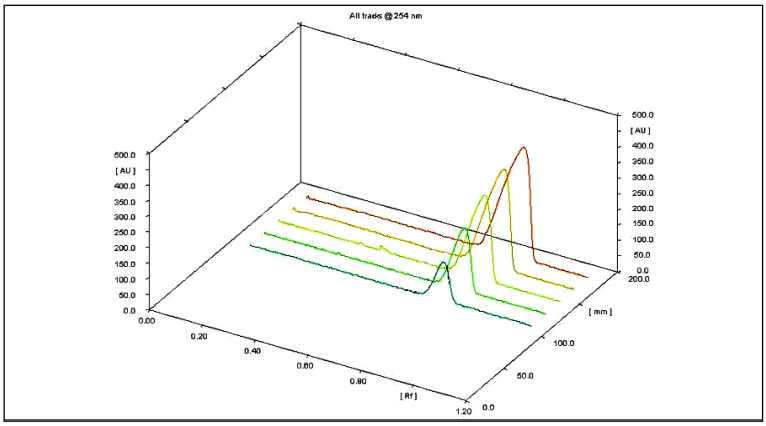

Fig. 4. Densitograma obţinută la evaluarea semicantitativă a metadonei în domeniul de concentraţii 10 – 80 µg/ml

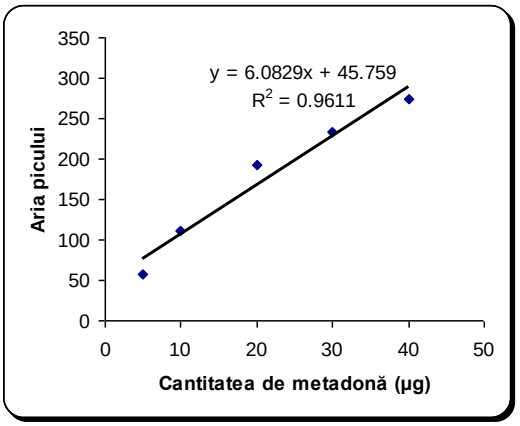

Fig. 5. Dreapta de regresie obţinută la determinarea semicantitativă a clorhidratului de metadonă în domeniul 5 – 40 µg

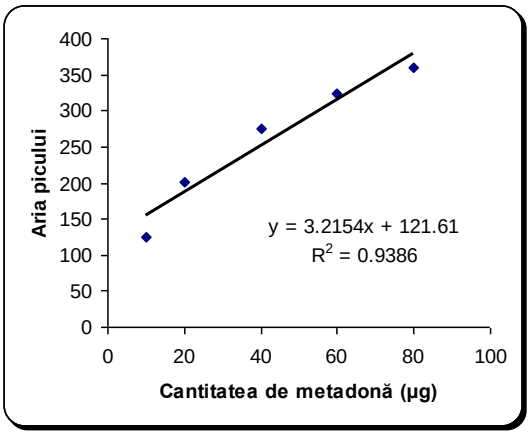

Fig. 6. Dreapta de regresie obţinută la determinarea semicantitativă a clorhidratului de metadonă în domeniul 10 – 80 µg

CONCLUZII

S-a demonstrat că metadona poate fi determinată semicantitativ în condiţiile cromatografice şi în domeniile testate, cu rezultate mai bune pe domeniul 5 - 40 µg.

Metoda de evaluare semicantitativă propusă de noi are ca principale avantaje rapiditatea, simplitatea şi costul relativ scăzut faţă de alte metode mai performante.

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NITRAȚII ŞI NITRIȚII DIN PREPARATELE DIN CARNE-PRECURSORI AI NITROZAMINELOR

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NITRATES AND NITRITES IN MEAT PRODUCTS – NITROSAMINES PRECURSORS (Abstract). **Aim:** To determine the content in nitrates and nitrites and the formation of two nitrosamines (*N*-nitrosodimethylamine – NDMA, and *N*-nitrosodiethylaamine- NDEA) in samples of chicken ham, dry Banat salami, dry French salami, traditional Romania sausages, and pork pastrami. **Material and methods:** Nitrites were determined by spectrophotometry with Peter-Griess reagent, and nitrates by the same method after reduction to nitrites with cadmium powder. High performance liquid chromatography with UV detection was used to determine nitrosamines. **Results:** The initial concentration of nitrates, nitrites, NDMA and NDEA in the samples ranged as follows: 14.10 – 60.40 mg NO_3/kg, 2.70 – 26.70 mg NO_2/kg, from non-detectable to 0.90 μg NDMA/kg, and from non-detectable to 0.27 μg NDEA/kg, respectively. After 28 days the concentrations were: 3.24 – 17.1 mg NO_3/kg, 0.04 – 1.87 mg NO_2/kg, 0.8-29 μg NDMA/kg, and 11.6 – 61.9 μg NDEA/kg, respectively. **Conclusions:** The decreased nitrate and nitrite and increased NDMA and NDEA concentrations prove that in food products nitrosamines are formed due to residual nitrite during their preservation. The determination of nitrasamines revealed levels much above the admitted maximal concentration for these food products. **Key words:** NITRATES, NITRITES, NITROSAMINES, MEAT PRODUCTS

Expunerea la nitrații şi nitriții din surse alimentare şi elemente de mediu reprezintă un risc major pentru sănătatea umană. Nitritul rezidual din produsele alimentare în care nitrații şi nitriții au fost adăugați drept conservanți pot forma în aliment sau în organismul consumatorului nitrozamine, compuşi clasificați de IARC International Agency for Research on Cancer (1) cancerigeni pentru om. Formarea nitrozaminelor în preparatele din carne este un proces complex, influențat de o serie de factori: cantitatea de nitriți respectiv nitrați adăugați inițial, concentrația nitritului rezidual, interacțiunea cu alți aditivi, natura procesului culinar, temperatura şi durata acestuia, condițiile de păstrare a alimentului (2). Mecanismul probabil al formării *in vivo* a dimetilnitrozaminei, una dintre cele mai frecvent întâlnite nitrozamine (2), la nivel gastric, este prezentat în figura 1.

Formarea nitrozaminelor în produsul alimentar are loc lent, progresiv, în funcție de nivelul nitritului rezidual şi de condițiile din mediul de reacție.

Determinarea nitrozaminelor în prepara-

te din carne se poate face prin diverse metode. În prezent sunt utilizate metodele HPLC cu detecţie prin fluorescenţă (3), HPLC cu fotohidroliză post-coloană şi detecţie UV-VIS (4) şi metodele gaz cromatografice (5).

$$NaNO_2 + H^+ \rightarrow HNO_2 + Na^+$$
$$2\ HNO_2 \leftrightarrow N_2O_3 + H_2O$$
$$2\ N_2O_3 + O_2 \rightarrow 2N_2O_4$$
$$2\ HNO_2 + H^+ \leftrightarrow H_2NO_2^+$$
$$H_2NO_2^+ + CH_3NHCOCH_3 \rightarrow CH_3N(NO)COCH_3 + H_2O + H^+$$
$$2NO + O_2 \rightarrow 2NO_2$$
$$NO + NO_2 \leftrightarrow N_2O_3$$
$$2NO_2 \leftrightarrow N_2O_4$$
$$N_2O_3 + (CH_3)_2NH \rightarrow (CH_3)_2N.NO + HNO_2$$
$$N_2O_4 + (CH_3)_2NH \rightarrow (CH_3)_2N.NO + HNO_3$$
$$N_2O_4 + 2(CH_3)_2NH \rightarrow (CH_3)_2N.NO + (CH_3)_2N.NO_2$$

Fig. 1. Mecanismul formării nitrozodimetilaminei (NDMA)

Cercetările efectuate au urmărit determinarea în dinamică a concentraţiilor nitraţilor, nitriţilor, nitrozodimetialaminei şi nitrozodietilaminei din preparate din carne, pe o perioadă de 28 de zile.

MATERIAL ŞI METODĂ

Reactivi

Toţi reactivii utilizaţi la realizarea cercetărilor au fost de puritate analitică şi au foat achiziţionaţi de la compania Fluka Chemika. Pentru determinările HPLC s-au folosit reactivi de puritate corespunzătoare achiziţionaţi de la Fisher Chemicals.

Aparatură

- spectrofotometru JASCO, model V-550 UV-VIS;
- sistem HPLC Shimadzu, detector UV.

Probe de analizat

Analiza în dinamică a conţinutului în nitraţi, nitriţi şi evidenţierea formării a două dintre nitrozamine (NDMA şi NDEA) au fost realizate pe probe de preparate din carne (şuncă de pui, salam bănăţean uscat, salam franţuzesc uscat, cârnaţi tradiţionali şi pastramă de porc), procurate direct de la producător, în prima zi în care puteau fi comercializate. Determinările s-au efectuat iniţial şi la intervale de 7 zile, timp de 28 de zile.

Metode

a) Determinarea nitriţilor

Determinarea conţinutului în nitriţi s-a realizat prin metoda spectrofotometrică cu reactiv Peter - Griess, după separarea acestora din probe prin extracţie în soluţie apoasă (6).

b) Determinarea nitraţilor

Determinarea nitraţilor s-a realizat prin aceeaşi metodă, după reducerea nitratului la nitrit cu pulbere de cadmiu (6). Calcularea conţinutului de nitrat s-a realizat prin diferenţa între nitritul total (după reducerea cu cadmiu) şi nitritul iniţial, determinat în acelaşi extract.

c) Separarea nitrozaminelor

Separarea nitrozaminelor din probele de analizat s-a realizat prin extracţie în tampon fosfat – citrat de pH 6,5 şi acid ascorbic. Purificarea extractelor s-a făcut

prin filtrare prin coloane de Celite 545. Eluatele au fost extrase cantitativ cu diclormetan. Faza organică s-a evaporat la sec la rotavapor şi a servit determinării prin cromatografie de lichide de înaltă performanță a nitrozodimetilaminei şi a nitrozodietilaminei.

d) Determinarea nitrozaminelor

Determinarea nitrozaminelor s-a realizat prin cromatografie de lichide de înaltă performanță; reziduul obținut în urma evaporării s-a reluat cu 1 ml de fază mobilă, acetonitril- acid acetic 1% (20:80 v/v) şi un volum de 20 µl a fost injectat în lichid cromatograf.

e) Condiții de cromatografiere

Instrument: sistem HPLC Shimadzu

Coloana: Grace 25 ×4,6 mm, 5 µm, ODS

Faza mobilă: acetonitril - acid acetic 1% (20:80, v/v)

Debit: 0,8 ml / minut

Volum injectare: 20 µl

Temperatura coloanei: 25°C

Detectia : UV (230 nm)

Timp de retenție NDMA: 4,56 min.

Timp de retenție NDEA: 9,11 min.

Timp de analiză / probă : 10 minute.

Curbele de etalonare pentru nitrit, nitrat, nitrozodimetilamină (NDMA), respectiv nitrozodietilamină (NDEA), sunt redate în figurile 2, 3 şi 4.

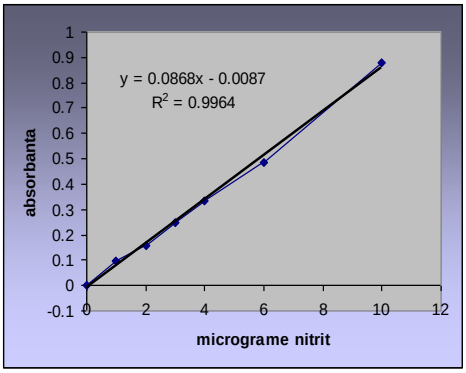


Fig. 2. Curba de etalonare pentru determinarea nitriților

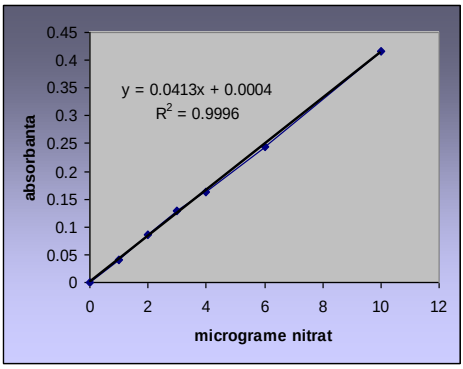


Fig. 3. Curba de etalonare pentru determinarea nitraților

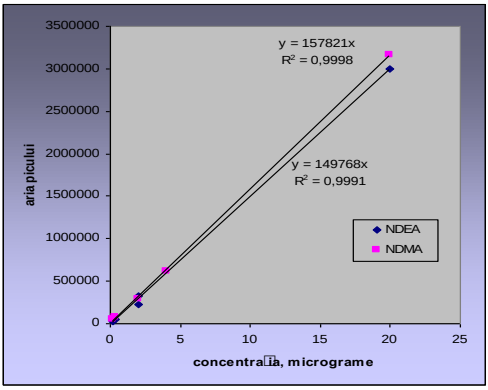


Fig. 4. Curbele de etalonare pentru determinarea NDMA şi NDEA

REZULTATE

Rezultatele obţinute la determinarea nitraţilor, nitriţilor, NDMA şi NDEA sunt redate în figurile 5, 6, 7, 8, 9.

DISCUŢII

Aşa cum era de aşteptat, pe perioada celor 28 de zile de experiment s-a constatat o scădere a concentraţiilor de nitraţi şi de nitriţi din probele analizate iniţial.

Preparatele tip jambon (şunca de pui-proba 1, respectiv pastrama de porc-proba 5) au un conţinut ridicat de nitrit (26,70 mg/kg, respectiv 25,82 mg/kg), fără a depăşi concentraţia maximă admisă pentru acet tip de preparate, de 100 mg/kg. Pe durata studiului concentraţia în nitrit rezidual a probelor scade marcant la toate preparatele analizate într-un procent de 96,17%.

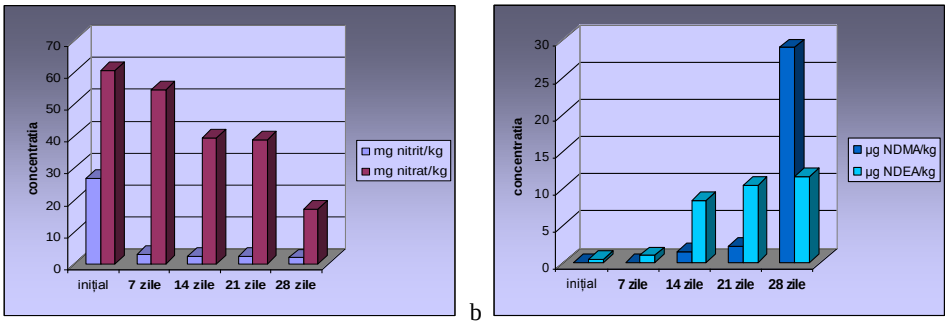


Fig. 5. Concentraţia nitriţilor şi nitraţilor (a), NDMA şi NDEA (b) în proba de şuncă de pui

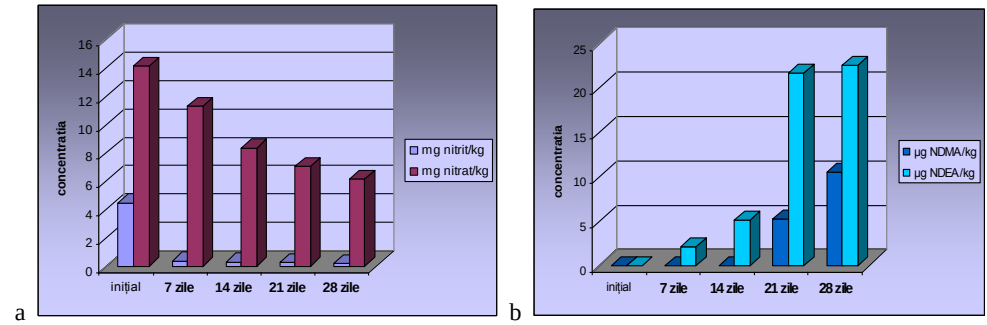


Fig. 6. Concentraţia nitriţilor şi nitraţilor (a), NDMA şi NDEA (b) în proba de salam bănăţean uscat

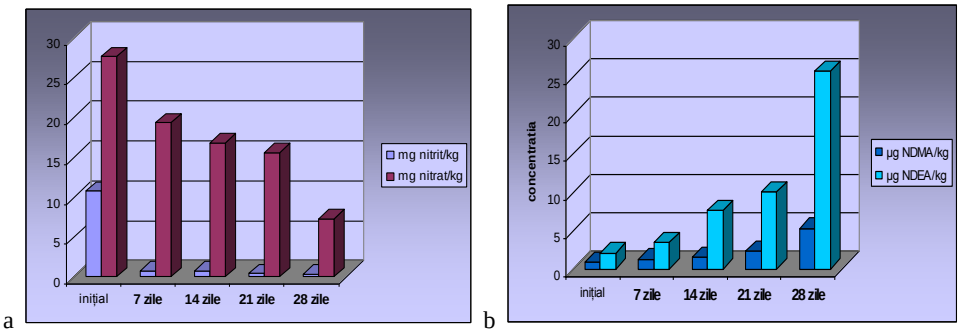
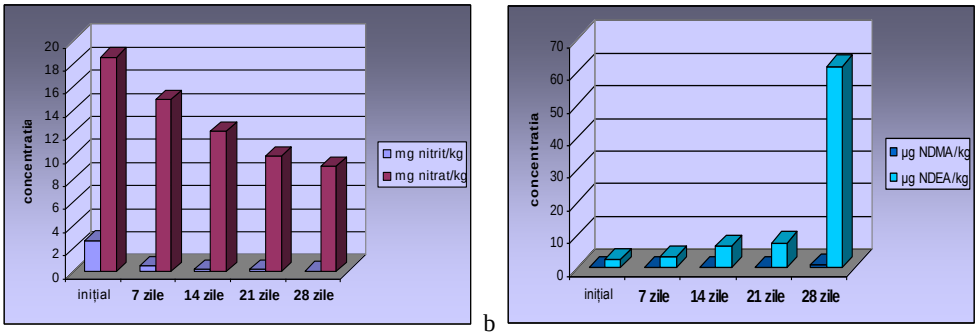
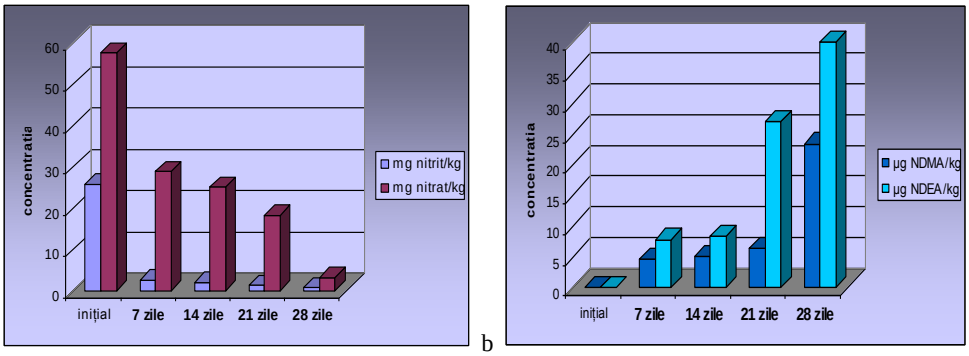


Fig. 7. Concentraţia nitriţilor şi nitraţilor (a), NDMA şi NDEA (b) în proba de salam franţuzesc uscat



a b

Fig. 8. Concentrația nitriților şi nitraților (a), NDMA şi NDEA (b) în proba de cârnați tradiționali



a b

Fig. 9. Concentrația nitriților şi nitraților (a), NDMA şi NDEA (b) în proba de pastramă de porc

Cantități crescute de nitrați au fost determinate în preparatele tip jambon (probele 1 şi 5), dar şi în proba 3-salam franțuzesc uscat (produs fiert, dublu afumat şi uscat). Pe durata studiului concentrațiile în nitrat au scăzut la toate probele, într-un procent.de 58,14%. Scăderea semnificativă a cantității de nitrat rezidual, în procent de 80 %, s-a observat în ultimul interval de 7 zile a studiului pentru trei din cele cinci probe analizate (probele 1, 3 şi 5).

În ceea ce priveşte prezența nitrozaminelor şi evoluția în dinamică a concentrațiilor acestora, s-a constatat că numai în proba 3–salam franțuzesc uscat, NDMA a fost prezentă inițial într-o cantitate de 0,9 µg/kg. Aceasta ar putea fi explicată prin existența perioadei de maturare a produsului, care în acest caz, a fost de 45 de zile. Pentru celelalte probe conținutul în NDMA a fost sub limita de detecție a metodei HPLC aplicate.

După 7 zile NDMA a fost detectată în proba 5–pastramă de porc, după 14 zile s-a evidențiat în proba 1-şuncă de pui şi după 21 de zile s-a evidențiat în proba 2–salam bănățean uscat; formarea NDMA a avut loc cel mai lent şi în cantitatea cea mai mică (0,8 µg/kg) în proba 4–cârnați tradiționali.

La finalul studiului, probele cu concentrația cea mai mare de NDMA au fost preparatele din carne de tip jambon (proba 1-şuncă de pui şi proba 5- pastramă de porc) – 29 µg/kg, respectiv 23,4 µg/kg.

Remarcăm o proporționalitate directă între conținutul inițial în nitrați şi nitriți şi intensitatea procesului de formare a NDMA; astfel proba 1 a avut inițial o concentrație de 26,7 mg NO_2^-/kg, respectiv 60,4 mg NO_3^-/kg, iar în proba 5 s-a determinat o concentrație de 25,82 mg NO_2^-/kg, respectiv 57, 56 mg NO_3^-/kg. Concentrații-

le de NDMA, respectiv NDEA din probele menţionate au fost, la finalul studiului, de 29 µg NDMA/kg şi 11,6 µg NDEA/kg în proba 1, respectiv de 23.4 µg NDMA/kg şi 40 µg NDEA/kg în proba 5.

Nitrozodietilamina (NDEA) nu a fost evidenţiată la începutul studiului în proba 2-salam bănăţean uscat şi în proba 5-pastramă de porc, în timp ce proba 4-cârnaţi tradiţionali a avut cea mai mare concentraţie -2,7 µg NDEA/kg; după 7 zile în proba 5 s-a determinat cea mai mare cantitate de NDEA, 7,8 µg/kg; la finalul studiului concentraţia de NDEA a crescut semnificativ, atingând valori de 22,5 µg/kg (în proba 2), 25,7 µg/kg (în proba 3), 40 µg/kg (în proba 5), respectiv 61,9 µg/kg (în proba 4).

Rezultatele obţinute sunt în concordanţă ce cele obţinute de alţi autori; astfel în probe de carne şi preparate din carne concentraţia de NDMA a fost între 0,16-1,44 µg/kg, iar concentraţia de NDEA a fost între 0–0,69 µg/kg (2, 7, 8, 9).

CONCLUZII

Cercetările efectuate au scos în evidenţă faptul că nitraţii şi nitriţii adăugaţi în preparatele din carne ca şi conservanţi sunt prezenţi în aliment şi după o perioadă mare de păstrare a acestuia (în concentraţii mai mici decât cele permise de legislaţie) şi pot explica formarea în timp şi creşterea concentraţiei nitrozaminelor în preparatele din carne.

Scăderea concentraţiilor ionilor nitrat şi nitrit şi creşterea concentraţiilor de NDMA şi NDEA dovedesc formarea nitrozaminelor în preparatele din carne pe seama nitritului rezidual, în timpul păstrării acestora.

Determinarea conţinutului în nitrozamine a evidenţiat valori care depăşesc cu mult concentraţia maximă admisă pentru acest tip de produse alimentare.

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ISTORIA MEDICINEI

INSTRUMENTS MEDICAUX ROMAINS PROVENUS DE LA PROVINCE MÉSIE INFÉRIEURE

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ROMAN MEDICAL INSTRUMENTS FROM LOWER MOESIA (Abstract) : To elucidate the evolution of society over time, ancient medicine is a very interesting and important researching field. Archaeological discoveries, such as the objects described by this article, but other ancient sources, also, are able to provide a complex framework of medical practice in Roman times. The geographic area that we have like target in this material is the province of Lower Moesia, which includes the territory between Danube and Black Sea (Romanian Dobrodja) and northern Bulgaria. In the present study we present nine ancient medical instruments, from a private collection : two tweezers, two ear probes, a probe-spatula, a probe-spoon, a spoon for pharmacy and two fragments of some kind of hooks used in surgical operations. Most likely, we have a mixed medical kit with tools used in general medicine, surgery, in preparation of the pharmaceutical treatments, but very possible, also, in cosmetic practices. Publication of these archaeological materials is, in addition to an extra page in the history of ancient medicine, a pretext for stepping up in a research field that, in other regions of the former Roman Empire, it is a great interest for researchers. **Key words** : ANCIENT MEDICINE, MOESIA INFERIOR, MEDICAL INSTRUMENTS

Les civilisations antiques associaient le corps et la raison de l'homme au mythique et à la religion. Les causes des défauts physiques et des maladies étaient cherchées, surtout dans l'Orient antique, au monde divin. Ce sont les Grecs ceux qui ont essayé concilier une vision rationnelle sur les concepts de santé et de maladie avec la foi pour les Dieux (1,2,3,4,5). L'école d'Alexandrie a représenté un moment clé pour l'évolution de la médecine „intuitive" vers la médecine scientifique (6,7). Les pratiques anatomiques et physiologiques sont devenues scientifiques, grâce à la pléiade de médecins y venus de Grèce entière (8).

L'étape suivante pour le cours de la médecine antique, la plus importante, a été le «transplant» des connaissances grecques dans le monde romain (9). Sans cette transposition, la médecine grecque serait restée, probablement, au niveau de celle d'Egypte ou Babylone (10). Dans une première phase, qui correspond à l'époque républicaine, le respect pour des médecins manque. Les esclaves et les affranchis exerçaient ce métier, mais sans bénéficier de la protection de l'Etat (11,12).

César a accordé des droits à ceux qui pratiquaient des professions libérales (11). Suétone en parle : "*Omnes professos et liberalium artium doctores, quo libentius et ipsi urbem incolerent, et coeteri appeterent, civitate donavit"* (13). Ce moment a marqué le véritable essor des médecins et, implicitement, leur intérẹt pour pratique et recherches a grandi (14). Excepté les médecins généralistes, nommés dans des inscriptions *medici clinici*, il y en avait des médecins spécialisés pour chaque partie du corps (14,15,16,17,18,19,20,21). Leurs connaissances deviennent mises en pratiques dans tous les milieux sociaux. Les médecins des

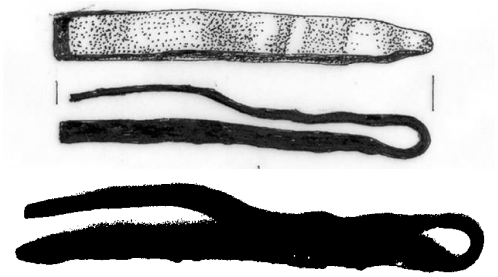

Fig. 1. Pincette

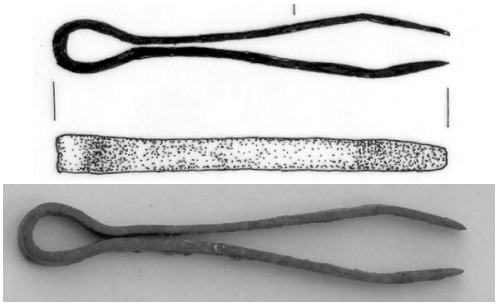

Fig. 2. Pincette

cours impériales sont devenus indispensables (12) ; c'est, aussi, le cas des unités militaires (5,22,23,24,25), des grandes cités, ou des petites communautés. Ils y étaient męme venus d'autres villes (26). Les instruments médicaux utilisés, les diverses branches de la médecine (2) et les spécialisations des médecins (27) ont pris se diversifier. En ce qui concerne les instruments médicaux, ils ont été découverts dans tout le monde romain, surtout dans des sépulcres (28,29). Gallia Belgica, Germania Inferior et Germania Superior sont les provinces le mieux représentées (30).

Quant aux pratiques médicales en Moesia Inferior, l'historiographie s'en est occupée, sans épuiser le sujet (31,32,33,34,35, 36,37,38,39). Assez souvent, les artefacts médicaux sont publiés dans un contexte général, sans une description complexe et en omettant des détails sur leur utilité (40, 41,42,43). Les inscriptions et les instruments médicaux y découverts jusqu'à ce moment prouvent que la médecine était réservée aux spécialistes (26,44,45,46,47).

Cette étude propose une analyse des nouveaux instruments médicaux et de pharmacie (provenus d'une collection privée) découverts en Dobroudja (fig. 10). Il s'agit de neuf pièces en bronze, d'emploi divers, du Nord de cette région.

1. Pincette (gr. λαβιζ, lat. *vulsella, volsella*) (48) à un bras droit et l'autre courbé, confectionnée d'une seule bande de bronze pliée. Dimensions : L= 62 mm ; poids= 9,40 g. (fig. 1) ; Ier –IIe siècle ap. J.- Ch.

2. Pincette à deux bras courbés (gr. λαβιζ, lat. *vulsella*, *volsella*) ; bronze. Dimensions : L= 59 mm ; poids = 5,20 g. (fig. 2) ; Ier –IIe siècle ap. J.- Ch.

Utilisées dans les pratiques chirurgicales, la médecine générale ou la pharmacie, les pincettes, fréquemment découvertes dans des trousses médicales, avaient des formes les plus diverses (22,48). Il y avait aussi des pinces à forme de pincette, mais avec des extrémités prévues des dents pour empoigne et fixation.

3. L'état assez précaire de ce fragment rend son identification difficile. La partie

Fig. 3. Fragment d'instrument utilisé en chirurgie

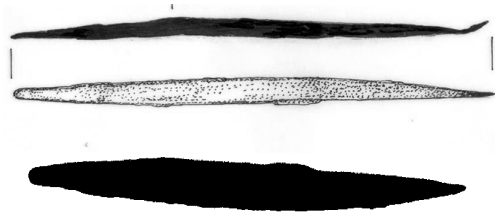

Fig. 4. Fragment d'instrument utilisé en chirurgie (crochet ?)

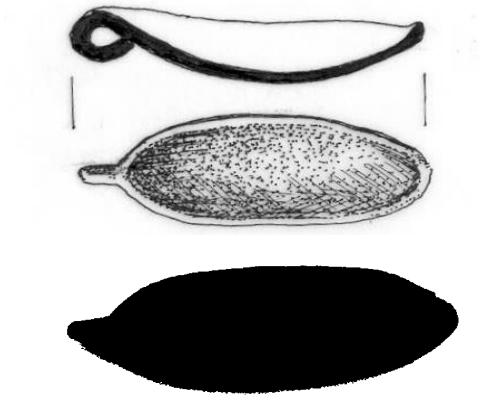

Fig. 5. Cuillère

pointue pouvait aider en stomatologie ou pour les interventions ophtalmologiques ; bronze ; Dimensions : L= 100 mm, poids = 7,50 g. (fig. 3) ; Ier–IIe siècle ap. J.- Ch.

4. Comme le fragment antérieur, l'emploi de cette pièce nous reste assez ambigu (peut ętre un crochet pour des opérations chirurgicales simples) (49) ; bronze ; Dimensions : L = 93 mm, poids = 7,00 g. (fig. 4) ; IIe siècle ap. J.- Ch.

5. Petite cuillère pharmaceutique ovale, prévue d'un crochet dont s'attache la manche ; bronze ; Dimensions : diamètre de la circonférence verticale = 40 mm ; diamètre de la circonférence horizontale = 12 mm ; poids = 3,30 g. (fig. 5) ; IIe siècle ap. J.- Ch.

Ces petites cuillères pharmaceutiques sont de types et dimensions diverses et différents moyens pour la fixation de la manche (48). Cette pièce ne garde pas sa manche, mais le système était classique : ancrée par une tige dans l'oreille de la base (50).

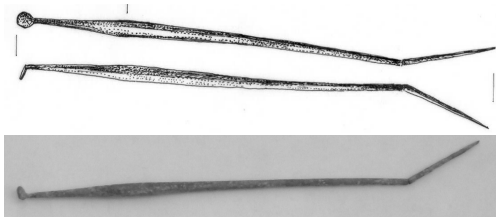

Fig. 7. Sonde auriculaire

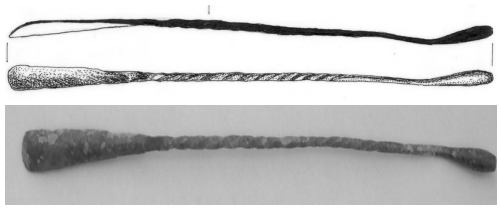

Fig. 6. Sonde-cuillère

6. Cuillère sonde (gr. Κυαθομηλη, lat. *cyathiscomela*) ; bronze ; Dimensions : L= 152 mm ; poids = 4,10 g ; L cuillère = 35 mm (fig. 6) ; IIe siècle ap. J.- Ch.

Nommée en Antiquité μηλη, μηλωτιζ/ μηλωριζ ou *specillum* (51), l'instrument est rond en section, s'amincit à un bout et se prolonge avec une petite cuillère allongée et aplatie à sa pincée. L'autre bout se termine en noyau d'olive (48). La partie à manoeuvrer, notamment le centre de la sonde, est tordu pour une meilleure prise et pour une utilisation rapide et facile.

Utilisée dans les pratiques pharmaceutiques, une extrémité pour combiner les divers ingrédients et l'autre pour peser les quantités de médicaments recommandés, la petite cuillère servait comme curette pour de divers tissus. Un tel instrument, qui se remarque par son ornement, a été découvert à Callatis, dans un tombeau daté à la moitié du IIe siècle ap. J.- Ch. (32,41). On en connaît autres pièces aux environs (43,52).

7. Sonde auriculaire (gr. μηλωτιζ, lat. *specillum oricularium* ; *auriscalpium*) ; bronze ; Dimensions : L= 135 mm ; poids = 4,10 g (fig. 7) ; Ier–IIe siècle ap. J.- Ch.

Sa partie inférieure est bien pointue et polie. La petite cuillère de l'autre coté, *ligula*, est plate et de petites dimensions.

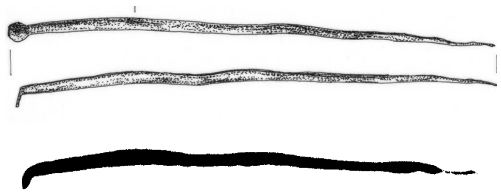

Fig. 8. Sonde auriculaire

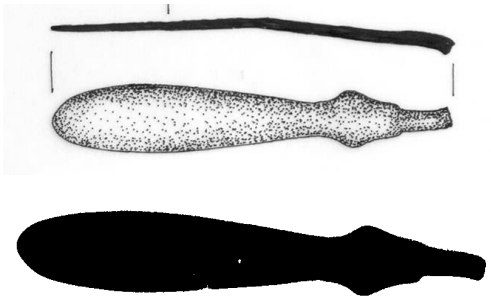

Fig. 9. Fragment de sonde - spatule „poisson"

La courbure de l'extrémité pointue nous frappe, étant, probablement, employée dans autres buts. Dans la région Radomir (province Thrace), bien possible un centre de production pour de petits objets en bronze, ont été découvertes 20 sondes, environ (53).

8. Sonde auriculaire (gr. μηλωτιζ, lat. *specillum oricularium* ; *auriscalpium*) ; bronze ; Dimensions : L=127 mm, poids = 4,40 g (fig. 8) ; Ier –IIe siècle ap. J.- Ch.

Comme la pièce antérieurement présentée, elle a une extrémité bien pointue, mais droite. L'extrémité de l'autre coté se finit par la męme petite cuillère très fine et aplatie (*ligula*) (48).

Les médecins spécialistes, nommés *medici auricularii* (14), s'aidaient des sondes d'oreilles ou auriculaires (*auricullarium specillum)* pour éloigner les intrus et les parasites (54).

En Asie Mineur, ces instruments servaient comme des sondes mais aussi comme des cautères (gr. Καυτηριον, lat. *ferrum candens*) (55). Leur grand nombre prouve qu'ils étaient indispensables dans chaque trousse médicale, grâce aux emplois multiples. Leurs dimensions étaient variables.

9. Fragment de sonde spatule (gr. σπαθομηλη, lat. *spathomela*) de type „poisson" (29,48) ; bronze ; Dimensions : L de la spatule = 69 mm, poids = 6, 30 g (fig. 9). Ier –IIe siècle ap. J.- Ch.

Une large gamme d'instruments chirurgicaux la représente ceux pour exploration, dilatation et inspection des cavités et encore les sondes nommées *specillum*. Ces sondes représentaient des outils pour les pratiques médicales, mais encore pour le ménage (56). Hippocrate les mentionne comme instruments pour soigner les blessures de la tęte. Leur utilité est largement décrite par Celse (54,57) et Galien (58). Certains types de sondes sont en usage męme à nos jours (59).

Cet objet y présent a une manche en forme de trapèze d'où commence la partie à manoeuvrer, en se prolongeant, le plus probable, par un pommeau en forme de noyau d'olive (fig. 10). Elle apparaît souvent dans les trousses d'ophtalmologie (60). D'ailleurs, c'est le type de sonde le plus connu. A l'une de ses extrémités pouvait avoir, au lieu d'une spatule, une palette ou une cuillère. Ces modifications étaient faites pour simplifier la trousse.

Ces sondes servaient en pharmacie et, en męme temps, dans les pratiques de la médecine générale. L'extrémité ronde était employée comme sonde, dirigeant du bistouri (gr. μαχαιρα, σμιλμ, lat. *culter, scalper, scalpellus*), et pour cautériser. L'extrémité spatule servait pour écraser et combiner les poudres, les liquides et les onguents et pour saupoudrer, pour dégoutter les

Fig. 10. Des instruments médicaux en bronze de la province romaine Mésie Inférieure (Ier – IIe siècle ap. J.- Chr.)

lotions dans les yeux. Dans les cuillères, étaient dissoues les diverses poudres (60). Elles étaient utilisées, sans doute, pour la séparation des colorants dans la cosmétique (29,49,56,61). Ces emplois multiples expliquent leur grand nombre (27,49,62).

On ne dispose pas d'informations concernant le cadre archéologique ou autres preuves (objets associés qu'on puisse dater). Pourtant, on sait bien qu'il n'y a pas une évolution importante quant à la forme des ustensiles appartenant aux premiers siècles de l'ère chrétienne. Prenant pour repère des objets similaires de la même région ou de l'Empire, on pourrait placer cette trousse médicale, cosmétique ou toutes les deux, dans les premiers deux siècles ap. J. - Ch.

Ces instruments ont probablement appartenu à un médecin pratiquant de la médecine générale, mais qui possédait des connaissances nécessaires pour de simples interventions chirurgicales. La diversité de leur utilisation nous fait croire qu'il n'était pas un spécialiste et pouvait guérir plusieurs maladies. Cette situation est valable pour le monde romain où on connaît des médecins avec multiples spécialisations (27).

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NOUTĂŢI

Cryptochrome - legătura dintre ceasul biologic şi diabetul zaharat

Creşteri ale nivelelor circulante de glucagon şi adrenalină, doi hormoni care activează gluconeogeneza hepatică, declanşează fosforilarea mediată de AMPc a factorului de transcripţie Creb *(cAMP response element–binding protein)* şi defosforilarea co-activatorului 2 al Creb *(Crtc2, Creb-regulated transcription coactivator-2)*. Prin controlul sintezei de AMP ciclic, Cryptochrome reglează activitatea Creb în ficat. În acest fel, sinteza de glucoză în ficat este legată prin intermediul perioadelor interprandiale şi a celor de somn de funcţionarea ceasului biologic. Activitatea Creb în timpul perioadelor interprandiale este modulată de Cry1 şi Cry2, ritmic exprimate de către ficat. Expresia Cry1 creşte în timpul tranziţiei de noapte-zi, atunci când se reduce expresia genelor implicate in gluconeogeneză prin blocarea creşterii mediate de glucagon a concentraţiei AMPc intracelular şi a fosforilarii Creb mediată de Protein kinaza A. În studiile biochimice, s-a constatat că, Cry1 a inhibat acumularea de cAMP ca răspuns la activarea unei proteine G cuplate la receptor, dar nu şi la forskolin, un activator direct al adenilat ciclazei. Proteinele Cry par să moduleze direct activitatea proteinelor G cuplate la receptori prin intermediul subunităţii Gsá. Supraexpresia hepatică a Cry1 a scăzut nivelul glucozei din sânge şi a îmbunătăţit sensibilitatea la insulină la şoarecii insulino-rezistenţi db/db, ceea ce sugerează că acei compusi care amplifică activitatea Cryptocrom pot oferi beneficii terapeutice in DZ de tip 2 (Zhang EE, Liu Y, Dentin R, Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat Med* 2010 ; 16 (10) : 1145-1151).

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CERCETĂRI PRIVIND PRODUCEREA DE BETA-LACTAMAZE CU SPECTRU EXTINS LA TULPINI DE ENTEROBACTERII

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RESEARCHES REGARDING EXTENDED-SPECTRUM BETA-LACTAMASES PRODUCE BY ENTEROBACTERIA STRAINS (Abstract): **Aim**: To evaluate the susceptibility of extended spectrum beta-lactamase (ESBL) producing organisms among *Escherichia coli* and *Klebsiella pneumoniae*, isolated between January-October 2009 from hospital and community. **Materials and methods**: From 611 enterobacteria strains, for 112 strains (*E. coli*, n=84 and *K. pneumoniae*, n=28), the ESBL phenotypic confirmation was performed with double disk test (DDT) and the replacement disk method. The minimum inhibitory concentrations (MICs) for next antimicrobial agents tested: ceftazidime, cefotaxime, ceftriaxone, cefepime, cefpirome, imipenem, meropenem, ertapenem, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin were performed by agar dilution method. Resistance rates were reported using the *Clinical Laboratory Standard Institute* (CLSI) breakpoints for the fully susceptible category; moderately susceptible isolates were classified as resistant. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used for quality control. **Results**: Resistance rates for ceftazidime, cefotaxime, ceftriaxone and aztreonam among tested strains were very high: 84.5%, 100%, 100% and 96.4% for *E. coli* and 98.8%, 98.8%, 97.6% and 89.2% for *K. pneumoniae*, with the MIC90 values > 256 mg/L for either antimicrobial tested agent. The MICs for fourth generation cephalosporins ranges from 4 mg/L to 512 mg/L. The MICs for tested quinolones were > 2 mg/L for 89.2% strains. Using breakpoint for susceptibility, all isolates were sensitive to ertapenem, imipenem and meropenem. **Conclusions**: We noticed a high resistance rate for ceftazidime, cefotaxime, ceftriaxone and aztreonam. In this study, carbapenems demonstrated the highest degree of activity. **Key words**: EXTENDED SPECTRUM BETA-LACTAMASE, ENTEROBACTERIA, ANTIMICROBIAL RESISTANCE

Tulpinile de *Escherichia coli* şi *Klebsiella pneumoniae* producătoare de beta-lactamaze cu spectru extins (BLSE) constituie cele mai comune bacterii gram-negative cu rezistenţă multiplă la antibiotice (1). Beta-lactamazele cu spectru extins sunt enzime care conferă rezistenţă la cefalosporinele de generaţia a treia (e.g., ceftazidim, cefotaxim şi ceftriaxonă) şi monobactame (e.g., aztreonam), dar care nu afectează cefamicinele şi carbapenemele. Deşi aceste enzime conferă rezistenţa la cefalosporinele cu spectru extins şi la aztreonam, diversitatea BLSE determină profiluri variate de sensibilitate pentru alte beta-lactamine (2). Din acest motiv, este absolut necesar ca laboratoarele clinice să fie capabile să depisteze rezistenţa determinată de BLSE (3).

Scopul studiului a fost de a evalua rezistenţa la agenţi antimicrobieni a unor tulpini de *Escherichia coli* şi *Klebsiella pneumoniae* producătoare de beta-lactamaze cu

spectru extins în vederea descrierii profilului de rezistenţă şi al selectării tulpinilor pentru studii viitoare de biologie moleculară.

MATERIAL ŞI METODE

Tulpini bacteriene

Am testat 112 tulpini de *Enterobacteriaceae* (84 tulpini de *Escherichia coli* şi 28 tulpini de *Klebiella pneumoniae*, selectate dintre 611 enterobacterii pe baza antibiogramelor prin metoda difuzimetrică, ca test *screening* iniţial recomandat de *Clinical Laboratory Standard Institute* (CLSI) (4). Tulpinile testate, identificate prin teste convenţionale şi cu sistemul automat mini-API (bioMerieux), au fost izolate de la pacienţi şi purtători sănătoşi în diverse produse patologice (urină, abcese, materii fecale), în perioada ianuarie-octombrie 2009.

Pentru controlul de calitate am utilizat tulpinile martor pozitiv, respectiv negativ pentru producerea de BLSE: *K. pneumoniae* ATCC 700603 şi *E. coli* ATCC 25922.

Agenţi antimicrobieni

Am determinat concentraţia minimă inhibitorie (CMI) pentru următorii agenţi antimicrobieni: ceftazidim, cefotaxim, ceftriaxonă, cefepim, cefpirom, aztreonam, imipenem, meropenem, ertapenem, moxifloxacin, pefloxacin, levofloxacin şi gatifloxacin, obtinuţi din surse comerciale şi testaţi în 12 concentraţii diferite, cuprinse între 0,06 şi 512 mg/L.

Testarea sensibilităţii

Concentraţiile minime inhibitorii au fost determinate prin metoda diluţiilor în agar Mueller-Hinton, conform recomandărilor CLSI (4). După inocularea cu aproximativ 10^5UFC/spot, plăcile au fost incubate peste noapte la 37^0C. Concentraţia minimă inhibitorie (CMI) a fost considerată ca cea mai mică diluţie de antibiotic fără creştere per spot. Categoriile de sensibilitate le-am stabilit conform punctelor de ruptură (*breakpoints*) recomandate de CLSI (4) pentru categoria total sensibil (izolatele moderat rezistente au fost clasificate în acest studiu, ca fiind rezistente).

Analiza statistică

Analiza statistică am efectuat-o prin stabilirea procentelor tulpinilor inhibate la o anumită valoare a CMI, calcularea valorilor CMI 50 şi CMI 90 şi a ratei de rezistenţă faţă de agenţii testaţi. Valorile CMI 50 şi CMI 90 reprezintă concentraţia minimă inhibitorie care inhibă 50%, respectiv 90% din izolatele investigate.

REZULTATE ŞI DISCUŢII

Tabelele I şi II însumează datele privind sensibilitatea tulpinilor studiate faţă de agenţii antimicrobieni testaţi, prezentate ca valori ale CMI 50, CMI 90, limite ale CMI şi rata rezistenţei.

Rata de rezistenţă a tulpinilor de *E. coli* la cefalosporinele de generaţia a 3-a a fost foarte crescută: 84,5%, pentru ceftazidim şi 100%, pentru cefotaxim şi ceftriaxonă. Pentru tulpinile de *K. pneumoniae*, rata de rezistenţă a fost cuprinsă între 97,6% (la ceftriaxonă) şi 98,8% (la ceftazidim şi cefotaxim). Rezistenţa pentru aztreonam a fost detectată la 96,4% dintre tulpinile de *E. coli* şi 89,2% dintre tulpinile de *K. pneumoniae* testate. În studiul nostru, valorile CMI 90 pentru fiecare cefalosporină de a 3-a generaţie testată au fost >256 mg/L, iar pentru cefalosporinele de generaţia a 4-a, >128 mg/L. Valorile CMI pentru fluoroquinolonele testate au fost >2 mg/L pentru > 89,2% dintre tulpinile de *E. coli* şi >71,43% dintre tulpinile de *K. pneumoniae* investigate. Microorganismele producătoare de BLSE pot fi selectate în urma unor greşeli în terapia cu antibiotice. Beta-lactamazele cu spectru extins apărute pot fi detectate cu dificultate, pentru că ele au niveluri diferite de activitate asupra cefalosporinelor.

Conform standardului CLSI (4), tulpinile care au valori ale CMI pentru cefpodoxim, ceftazidim, aztreonam, cefotaxim sau

TABELUL I

Activitatea *in vitro* a agenţilor antimicrobieni testaţi asupra tulpinilor de *E. coli* (n=84)

Agent antimicrobian	CMI (mg/L)			Procent de rezistenţă
	50%	90%	Limite	
Ceftazidim	64	256	0,25-512	84,52
Cefotaxim	128	512	32-512	100
Ceftriaxonă	256	512	16-512	100
Cefpirom	128	256	64-512	100
Cefepim	64	128	8-256	96,4
Aztreonam	128	256	4-512	96,4
Imipenem	0,06	0,06	0,06-0.5	0
Meropenem	0,06	0,06	0,06-0,06	0
Ciprofloxacin	32	64	0,06-128	90
Moxifloxacin	32	64	0,06-128	89,2
Pefloxacin	32	64	0,25-128	89,2
Levofloxacin	32	64	0,06-128	89,2
Gatifloxacin	16	32	0,125-64	89,2

TABELUL II

Activitatea *in vitro* a agenţilor antimicrobieni testaţi asupra tulpinilor de *Klebsiella* spp. (n=28)

Agent antimicrobian	CMI (mg/L)			Procent de rezistenţă
	50%	90%	Limite	
Ceftazidim	64	256	8-512	98.8
Cefotaxim	128	512	8-512	98,8
Ceftriaxonă	256	512	4-512	97,61
Cefpirom	128	256	64-256	100
Cefepim	32	128	4-256	89,2
Aztreonam	128	512	4-512	89,2
Imipenem	0,06	0.06	0,06-0,5	0
Meropenem	0,06	0,06	0,06-0,06	0
Ciprofloxacin	32	64	0,25-128	72
Moxifloxacin	16	64	0,06-128	71,43
Pefloxacin	32	64	0,25-128	71,43
Levofloxacin	16	32	0,06-128	71,43
Gatifloxacin	16	32	0,25-128	71,43

ceftriaxonă ≥2 mg/L trebuie suspectate ca fiind producătoare de BLSE.

La pătrunderea cefalosporinelor de generaţia a 3-a pe piaţa românească în anii '90, fenomenul de rezistenţă a tulpinilor de *Enterobacteriaceae* era absent. Primele tulpini rezistente la aztreonam au fost semnalate în perioada 1991-1995; procentul acestora crescuse semnificativ de la 0,6% la 13% în anul 2002 (5).

Unul dintre cele mai îngrijorătoare aspecte ale rezistenţei producătorilor de BLSE constă în rata crescută de rezistenţă la antibiotice non beta-lactamice, în special la chinolone, trimetroprim-sulfametoxazol şi aminoglicozide (6).

În studiul nostru, rata de rezistenţă la fluoroquinolone a fost remarcabilă: 89,2% pentru tulpinile de *E. coli* şi 71,43% pentru tulpinile de *K. pneumoniae* testate. Într-un alt studiu, rata de rezistenţă la ciprofloxacin a fost de 84% la tulpinile de *E. coli* BLSE pozitive (6). Hal Jones et al. (7) au înregistrat corezistenţă la levofloxacin de 42%. Diferite ţări europene (Grecia, Italia, Spania, Marea Britanie) au raportat un trend

alarmant al rezistenţei asociate cu alte clase de agenţi antimicrobieni între tulpinile producătoare de BLSE (8,9,10,11). Raportat la valorile *breakpoint* pentru sensibilitate, toate tulpinile testate au fost sensibile la carbapeneme. În literatura recentă, carbapenemele au fost cei mai activi agenţi antimicrobieni asupra enterobacteriilor producătoare de BLSE, astfel încât sunt indicate ca tratament în infecţiile cauzate de tulpini de *E. coli* şi *K. pneumoniae* producătoare de BLSE (1, 6). Fenomenul îngrijorător semnalat impune continuarea studiilor privind sensibilitatea la antibiotice a tulpinilor de *Enterobacteriaceae*, în vederea ajustării schemelor terapeutice şi a realizării ghidurilor de tratament conform rezultatelor testărilor prin metode standardizate.

CONCLUZII

Tulpinile studiate prezintă valori crescute ale CMI50 şi CMI90 pentru cefalosporinele de generaţia a treia şi aztreonam, dar nu sunt afectate de carbapeneme, fapt care confirmă supoziţia de producere a BLSE.

Rezultatele noastre demonstrează necesitatea urmăririi, în condiţii standardizate, a sensibilităţii la antibiotice a tulpinilor de *Enterobacteriaceae*.

NOTA

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FORMULATION OF CREAMS WITH BETAMETHASONE DIPROPIONATE: EVALUATION OF CHEMICAL STABILITY

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FORMULATION OF CREAMS WITH BETAMETHASONE DIPROPIONATE: EVALUATION OF CHEMICAL STABILITY (Abstract): The aim of this study was to investigate how the different excipients influenced the chemical stability of betamethasone dipropionate in creams. The chemical stability was evaluated by analyzing betamethasone related substance content. Transcutol is the excipient ensuring maximum stability to betamethasone. Incompatibilities between betamethasone dipropionate and hexylene glycol were observed. The pH values in the weak acid range confer chemical stability to betamethasone. **Key words**: BETAMETHASONE DIPROPIONATE, EXCIPIENTS, STABILITY, PH

LAUDATIO

PROFESSOR ALLA VÂȚĂ, M.D., Ph.D.

A CAREER OF SUCCESS

Prof. Alla Vâță, PhD is one of the most important Romanian specialists in the field of infectious diseases. A genuine and dedicated scholar, she is endowed with a rare intelligence. She has always enjoyed the respect and admiration of the whole medical community. She is admired for her excellent professional training that became obvious whenever she presented her opinions about a diagnosis or whenever she taught her undergraduate and graduate students. Her lectures captivated the audience. She is a modest lady and an exact and patient scientist. Prof. Alla Vâță led with a firm hand the Iasi Infectious Diseases Hospital. She was also a remarkable researcher in her field.

She was born in 1920 in Kishinew, where she completed her secondary studies. In the period 1940-1945 she attended the Iaşi Faculty of Medicine**.** Her professors were among others**:** Al. Țupa, C. C. Dumitriu, I. Enescu, I. Tănăsescu, D. Cornelson, all of them outstanding scientists in their field.

In 1945, after a competition she was accepted in the externship program at the Hospital of Infectious Diseases, led by prof. Maria Franche, PhD, who became Mrs Vâță"s model throughout her life. She started her academic career in 1946 at the same clinic. A brilliant career followed: junior teaching assistant, assistant lecturer, lecturer (1952), associate professor (1961), professor (1970). In 1963 she defended her PhD in medical sciences, complemented in 1972 by the highest Romanian academic degree that was awarded after defending the second doctoral dissertation. Prof. Dr. Alla Vâță has been an honorary Member of the Academy of Medical Sciences since 1996.

In 1981 she became consultant on the staff of the Hospital of Infectious Diseases. During her teaching career she supervised 16 doctoral dissertations at the Iasi ″Gr. T. Popa″ University of Medicine and Pharmacy. During this prodigious career she was driven by the desire for continuous improvement despite the isolation of Romania from the western world.

Prof. Dr. Alla Vâță enjoyed a remarkable teaching career**.** She used to teach the 6th year medical students and postgraduates, being admired and respected by all her students and colleagues.

She taught a course of lectures on infectious diseases. Moreover, she managed to create a school of highly trained specialists in infectious diseases, many of them becoming in their turn professors in this field (St. Dumitriu, E. Brauner, V. Luca), senior consultants (dr. E. Beşleagă, dr.C. Scurtu), and heads of department from of hospitals all over Romania. All of them were able to solve the most difficult problems this specialty was faced with at that time.

She was a remarkable manager, leading

the most crowded hospital in Moldova during a very difficult period. She managed to make useful changes in the hospital: restructured the patient reception area; had an amphitheater built (1964-1965), upgraded with new equipment the radiology unit; the hospital mortuary (1965), had a new building built for laboratories of bacteriology and chemistry laboratories (1969); the follow-up care unit for epidemic hepatitis patients; complete renovation of the first pavilion, the rooms and entry spaces on the ground floor being turned into a library and reading room for students, doctors and hospital staff.

Prof. Vâță always distinguished herself for the quality of her performance, whether it was about lectures, oral presentations, conferences, roundtables, which she always turned into events exceeding the frame of lectio magistralis and becoming examples of sharing medical practice and highly ethical conduct.

An exquisite clinician, Prof Alla Vâță used to amaze those whom she taught or colleagues by the accuracy of clinical reasoning, amount of knowledge and therapeutic strategy. Besides being a wonderful clinician she was the best and most compassionate doctor, who knew how to approach her patients often with poverty-related diseases and to care for them devotedly. When doctors in other areas were faced with a difficult case she was always the one who was asked for an interdisciplinary examination.

Prof. Dr. Alla Vâță has a very rich scientific activity. She published more than 250 scientific papers. Her research was concentrated on infectious diseases, focusing primarily on issues of utmost importance to public health: viral hepatitis**;** ECG changes in some bacterial and viral diseases (exanthematic typhus was a national priority); studies on measles, mumps and typhus**;** zooanthroponoses **(**anthrax, tularemia, Q fever, and leptospirosis); studies on bacterial and viral meningitis; sepsis. She published the first treatise on infectious diseases in 1971, reprinted in 1978, "Infectious Diseases Update", Junimea Publishing House, Iaşi, 1975, and also the chapter "Therapy of infectious diseases" in the Therapeutic Guidelines" (three editions).

She is an active member in the medical life of Romania. She attended numerous national infectious disease congresses, conferences, symposia in Bucharest, Iasi, Constanta, Cluj, Tg. Mures, and in the annual scientific sessions organized by the University of Medicine and Pharmacy and the Society of Physicians and Naturalists from Iasi. Her presentations of the results of her research work have always been well received by other specialists. She also contributed directly to the organization of many scientific events in Moldova. She attended various scientific meeting abroad: The Balkan Medical Week in Istanbul (1968), Belgrade (1970), Athens, etc. She was elected president and vice-president of national congresses on infectious diseases. She presented her papers at the Congresses of the International Society for Infectious Diseases and Parasitology in Vienna (1970), Warsaw (1974), (at this congress she was elected member of the International Society Committee), Varna (1978), Cairo (1985). She also presented papers at the International Congress of Infectious Diseases, held in Jerusalem (1989). Last but not least, the syntheses of her researches at conferences held in Copenhagen and Lund as part of the experience exchange programs (1971).

She is a member of important national and international scientific societies: Presi-

dent of the Romanian Society for Infectious Diseases, Microbiology and Epidemiology (1972-1985), Society of Physicians and Naturalists of Iaşi (secretary1972 – 1974, president 1974- 1979, vice-president 1979-1990, honorary president (1990 to the present), the Balkan Medical Union (secretary of the national committee), member of the Romanian Association of Scientists, Iasi Branch, since 1981, International Society for Infectious Pathology and Parasitology (member since 1972, member of society committee 1976-1982).

Professor Alla Vâță was member of the editorial board of many important medical publications (*Medical Surgical Journal, Bacteriology, Virology, Parasitology, Epidemiology,* and *Journal of Preventive Medicine*).

She served with honor and efficiency various administrative roles at the Gr. T. Popa University of Medicine and Pharmacy and the Ministry of Health. In this respect, it is worth mentioning that in 1972-1973 she was appointed deputy rector.

For the entire activity she was awarded diplomas of honor and excellence: Medal of Labor (1969), Sanitary Merit Medal (1971), Scientific Merit Order Class III (1971), "Gold Diploma" for outstanding achievements in the Red Cross activity (1972), Medal "25 Since the Proclamation of the Republic" (1972), Physician emeritus (1973), Order of Labor class III (1976), Professor emeritus (1980), Member emeritus of the Academy of Medical Sciences (1993), Honorary Member of the Romanian Association of Scientists (1996), Honorary member of the academic staff of the Iasi "Gr. T. Popa" University of Medicine and Pharmacy (1997), Diploma of Excellence of the Iasi College of Physicians (2000), Medical Merit Order in the rank of Commander (2004).

Along with other great personalities of Iasi medicine, Professor Alla Vâță is included in the gallery of the personalities who contributed to the progress and modernization of health care in Romania.

Happy Anniversary our Dear Professor Alla Vâță!

E. Târcoveanu

O POSIBILITATE DE DETECȚIE A POTENȚIALULUI P300 CU AJUTORUL TRANSFORMATEI WAVELET MULTIREZOLUȚIE

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THE POSSIBILITY OF A MULTIRESOLUTION WAVELET APPROACH FOR DETECTING THE P300 EVENT RELATED POTENTIAL (Abstract): The main objective is to highlight the P300 potential on certain electroencephalographic signals. P300 occurs at a relatively well defined time in relation to a stimulus and it represents a signal with a specified band frequency. **Method:** The electroencephalographic (EEG) was recorded with 4 wet electrodes by means of g.MOBIlab+ module, a g.tec acquisition system. The multiresolution wavelet transform was chosen to extract the P300 potential from the EEG signal because it provides information on both time and frequency domains. **Results:** The multiresolution wavelet transform decomposes the signal in sub-bands and it helps to highlight the P300 potential. The spectrum of the P300 potential is around 3Hz. For the multiresolution wavelet decomposition this corresponds to coefficients of approximation of order 4 according to 0 to 60 Hz band of the original EEG signal. The representation of these coefficients emphasizes a better detection of P300 potential then in the original signal. **Conclusion:** It is shown to be a more appropriate method than the direct analysis of the signal because it works with lower dimensional signals. This method of detection of the P300 potential can be used successfully in the implementation of a Brain Computer Interface (BCI). **Key words**: BRAIN COMPUTER INTERFACE, EEG, P300, MULTI-RESOLUTION WAVELET TRANSFORM.

Posibilitatea de a comunica cu alte persoane prin intermediul vorbirii, al gesturilor sau al scrisului reprezintă unul dintre factorii care fac ca fiecare ființă umană să se poată bucura de viață. Persoanele care au anumite afecțiuni motorii (cei ce suferă de aşa-numitul sindrom al imobilizării - „locked-in syndrome") nu au posibilitatea să exprime idei, dorințe sau sentimente. Afecțiunea (de exemplu scleroză amiotrofică laterală, atac cerebral sau polineuropatie severă) se manifestă prin pierderea completă a controlului voluntar asupra muşchilor. Pacienții ce prezintă acest sindrom sunt deplin conştienți de ceea ce se întâmplă în mediul înconjurător şi prezintă o activitate cerebrală normală (electroencefalogramă normală), dar nu sunt capabili să comunice nici măcar nevoile de bază sau să se mişte, cu alte cuvinte sunt prizonieri în propriile corpuri.

O modalitate de a le oferi un anumit grad de autonomie şi posibilități minimale de comunicare o constituie integrarea într-un sistem alternativ pentru comunicare şi control, cunoscut sub denumirea de „Inter-

faţă Creier-Calculator". Ideea de bază a unui sistem BCI este aceea de a măsura activitatea electrică, magnetică sau orice alte manifestări fizice ale activităţii creierului şi de a le transla în comenzi pentru un calculator sau orice alt dispozitiv (scaun cu rotile, neuroproteză). Altfel spus, scopul BCI este acela de a traduce intenţiile umane - convertite în semnale convenabil alese - în semnale de control pentru un dispozitiv de ieşire.

Potenţialele evocate ERP ("Event related potentials") reprezintă segmente EEG generate de creier atunci când o persoană recepţionează un stimul şi răspunde în mod voluntar la acesta. Aceste tipuri de potenţiale sunt utilizate pentru BCI şi pentru detectarea unor boli neurologice.

Unul dintre aceste potenţiale este potenţialul P300, care este o deviaţie pozitivă în EEG-ul uman care apare la aproximativ 300-400ms după prezentarea neaşteptată a unor stimuli relevanţi. Timpul de apariţie a acestei componente poate varia de la 250 ms chiar până la 900 ms cu amplitudini de la minimum 5μV până la limita uzuală de 20μV pentru potenţialele auditive şi cele evocate vizual. Spectrul de frecvenţă al potenţialul P300 este situat la frecvenţe joase, în jur de 3 Hz (1).

Farwell şi Donchin au demonstrat că potenţialul P300 poate fi utilizat pentru citirea cuvintelor atunci când se utilizează aşa numita paradigmă "odd-ball" (2).

Întrucât P300 apare la un moment relativ bine determinat de timp în raport cu un stimul şi de asemenea reprezintă un semnal cu frecvenţa situată într-o bandă foarte îngustă şi de asemenea destul de bine precizată, pentru extragerea acestuia din semnalul EEG s-a optat pentru transformata wavelet, ce oferă informaţii legate atât de timp, cât şi de frecvenţă.

MATERIALE ŞI METODĂ

Analiza wavelet multirezoluţie

Transformata wavelet multirezoluţie oferă posibilitatea analizei semnalelor nestaţionare. Transformata wavelet poate analiza aceste semnale la diferite rezoluţii prin descompunerea acestora în benzi de frecvenţă. Această metodă a fost dezvoltată pentru a rezolva deficienţele transformatei Fourier scurte (STFT Short time Fourier Tranfform).

În cazul transformatei wavelet continue, semnalele sunt analizate prin scalarea si translarea unei funcţii wavelet mamă (3). Tranformata wavelet discretă (DWT) este reprezentarea timp-scală bazată pe metode digitale de filtrare. Transformata wavelet discretă analizează semnalele la diferite rezoluţii (analiză wavelet multirezoluţie), prin descompunerea semnalului în benzi diferite de frecvenţă. În analiza wavelet, se vorbeşte despre aproximări şi detalii. Aproximările sunt componentele la scale ridicate şi frecvenţe joase. Detaliile sunt componentele la scale joase şi frecvenţe ridicate.

DWT se calculează prin filtrări complementare trece-jos şi filtrări trece-sus succesive şi subeşantionări, metodă cunoscută sub denumirea de algoritmul Mallat (4).

Răspunsul la impuls al filtrului trece-sus (FTS) este notat cu h(n), iar al filtrului trece-jos (FTJ) cu g(n). La fiecare nivel, FTS produce un semnal d(n) al detaliului, în timp ce FTJ asociat cu funcţia de scalare produce un semnal a(n) ce reprezintă o aproximare grosieră. După filtrare FTJ şi FTS şi subeşantionare cu 2 se obţin următoarele semnale:

$$y_J(n) = x(n) * g(n) = \sum_{k=-\infty}^{\infty} x(k) g(2n-k)$$

$$y_S(n) = x(n) * h(n) = \sum_{k=-\infty}^{\infty} x(k) h(2n-k)$$

REZULTATE

Datele au fost înregistrate personal, în laboratorul de Procesare de biosemnale din cadrul Facultății de Bioinginerie Medicală Iași, cu ajutorul sistemului de achiziție de semnale EEG de la firma g.tec din Austria și a platformei BCI2000 (5). Sistemul este format din amplificatorul g.GAMMAbox, g.GAMMAcap cu electrozii și din sistemul de achitiție g.MOBIlab (6). Produsul folosit oferă un sistem de cercetare/dezvoltare complet, sub mediul de programare MATLAB. BCI2000 este o platformă de uz general pentru cercetare și dezvoltare ce dă posibilitatea să se implementeze, evalueze și compare diferite sisteme. S-au făcut înregistrări de la mai mulți subiecți, dar rezultatele prezentate sunt numai de la un singur subiect.

Pacientului -R.A, 25 ani, sănătos- îi este măsurată distanța între inion și nasion și i se așează cap-ul cu electrodul de masă (plasat pe Fpz) la 10% din distanță măsurată, în cazul nostru la 3,9 cm de nasion (conform standardului internațional 10-20).

S-au ales canalele Fz, Cz, Pz și Oz pentru plasarea electrozilor activi, iar electrodul de referință a fost plasat pe urechea dreaptă.

În timpul calibrării, subiectul trebuie să-și fixeze atenția asupra literelor unor cuvinte dintr-o matrice, conform paradigmei propusă de Farwell și Donchin. În varianta actuală a paradigmei, subiectului uman îi este prezentată o matrice cu dimensiunea 6x6 având diverse caractere, evidențiate succesiv, prin modificarea culorii (sau a luminozității) unei linii și/sau coloane. Ori de cate ori o litera, pe care subiectul o cunoaște în prealabil, se află în linia (coloana) evidențiată, în semnalul EEG se generează un potențial P300. Printr-o astfel de corespondență este posibilă comunicarea creier - calculator.

Cu ajutorul sistemului BCI2000, s-a realizat analiza offline a acestor date achiziționate. Analiza offline oferă două rezultate: reprezentarea coeficientului de determinare, r^2, în funcție de timp și canale (7) și afișarea formelor de undă utile pentru investigarea canalelor specifice.

Este reprezentat coeficientul de determinare (fig. 1), unde se poate observa că valorile cele mai ridicate sunt la aproximativ 400 ms față de aplicarea stimulului. Cea mai ridicată valoare corespunde electrodului 3, adică Pz.

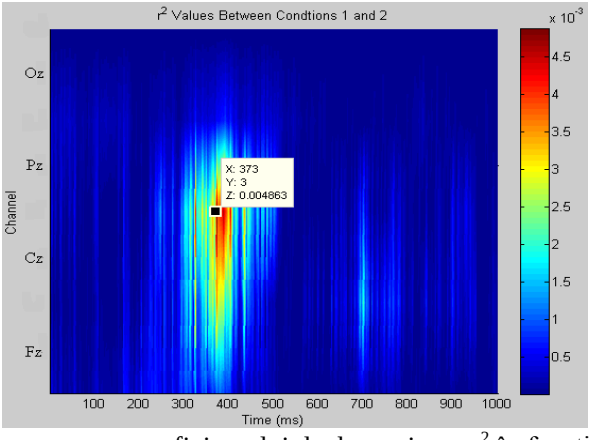

Fig. 1. Reprezentarea coeficientului de determinare r^2 în funcție de timp, pentru canalele Fz, Cz, Pz și Oz

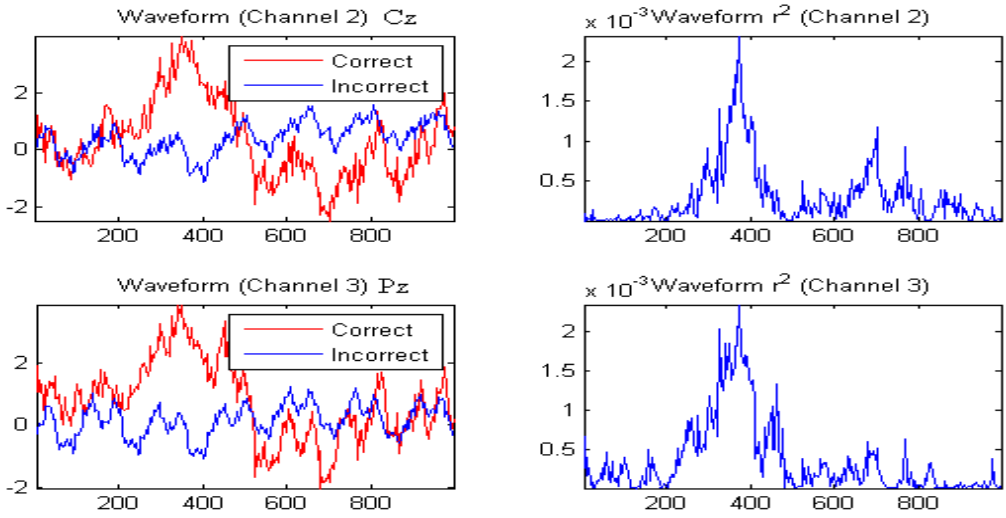

Fig. 2. Reprezentarea EEG pe canalele Cz şi Pz pentru cazul în care se iluminează (corect) şi în care nu se iluminează caracterul dorit (incorect) (partea stângă) şi coeficientul de determinare r^2 (partea dreaptă)

Se observă că potențialul P300 apare la aproximativ 400 ms (fig. 2), ceea ce este în concordanță cu morfologia acestuia. Semnalul notat "Corect" reprezintă semnalul rezultat atunci când litera luminată corespunde cu cea asupra căreia se concentrează subiectul, iar semnalul notat cu "Incorect" semnalul când litera de interes nu este şi cea luminată. În graficul lui r^2 se observă că valorile sale determină un vârf de asemenea la aproximativ 400 ms. Ca formă de undă pentru potențialul P300 s-ar părea că cele mai apropiate rezultate față de cele din literatură s-au obținut pe electrodul Cz. Cele mai relevante valori pentru coeficientul r^2 s-au obținut pentru canalele Cz şi Pz.

Pentru a pune mai bine în evidență potențialul P300 s-a utilizat transfomata wavelet şi descompunerea multirezoluție aplicate pe semnale obținute prin medierea pe linie şi coloană pentru fiecare literă a cuvintelor ce au fost afişate şi urmărite. S-a reprezentat coeficientul de aproximare de ordin 4 (corespunzător benzii 0-4 Hz) pentru literele cuvântului QUICK pe canalele 2 şi 3, respectiv Cz şi Pz. Am reprezentat descompunerea multirezoluție cu waveletul db2 pe 4 nivele pentru media pe linie şi coloană a literei Q pe canalul 3 (Pz) (fig. 3). Mediul de programare folosit a fost MATLAB.

În al doilea grafic (fig. 3), corespunzător coeficientului de aproximare a4 (0-4Hz), pe canalul Pz, se observă o formă asemănatoare unui P300 la aproximativ 450 ms, ceea ce este considerat a fi corect. Pentru observarea potențialului P300 am extras doar coeficientul de aproximare de ordin 4 (fig. 4).

De menționat este faptul că sistemul de achiziție amplifică înregistrările cu 100.

Rezultatele obținute pentru coeficientul r^2 (fig. 2) arată că avem rezultate destul de bune şi pe canalul Cz. Pe acest canal cele mai bune rezultate sunt pentru medierile pe linii şi coloane a literelor U şi I (fig. 5, 6).

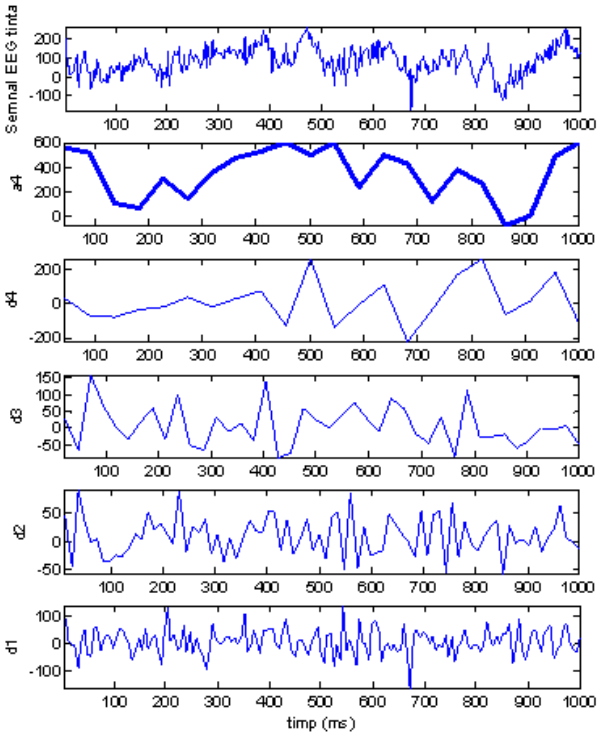

Fig. 3. Reprezentarea descompunerii wavelet (db2) multirezoluție pe 4 niveluri pentru litera Q pe canalul Pz

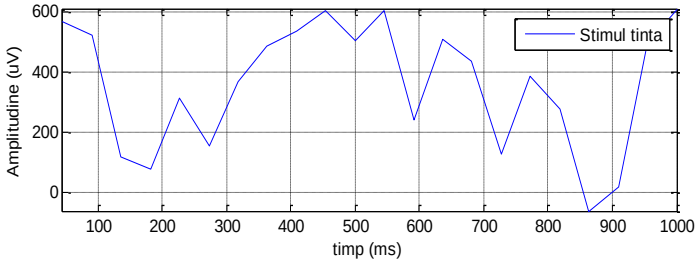

Fig. 4. Coeficienții de aproximare de ordin 4 pentru semnalul țintă (Litera Q, Canal Pz)

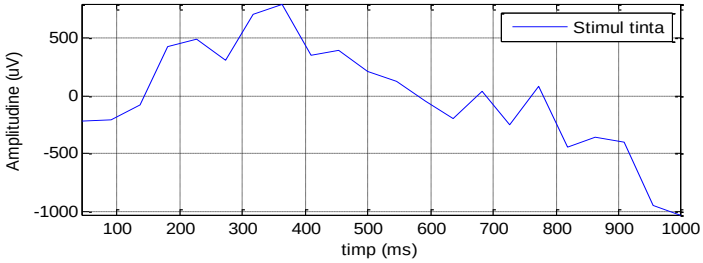

Fig. 5. Coeficienții de aproximare pentru semnalul țintă (Litera U, Canal Cz)

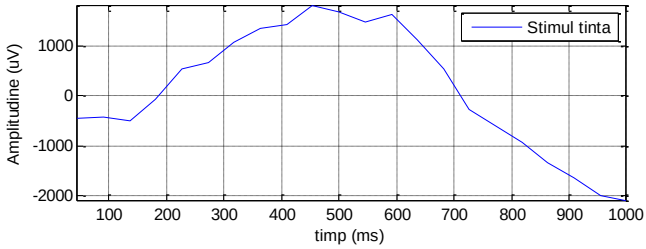

Fig. 6. Coeficienții de aproximare pentru semnalul țintă (Litera I, Canal Cz)

CONCLUZII

În concluzie, analiza wavelet multi-rezoluție, cu wavelet db2, este o metodă de luat în considerare pentru detecția potențialului P300, fiind în avantaj față de metoda directă de analiză a semnalului EEG, când semnalul nu este descompus pe sub-benzi. Metoda permite reducerea dimensiunii datelor, iar acest lucru constituie un câştig important.

S-au prezentat rezultate pentru date achiziționate cu sistemul furnizat de firma austriacă *g.tec* care foloseşte platforma open-souce, BCI2000, cea mai utilizată platformă de către laboratoarele de cercetare din comunitatea BCI. Cu ajutorul analizei offline permisă de această platformă s-a pus în evidență faptul că electrozii Pz şi Cz sunt cei mai potriviți a fi folosiți la detectarea potențialului P300. În majoritatea cazurilor, momentul de apariție este situat în jurul valorii de 400 ms. Rezultatele sunt în concordanță cu unele raportate în literatura de specialitate (8).

MULȚUMIRI

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EVALUAREA ACŢIUNII ANTIMICROBIENE A UNOR DERIVAŢI DE ACID CINAMIC

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ANTIMICROBIAL ACTIVITY OF SOME CINNAMIC ACID DERIVATIVES (Abstract): Due to drug-resistance phenomenon, there is a constant need for discovering new antiinfectious agents. A series of cinnamic acid derivatives was synthesized and then brominated with bromine in the presence of chloroform or acetic acid. The structure of the new compounds was confirmed by elemental and spectral data. Their antimicrobial activity was tested by disc-diffusion method. The tested compounds had mainly antifungal activity and were moderately active against Gram-positive bacteria. Bromination of the double bond determined the enhancement of the antimicrobial activity for all the tested compounds. **Key words**: CINNAMIC ACID DERIVATIVES, ANTIMICROBIAL ACTIVITY.

Terapia antimicrobiană reprezintă domeniul ştiinţific care a cunoscut cea mai amplă dezvoltare în secolul al XX-lea, dar cu toate acestea, afecţiunile infecţioase continuă să aibă o incidenţă ridicată în rândul populaţiei. Microorganismele patogene se adaptează la acţiunea antibioticelor şi chimioterapicelor existente, dezvoltând în timp rezistenţă, fenomen care face ca perspectivele de viitor să nu fie foarte promiţătoare. Din acest motiv, sinteza unor noi substanţe cu potenţial antimicrobian prezintă un mare interes.

Studiile efectuate pe derivaţii de acid cinamic au evidenţiat faptul că atât acidul în sine, cât şi derivaţii săi prezintă o activitate antimicrobiană (antibacteriană şi antifungică) importantă (1, 2, 3, 4). Având în vedere faptul că derivatul dibromurat la nivelul dublei legături al acidului cinamic prezintă proprietăţi antibacteriene şi antifungice superioare compusului nebromurat, ne-am propus să sintetizăm o serie de noi derivaţi dibromuraţi de acid cinamic şi să stabilim spectrul acţiunii antibacteriene şi antifungice pentru aceştia, urmărind şi eventuala corelaţie între acţiunea antimicrobiană şi natura chimică a grupărilor grefate pe nucleul aromatic.

MATERIAL ŞI METODĂ

Pentru obţinerea derivaţilor de acid cinamic notaţi I_1-I_9 am folosit reacţia de condensare dintre acidul malonic şi diferite aldehide aromatice (fig. 1) (1, 3).

Derivaţii acidului cinamic obţinuţi (I_1-I_9)

prezintă o legătură olefinică în catena laterală, fapt care permite bromurarea directă, cu obţinerea derivaţilor dibromuraţi vicinali corespunzători, notaţi II_1-II_9 (fig. 2) (5).

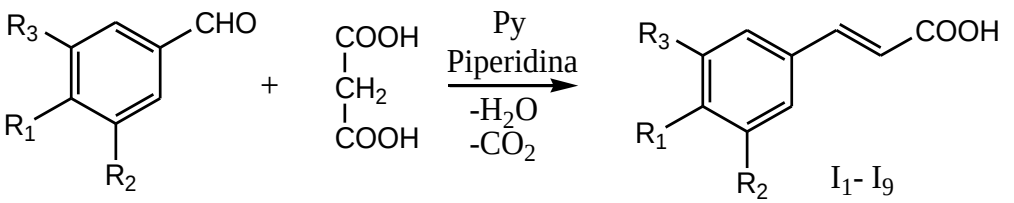

Fig.1. Schema de obţinere a compuşilor de tip I

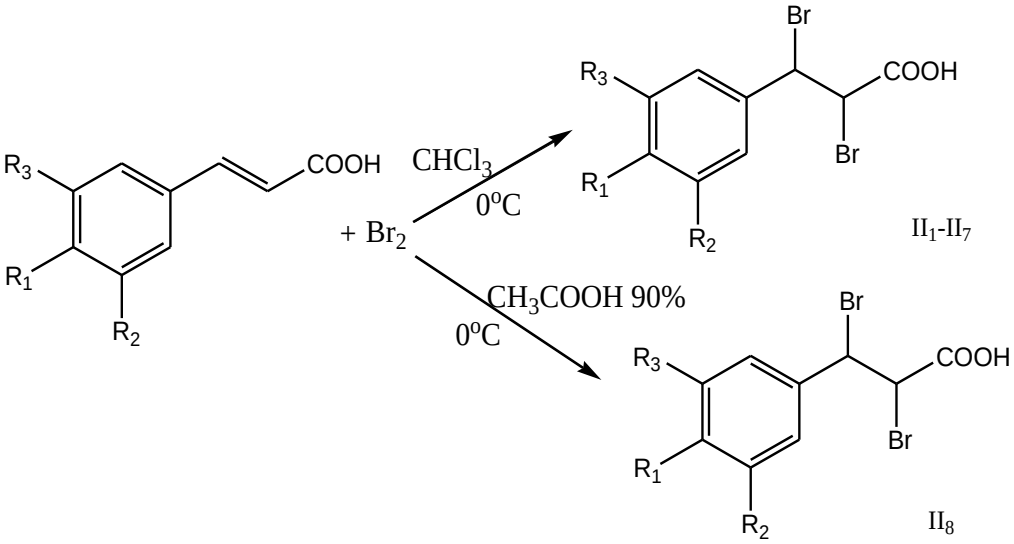

Fig. 2. Schema de obţinere a compuşilor de tip II

Derivaţii dibromuraţi ai acizilor cafeic şi p-hidroxi-cinamic nu au putut fi obţinuţi prin această metodă, produşii de reacţie având o stabilitatea foarte redusă, suferind probabil reacţii de oxidare.

Confirmarea structurii chimice a compuşilor sintetizaţi a fost realizată prin analiză elementală (Elemental Exeter Analytical CE 440 Analyzer) şi analiza spectrelor de vibraţie moleculară în IR (FT-IR Shimadzu Prestige 8400s).

Testarea activităţii antimicrobiene s-a realizat din punct de vedere calitativ prin metoda difuzimetrică folosind bacterii Gram pozitive, Gram negative şi 3 specii de *Candida*.

REZULTATE ŞI DISCUŢII

Compuşii obţinuţi sunt substanţe solide, colorate în alb, alb-gălbui, slab roz sau verde, solubile în alcool, acetonă sau DMSO.

Structura compuşilor de tip I şi II şi unele proprietăţi fizice (greutate moleculară, punct de topire) sunt indicate în tabelul I.

TABELUL I

Caracteristici fizice pentru compuşii de tip I şi II

Compus	R_1	R_2	R_3	Denumirea compusului	Masă moleculară (g/mol)	Punct de topire (ºC)
I_1	-H	-H	-H	acid cinamic	148	134
I_2	$-OCH_3$	-H	-H	acid p-metoxi-cinamic	178	167-8
I_3	-OH	$-OCH_3$	-H	acid ferulic	194	171-2
I_4	-OH	$-OCH_3$	-Br	acid 5-brom-ferulic	273	255-7
I_5	$-OCH_3$	$-OCH_3$	-H	acid 3,4-dimetoxi-cinamic	208	185-7
I_6	-OH	-H	-H	acid p-hidroxi-cinamic	164	216-8
I_7	$-CH_3$	-H	-H	acid p-metil-cinamic	162	198-9
I_8	-Cl	-H	-H	acid p-clor-cinamic	182,5	252-3
I_9	-H	-H	-Br	acid 3-brom-cinamic	227	180-2
II_1	-H	-H	-H	acid 3-fenil- 2,3-dibrom-propanoic	308	205-6
II_2	$-OCH_3$	-H	-H	acid 3-(p-metoxi-fenil)-2,3-dibrom-propanoic	338	154-5
II_3	$-OCH_3$	$-OCH_3$	-H	acid 3-(3′,4′-dimetoxi-fenil)-2,3-dibrom-propanoic	368	144-6
II_4	$-CH_3$	-H	-H	acid 3-(4′-metilfenil)-2,3-dibrom-propanoic	322	189-190
II_5	-Cl	-H	-H	acid 3-(4′-clorfenil)-2,3-dibrom-propanoic	342,5	200-1
II_6	-H	-H	-Br	acid 3-(4′-metil-fenil)-2,3-dibrom-propanoic	387	188-191
II_7	-OH	$-OCH_3$	-Br	acid 3- (3′-metoxi, 4′-hidroxifenil, 5′-brom)-2,3-dibrom-propanoic	433	182-3
II_8	-OH	$-OCH_3$	-H	acid 3- (3′-metoxi, 4′-hidroxifenil)-2,3-dibrom-propanoic	354	158-9

Spectrele în IR ale compuşilor sintetizaţi prezintă absorbţiile caracteristice principalelor elemente structurale ale moleculelor respective şi confirmă, alături de datele analizei elementale, structura compuşilor de tip I şi II dedusă din ecuaţiile reacţiilor de sinteză (tab. II).

TABELUL II

Caracteristicile fizico-chimice ale compuşilor sintetizaţi

Compus	%C (t/e)	%H (t/e)	%O (t/e)	υ (cm^{-1})
I_1	72,97/ 72,25	5,40/ 5,25	21,62/ 21,23	2960, 1698, 1620, 1450
I_2	67,41/ 67,09	5,61/ 5,52	26,96/ 26,20	2840, 1700, 1640, 1590, 1514
I_3	61,85/ 61,53	5,14/ 4,97	32,98/ 31,89	3425, 2945, 1710 1682, 1515, 1432
I_4	43,95/ 42,59	3,29/ 3,03	23,44/ 23,27	3430, 2938, 1745, 1683, 1510, 1290
I_5	63,46/ 62,59	5,76/ 5,24	30,76/ 29,98	2945, 1749, 1678, 1520, 1265
I_6	65,85/ 65,75	4,87/ 4,58	29,26/ 29,12	3475, 2974, 2665, 1749, 1685, 1205
I_7	74,07/ 73,09	6,17/ 5,97	19,75/ 19,04	3001, 1722, 1643, 1434, 1276
I_8	59,17/ 58,77	3,83/ 3,42	17,53/ 17,09	2930, 1722, 1665, 1490, 1269
I_9	47,57/ 46,98	3,08/ 3,01	14,09 13,66	3052, 2890, 1710, 1648, 1575, 1411
II_1	35,06/ 34,78	2,59/ 2,37	10,38/ 9,63	2980, 1705, 1490, 585
II_2	35,50/ 34,89	2,95/ 2,56	14,20/ 13,75	3004, 1720, 1606, 1259, 592
II_3	35,86/ 34,95	3,26/ 2,84	17,38/ 16,93	3111, 3004, 2844, 1753, 1517, 1265, 605
II_4	37,26/ 36,92	3,10/ 2,87	9,93/ 9,59	3033, 2918, 1716, 1612, 1431, 1276, 588
II_5	31,53/ 31,29	2,04/ 1,94	9,34/ 9,19	3035, 2910, 1722, 1593, 1429, 1269, 1091, 580
II_6	27,90/ 27,58	1,80/ 1,73	8,26/ 8,17	3002, 2885, 1708, 1569, 1411, 1076, 603
II_7	27,71/ 27,05	2,07/ 1,83	14,78/ 14,10	3429, 3002, 2935, 1716, 1602, 1502, 1045, 582
II_8	33,89/ 33,56	2,82/ 2,34	18,07/ 17,07	3429, 3004, 2918, 1714, 1609, 1515, 1433, 609

În continuarea cercetărilor noastre, compuşii sintetizaţi şi acidul cafeic (Fluka) – codificat I_{10} - au fost supuşi testării în vederea determinării din punct de vedere calitativ a activităţii antimicrobiene, prin metoda difuzimetrică folosind următoarele

microorganisme test: *Staphylococcus aureus, Sarcina lutea, Bacillus cereus, Bacillus subtilis, Escherichia coli, Candida albicans, Candida sake* şi *Candida glabrata* (6).

În tabelul III sunt prezentate microorganismele test folosite şi diametrele zonelor de inhibiţie a dezvoltării microorganismelor test, exprimate în mm.

TABELUL III

Diametrele zonelor de inhibiţie a dezvoltării microorganismelor test (mm)

Compus testat	***S. aureus* ATCC 25923**	***Sarcina lutea* ATCC 9341**	***B. cereus* ATCC 14579**	***B. subtilis***	***E. coli* ATCC 25922**	***C. albicans ATCC 10231***	***C. sake***	***C. glabrata***
I_1	16	25	10	15	0	30	29	21
I_2	10	20	8	10	0	29	29	10
I_3	13	20	10	10	0	30	30	0
I_4	14	20	11	20	0	0	0	0
I_5	0	9	10	10	0	0	0	0
I_6	14	20	10	10	0	30	30	0
I_7	12	15	10	10	0	14	20	20
I_8	10	13	12	12	0	9	0	0
I_9	14	15	17	17	0	9	0	0
I_{10}	10	26	10	10	0	30	30	0
II_1	17	26	13	17	10	30	30	16
II_2	18	20	17	17	12	30	28	10
II_3	10	12	10	10	0	25	10	20
II_4	12	15	12	8	9	20	0	12
II_5	19	18	18	19	0	20	20	20
II_6	30	30	16	20	10	29	21	25
II_7	21	20	20	18	11	29	26	24
II_8	32	29	26	20	17	30	30	30
Ampicilina 25 µg	39	35	16	30	16			
Cloramfenicol 30 µg	35	35	26	35	26			
Nistatin 100 µg						26	30	35

Compuşii sintetizaţi au prezentat o activitate antibacteriană moderată, în special asupra bacteriilor Gram pozitive (*Staphylococcus aureus, Sarcina lutea*) şi a bacililor sporulaţi (*Bacillus cereus* şi *Bacillus subtilis*). Niciunul dintre compuşii seriei I nu a prezintat activitate faţă de *Escherichia coli.*

Comparativ cu prima serie de derivaţi,

compuşii grupei II s-au dovedit mai activi. Derivaţii II_6 şi II_8 sunt foarte activi faţă de *Staphylococcus aureus* şi *Sarcina lutea,* având un diametru al zonei de inhibiţie de peste 25 mm în timp ce II_7 este activ faţă de aceleaşi microorganisme. Derivaţii II_7 şi II_8 sunt totodată activi şi faţă de *Bacillus cereus.* În plus, se observă că spectrul de acţiune este extins şi asupra *Escherichia coli*, în cazul compusului II_8.

Încercând să stabilim o interrelaţie structură-activitate antimicrobiană, putem observa că în cazul derivaţilor de acid cinamic, introducerea pe nucleul aromatic a unor grupe cu efect electromer respingător de electroni (de tipul $–OCH_3$, -OH, -Cl, -Br) influenţează în sens negativ activitatea antibacteriană asupra stafilococilor, dar reducerea potenţialului antistafilococic în comparaţie cu acidul cinamic nu este foarte semnificativă. Prezenţa halogenilor pe nucleu (-Cl, -Br), în special a bromului, determină o creştere a activităţii antibacteriene faţă de bacilii Gram pozitivi (*Bacillus cereus* şi *Bacillus subtilis*).

Compuşii sintetizaţi prezintă activitate antifungică remarcabilă, 9 derivaţi dintre cei sintetizaţi (I_1, I_2, I_3, I_6 şi respectiv II_1, II_2, II_6, II_7, II_8) şi acidul cafeic (I_{10}) sunt mai activi decât martorul nistatină, faţă de *Candida albicans*. În cazul tuturor substanţelor investigate, bromurarea dublei legături conduce la o creştere considerabilă a activităţii antifungice, determinând în acelaşi timp şi lărgirea spectrului de acţiune şi asupra speciei *Candida glabrata*, derivaţii de acid cinamic nebromuraţi fiind în general inactivi asupra acestei specii.

CONCLUZII

În cadrul studiilor efectuate s-au realizat următoarele:

- s-au sintetizat 9 compuşi de tip derivaţi de acid cinamic şi 8 compuşi dibromuraţi, derivaţi dibromuraţi ai primei serii;
- structura compuşilor a fost confirmată de rezultatele analizei elementale şi spectrale;
- s-a evaluat acţiunea antimicrobiană a compuşilor sintetizaţi, stabilind că în seria I cinci substanţe (I_1, I_2, I_4, I_5 şi I_7) prezintă activitate faţă de *Sarcina lutea* şi faţă de *Staphylococcus aureus*;
- 9 compuşi (4 de tip I şi 5 de tip II) sunt mai activi decât nistatina, martorul folosit pentru evaluarea acţiunii faţă de *Candida albicans*;

în cazul derivaţilor dibromuraţi la nivelul dublei legături, se remarcă o accentuare semnificativă a potenţialului antibacterian şi antifungic, tendinţă care s-a manifestat la toţi compuşii analizaţi.

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NOUTĂȚI

NIVELUL LIPOPROTEINELOR SERICE ÎN CARDIOMIOPATIA TAKOTSUBO VS INFARCTUL MIOCARDIC

Atitudinea recomandată de ghidurile curente la pacienţii cu infarct miocardic sau sindrom coronarian acut include terapia hipolipemiantă agresivă cu statine, indiferent de nivelul de bază al lipoproteinelor serice. Pacienţii cu cardiomiopatie Takotsubo prezintă o simptomatologie similară celei din infarct, beneficiind de aceeaşi atitudine terapeutică în decursul spitalizării. Cu toate acestea, fiziopatologia acestei boli nu include boala aterosclerotică coronariană, putându-se presupune că profilul lipidic este mai puţin modificat pentru aceste cazuri în comparaţie cu cel din infarctul miocardic. Un studiu retrospectiv american a evaluat nivelul seric al lipoproteinelor a jeun la zece pacienţi diagnosticaţi cu cardiomiopatie Takotsubo şi l-a comparat cu profilul lipidic de la 40 de pacienţi (similari din punct de vedere al vârstei şi sexului) cu infarct miocardic. Rezultatele nu au relevat nici o diferenţă între cele două grupuri în ceea ce priveşte valorile colesterolului total (174.5 mg/dL vs. 197.6 mg/dL, $p = 0.12$). Cu toate acestea, în grupul pacienţilor cu cardiomiopatie Takotsubo, valoarea medie a HDL-colesterolului a fost semnificativ mai mare (66.87 mg/dL vs. 36.5 mg/dL, $p = 0.008$) în timp ce valoarea LDL-colesterolului a fost semnificativ mai mică (89.7 mg/dL vs. 128.9 mg/dL, $p = 0.000$), la fel ca şi nivelul trigliceridelor (65.2 mg/dL vs. 166.8 mg/dL, $p < 0.0001$). Aceste rezultate sunt în acord cu mecanismele nonobstructive, non-aterosclerotice ale cardiomiopatiei Takotsubo, fiind recomandată o evaluare individualizată a profilului lipidic înaintea iniţierii terapiei hipolipemiante pe termen lung. (Gaddam S, Nimmagadda KC, Nagrani T, Naqi M, Wetz RV, Weiserbs KF, McCord D, Ghavami F, Gala B, Lafferty JC. Serum lipoprotein levels in takotsubo cardiomyopathy vs. myocardial infarction. Int Arch Med. 201;4:14)

Teodora Alexa

PROTECTIVE EFFECTS OF SELENIUM ON ACRYLAMIDE TOXICITY IN THE LIVER OF THE RAT. EFFECTS ON THE OXIDATIVE STRESS

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PROTECTIVE EFFECTS OF SELENIUM ON ACRYLAMIDE TOXICITY IN THE LIVER OF THE RAT. EFFECTS ON THE OXIDATIVE STRESS (Abstract): Acrylamide (AA), obtained for the first time by Moureu in Germany in 1893, is presently used as polyacrylamide in water treatment and wastewater treatment, paper and pulp processing, mineral processing, crude-oil production processes. Acrylamide is a chemical product formed when frying, roasting, grilling or baking carbohydrate-rich foods at temperatures above 120°C. Acrylamide is thus found in a number of foods, such as bread, crisps, French fries and coffee. Tobacco smoking also generates substantial amounts of acrylamide. Acrylamide administration is associated with significant increase of oxidative stress parameters; acrylamide caused disturbances in the oxidative status and enzyme activities and the effect was pronounced with the high doses. **Material and methods**: This study investigates the effect of selenium (as sodium selenite and as a selenium dietary supplements – Celnium) on the oxidative stress in Wistar rats which received high doses of acrylamide. **Results**: The administration of sodium selenite and selenium dietary supplements (Celnium) significantly increased GSH and GPx levels and decreased MDA compared to group which received only acrylamide. **Conclusions**: Our results show that sodium selenite and selenium dietary supplements (Celnium) can partially prevent the biochemical changes in the liver of the rats which received high doses of acrylamide. **Key words**: ACRYLAMIDE, OXIDATIVE STRESS, SODIUM SELENITE, DIETARY SUPPLEMENTS

Acrylamide is more commonly known as an industrial chemical used to prepare the polymeric material known as polyacrylamide, which is used as a flocculent for treating industrial wastewater and, to a lesser extent, drinking water, as well as having other industrial uses (1).

In April 2002, the Swedish National Food Administration announced their findings that the chemical, acrylamide, could be found in starch-containing foods cooked at high temperatures, such as fried or roasted

potato products and bread. The underlying mechanism of acrylamide formation is not yet known in detail (2). However, researches on food model systems concluded that the Maillard reaction is the main pathway of formation from amino acids (e.g.asparagine) and reducing sugars (e.g. glucose).

The biological consequences of acrylamide exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound (3).

Acrylamide is known to cause toxicity to the nervous system in animals and humans at very high levels of exposure, but the threshold for this effect is at a level of exposure that is far greater than the expected exposure from food. Consumption of food where acrylamide has been detected should not result in nerve damage (4-6).

The other health concern regarding acrylamide is its potential to cause genetic damage that may lead to cancer. Experimental exposure of rodents to acrylamide has also revealed a carcinogenic mode of action for this chemical (7).

Glycidamide, a metabolite of acrylamide, binds to DNA and can cause genetic damage. Prolonged exposure has induced tumours in rats, but cancer in man has not been convincingly shown. The International Agency for Research on Cancer (IARC) has classified acrylamide as "probably carcinogenic to humans" (8). Acrylamide is oxidized to glycidamide, a reactive epoxide, and undergoes conjugation with glutathione. DNA adducts from glycidamide have been reported following administration of acrylamide (9).

Kirman et al. (10) reported that the rate of acrylamide conversion to its epoxide metabolite glycidamide is higher during subchronic dosing conditions. Acrylamide caused disturbances in the oxidative status and enzyme activities, and the effect was pronounced with the high doses. The enhancement of lipid peroxidation is a consequence of depletion of glutathione to certain critical levels.

LoPachin et al. (11) suggested that acrylamide induced synaptic dysfunction which involved the adduction of presynaptic protein thiol groups and subsequent reduction in neurotransmitter release. The same author (11) reported that the neurological defects associated with acrylamide intoxication are mediated by impaired neurotransmission at central and peripheral synapses.

Free radicals are continuously produced *in vivo* and there are number of protective antioxidant enzymes (superoxide dismutase, catalase, glutathione S transferase, glutathione peroxidase) and antioxidant glutathione for dealing with these toxic substances (12 -14).

In animals, chronic acrylamide exposure causes neurotoxicity, morphological and biochemical damage in hepatic, renal, nervous and testicular tissues. Nowadays, there is an increased interest in dietary supplements with antioxidant properties that can reduce the effects of deleterious food constituents. Selenium plays an important biological role in living organisms, mainly through its presence in selenoproteins (such as glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases) (15).

Different toxic effects of acrylamide might be also caused by an increase in lipid peroxidation in case of disfunctions of endogenous antioxidant defence system. Components of this system such as enzymes (glutathione peroxidase, thioredoxin reductase) and reduced glutathione are known to protect biological systems against oxidative stress (16-18).

Selenium is an important biological antioxidant, action in which it is involved as a

part of gluthation-SH-peroxidase, the seleno-enzyme that catalyses reduction of lipid peroxides and of hydrogen peroxide and thus prevents the nocive effects of lipid peroxidation with a high content of unsaturated fatty acids and protects erythrocytes from haemolysis. Selenium protects cells and cell membranes from oxidative processes, facilitating reaction between oxygen and hydrogen and ions transfer at membrane level.

Selenium can have a double action: selenium compounds, sodium selenite among them, have direct pro-oxidant activity leading to acute toxicity but also antioxidant activity, through the process of embodying seleno-proteins and participating in controlling different biological processes within the body (19, 20). As a part of gluthation peroxidase (GSH/Px), selenium is a part of the first and the second level of cell's antioxidant protection.

Increased doses of sodium selenite may reduce inflammation through direct pro-oxidative effect and can increase cell's antioxidant capacity through selenium incorporation in seleno-enzymes (19).

This paper presents preliminary results regarding the protective potential of Selenium (as sodium selenite) and some selenium dietary supplements (Celnium) in the liver of the Wistar rats in experimental oral intoxication with increased dose of acrylamide.

MATERIAL AND METHODS

Chemicals

Acrylamide (C3H5NO, >99% purity) was used in the present study. Acrylamide and all other chemicals were purchased from Sigma Chemical Company.

Animal treatments

Adult Wistar male rats (Laboratory Animal Center of Cantacuzino Institute of Research, Bucharest, Romania), weighing 180-220 g, were housed in groups of five in plexiglas cages with the floor covered with sawdust. Animals were maintained in a controlled environment (12 hours light/dark cycle with lights on at 07.00, temperature of 20±1ºC) before and throughout the experimental period. The rats had free access to food (standard lab rat chow), except 18 hours before the experiment but tap water ad libitum. All experiments involving animals were approved by the University's Committee for Bioethics and animal experimentation according to international rules of conduct.

Studies were carried out using 5 groups of 10 Wistar male rats each which received as follows: group 1 (control) – saline = control group; group 2 – acrylamide (50 mg/kg/day), orally, 10 days = AA group; group 3 – acrylamide (50 mg/kg/day), orally and sodium selenite 1 mg/kg/day orally, 10 days = AA + Se 1 group; group 4 – acrylamide (50 mg/kg/day), orally and sodium selenite 0,2 mg/kg/day orally, 10 days = AA + Se 0,2 group; group 5 - acrylamide (50 mg/kg/day) and Celnium® (dietary supplement containing selenium from Saccharomyces cerevisiae) 0.50mg Se/kg/day orally, 10 days = AA + Ce group.

The previous studies also included groups of rats which received only sodium selenite or selenium from Celnium® (in above mentioned doses) but there were no significant differences compared to the control group.

At the end of treatment, rats were fasted for 12 h before being anesthetized and sacrificed by cervical dislocation. Liver tissues were isolated, washed with saline solution, and stored at -35°C for the biochemical studies. We determined MDA (malondialdehyde), GSH (reduced glutathione), GPx (glutathione peroxidase) as well as TAS (total antioxidant status).

Biochemical study

Liver homogenates analysis

Half-gram sample of liver tissues were homogenized in 4.5 ml KCl 1.5%; the homogenates were used in determination of the hepatic lipid peroxidation end product malondialdehyde (21). MDA level is expressed as µmol/g tissue.

One gram samples of liver tissues were homogenized in 5ml trichloroacetic acid (5%) containing 0.003M EDTA. The homogenates were centrifuged at 2500×g for 15 min. Reduced glutathione was measured in the clear supernatant by spectrophotometric method (22). GSH level is expressed as mmol/g tissue.

In order to investigate the response of the antioxidant systems against high levels of acrilamyde, the activities of glutathione peroxidase (GPx) in whole blood and total antioxidant status (TAS) in plasma were measured using the Randox kit for manual use.

The GPx activityis expressed as U/L. Total antioxidant status is expressed as mmol/L of all antioxidant circulating species in plasma. The statistical analysis was performed using analysis of variance (ANOVA) one way. Tukey test was used for multiple comparisons (software Stats Direct version 2.6). Results are presented as means of three determinations.

RESULTS

The data obtained from the experiments are presented in charts (fig 1-4), representing the level of the determined parameter for each study group.

DISCUSSION

In our study the acrylamide administration (AA group) was associated with statistically significant increase of MDA levels in liver homogenate compared to control group. Furthermore, compared to control group, acrylamide (AA group) caused decrease of glutation in liver homogenate, glutation peroxidase, and total antioxidant status (TAS) in whole blood respectively in plasma. The co-administration of sodium selenite and Celnium with acrylamide was associated with significant increased level of the oxidative stress parameters in the liver omogenate, compared to AA group. The level of MDA in liver homogenate slightly increased in the group receiving acrylamide simultaneously with sodium selenite and Celnium respectively, as hepatoprotective agents.

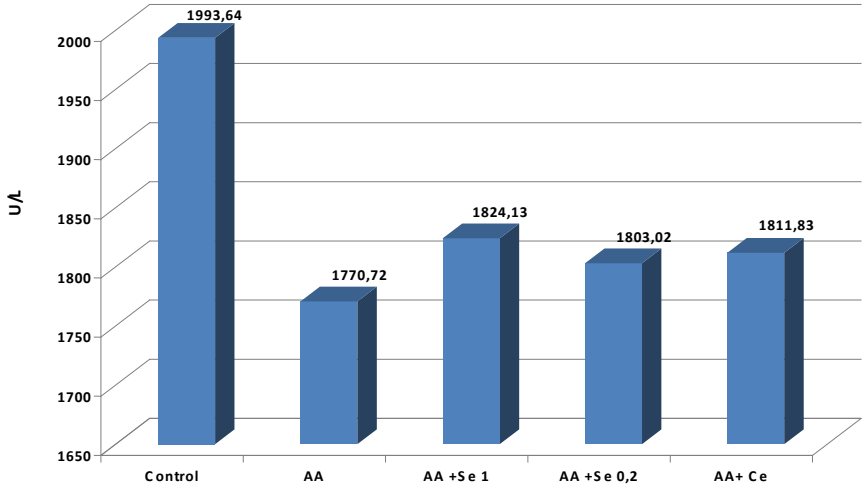

Fig.1. The values of GPx in whole blood

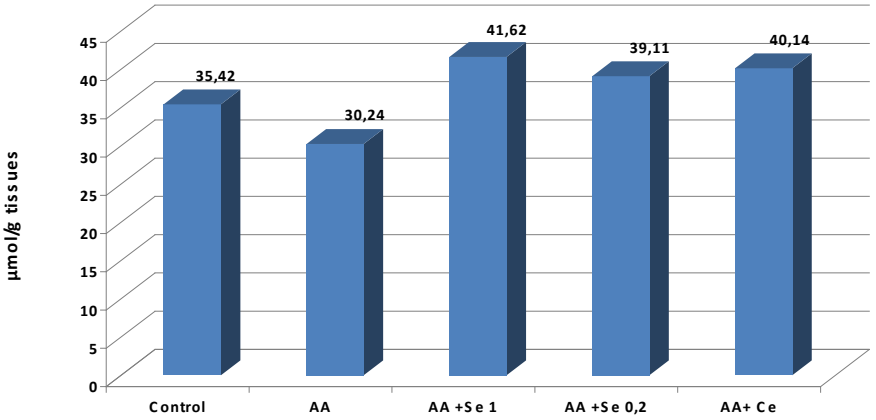

Fig. 2. The values of GSH in liver homogenate

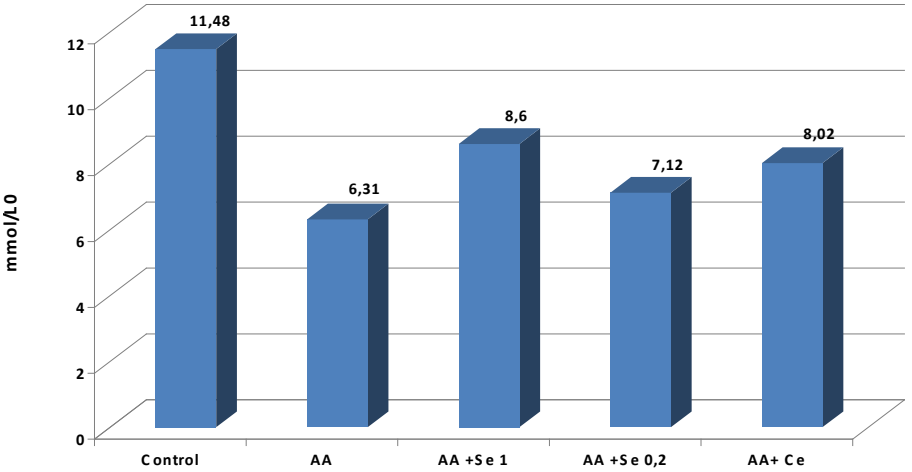

Fig.3. The values of TAS in plasma

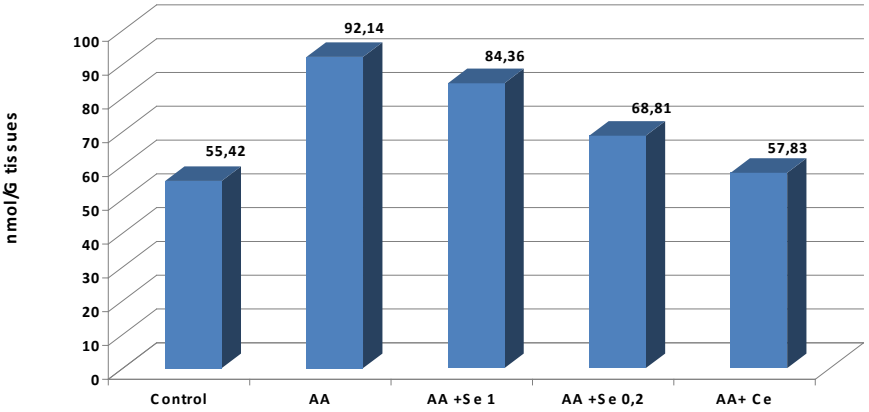

Fig. 4. The values of MDA in liver homogenate

Glutathion peroxidase levels significantly decrease when compared all groups that received AA to control group, sugesting that acrylamide triggers the decrease of the antioxidant defence activity in cell. In groups that received acrylamide alone or with different dose of selenium the most severe decay is observed in group AA. Within groups that received selenium (AA+ Se1) there was noticed the smallest decay suggesting that in high dose this element may supply some protection against oxidative aggresion.

Gluthation levels present a significat decrease when compared the AA group to control one suggesting for a significant amount of reactive species that consume that reducing agent.

An unexpected increase was observed when compared all selenium receiving groups with control group. These data might suggest that selenium has an antioxidant activity by itsself, added to the antioxidant gluthation activity.

Total antioxidant status present a significant decrease when compared all groups that received AA to control group. These data suggest that all antioxidant capacity decrease as a consequence of acrylamide administration. The most severe decrease in total antioxidant capacity is found in group AA, in the remaining other groups the decay was less severe. It is suprising that even if GSH registered an increase in group AA+Se1, the total antioxidant capacity decreased.

Malondialdehyde concentrations are, as expected, significantly higher when compared group AA with control. In groups that received different concentration of selenium, generated malondialdehyde varied as follows: the group with the high dose present an increase compared with group which received 0,2 mg/kg/day.

CONCLUSIONS

The present results showed that acrylamide caused disturbances in the oxidative status, enzyme activities and lipid peroxidation; the results show that a 50 mg/kg acrylamide intake in laboratory animals (Wistar rats) resulted in severe perturbation of some biochemical parameters. The simultaneous intake of selenium as sodium selenite or as a dietary supplement (Celnium) partially prevented some of the biochemical changes in rats which received high doses of acrylamide. We may assume that Selenium has a benefical rol in acrylamide intoxication.

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EVALUATION OF SOME PHARMACEUTICAL FORMULATIONS OF LISINOPRIL THROUGH DISSOLUTION TESTING

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EVALUATION OF SOME PHARMACEUTICAL FORMULATIONS OF LISINOPRIL THROUGH DISSOLUTION TESTING (Abstract): **Aim**: Lisinopril is a drug of the angiotensin converting enzyme (ACE) inhibitor class that is primarily used in treatment of hypertension, congestive heart failure, heart attacks and also in preventing renal and retinal complications of diabetes. We compared the dissolution profiles of Lisinopril 20 mg tablets (Antibiotice S.A. Iaşi) and Zestril 20 mg tablets (Astra Zeneca). **Material and method**: Because lisinopril is a third class active substance, we performed dissolution tests in standard media at three pH values: 1.2, 4.5 and 6.8 using the paddle apparatus at 75 rpm. **Results**: Both pharmaceutical formulations present a dissolution percentage more than 85% (Q) of the labeled amount. **Conclusion**: Both pharmaceutical formulations present similar dissolution profile. **Key words**: LISINOPRIL, TABLETS, DISSOLUTION TEST

Lisinopril, a lysine analogue of enalaprilat, is a long-acting angiotensin converting enzyme inhibitor witch differs from captopril by lacking the sulhydril group. Lisinopril, discovered and developed by the Merck Sharp & Dohme Research Laboratories, is indicated for the treatment of hypertension, congestive heart failure heart attacks and also in preventing renal and retinal complications of diabetes (1,2).

According to World Health Organization lisinopril is (s)-1-[1–carboxy-3 phenylpropyl) -L-lysyl]–L-proline dyhydrate (fig. 1) (2).

Lisinopril is a white to off–white crystalline powder. It is soluble in water (1 to 10) and methyl alcohol (1 to 70), but it is practically insoluble in ethyl alcohol, acetone, chloroform and ether. Log P (octanol/water) was reported to be $=1.22$; log P (phosphate buffer 0.1 M, pH 7/octanol) $=10.2\pm0.5$ (25^0C), pKa values 2.5, 4.0, 6.7, 10.1 (25^0C) (2, 3).

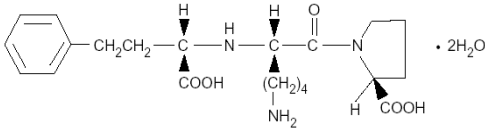

Fig. 1. Lisinopril – chemical structure

Lisinopril can be expected to be "highly soluble" at 37^0C over the entire pH-range 1-7.4. The low bioavailability of lisinopril is in line with its low permeability (2,3,4, 5,6,7,8,9).

Nowadays, the study of dissolution *in vitro* is considered a fundamental requirement in the pharmaceutical industry in order to assure the quality of solid pharmaceutical forms for oral use, guarantee the quality from lot to lot, orientate the development of new formulations and secure the uniformity, quality and performance of the drug even after modifications. On a parallel basis, this allows formulation, optimization in the development phase and, in the same

way, it allows stability studies, manufacturing process monitoring, and the establishment of *in vivo/in vitro* correlations.

The USP 29 dissolution specification for lisinopril tablets is not less than 80% (Q) dissolved in 30 minutes in 900 mL HCl 0.1M (2).

MATERIAL AND METHOD

Dissolution tests were performed according to USP 29 using :

- dissolution tester Erweka DT 808 LH - Apparatus no. 2 (paddle) ;
- medium : - HCl 0.1N, pH 1.2 buffer, pH 4.5 buffer and pH 6.8 buffer ;
- HPLC 1100 Agilent, detector DAD/ MWD and fluorescence.

The dissolution profile of the pharmaceutical formulations was studied using various dissolution medias. First, the comparative dissolution profiles of the drugs were performed using USP dissolution media (HCl 0.1N) at stirring speed of 50 rpm, then the dissolution profiles were obtained using three different dissolution media : pH 1.2 buffer, pH 4.5 buffer and then pH 6.8 buffer at a stirring speed of 75 rpm. The dissolution media volume was maintained at 900 mL in all the cases (3).

Determination of dissolved active substances was performed using a HPLC method. Lisinopril was separated on a C18 column using a mobile phase consisting of a mixture of phosphate buffer pH 2 containing 0.125% sodium hexanesulphonate and acetonitrile (72 : 28, v/v), at a flow rate of 1 mL/ min. Detection was performed at 215 nm on an Agilent 1200 HPLC system. The injection volume was 20 μL. In these chromatographic conditions, the retention time of lisinopril was 1.9 min.

RESULTS AND DISCUSIONS

Comparative dissolution profile of Lisinopril 20 mg tablets produced by Antibiotice S.A. Iaşi (A) and Zestril 20 mg tablets produced by Astra Zeneca (B) gave the results shown in tab. I when performed accordingly to USP 29. The results show that both products have similar dissolution profile as it can also be seen from the graphical representation in fig. 2.

While comparing dissolution profile of the pharmaceutical formulations with lisinopril using 900 mL pH 1.2 buffer as disso-

TABLE I
Dissolution Test in USP dissolution media

	% released lisinopril			
Time (min.)	10	15	20	30
A	89.1054	96.7536	95.7468	97.1445
B	80.5794	93.8223	94.0334	97.5769

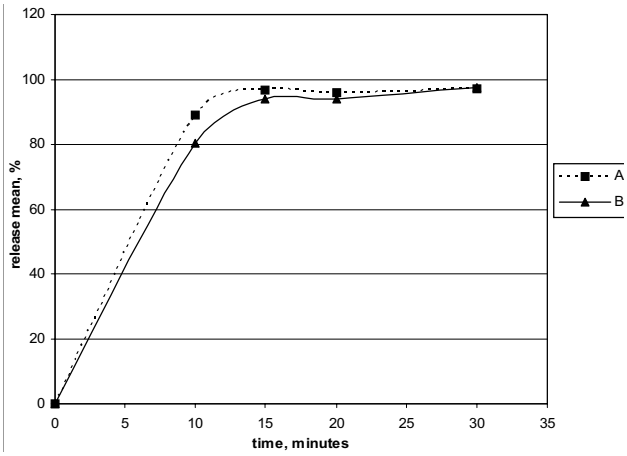

Fig. 2. Comparative dissolution profiles in USP dissolution media

lution media and stirring speed of 75 rpm, the results shown in tab. II prove that both products released more than 85 % within 15 min. of testing and that they have similar dissolution profiles. This can also be observed from fig. 3.

Each lisinopril product released a percent greater than 90% when tested in a pH 4.5 buffer as dissolution media and at stirring speed of 75 rpm. The percent increases when the released lisinopril is quantified after 30 min. – over 98%. The results are shown in tab. III and they are graphically represented in fig. 4.

The last dissolution test was performed at an almost neutral pH value (pH 6.8 buffer

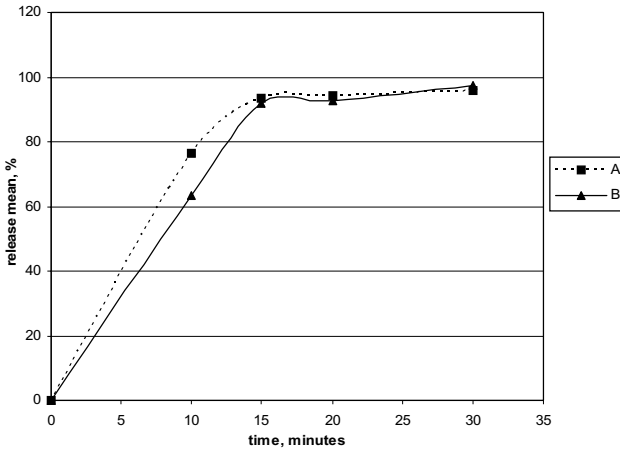

Fig. 3. Comparative dissolution profiles in pH 1.2 dissolution media

TABLE II

Dissolution Test in pH 1.2 dissolution media

% released lisinopril				
Time (min.)	10	15	20	30
A	76.5515	93.5989	94.0958	95.8547
B	63.3411	91.8680	92.7665	97.3693

TABLE III

Dissolution Test in pH 4.5 dissolution media

% released lisinopril				
Time (min)	10	15	20	30
A	51.1896	96.7726	97.7318	98.5634
B	75.5164	93.0365	95.4984	98.3925

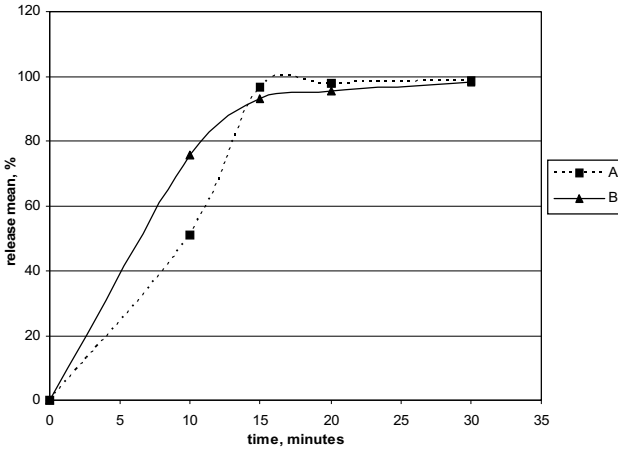

Fig. 4. Comparative dissolution profiles in pH 4.5 dissolution media

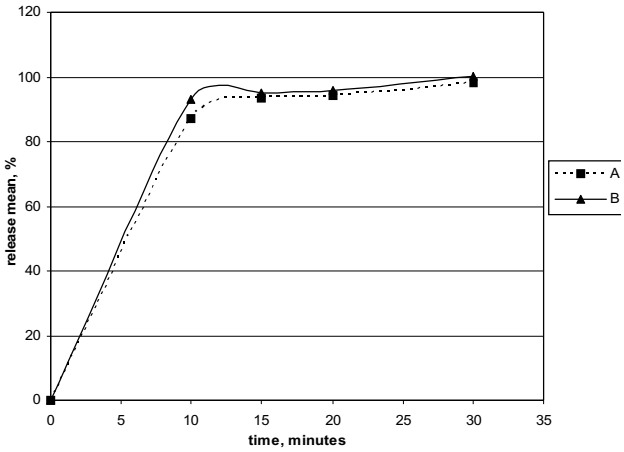

Fig. 5. Comparative dissolution profiles in pH 6.8 dissolution media

TABLE IV

Dissolution Test in pH 6.8 dissolution media

% released lisinopril				
Time (min)	10	15	20	30
A	87.1772	93.5230	94.1995	98.3643
B	93.1250	95.2389	95.9041	100.1474

was used as dissolution media). Both products released more than 85% lisinopril in the first 15 min. as it can be seen from tab. IV and fig. 5.

The dissolution rate for both products was over 85 % in 15 min. when testing their dissolution profiles at different pH values, thus it was not necessary to calculate the similarity factors.

CONCLUSIONS

Our studies led to the following results : both products present a dissolution percentage more than 85% (Q) of the labeled amount after 30 min. for lisinopril. The dissolution profiles of the studied products are almost identical.

The products develop similar dissolution profiles, thus the bioequivalence of Lisinopril 20 mg tablets (Antibiotice S.A. Iaşi) and Zestril 20 mg tablets (Astra Zeneca) was demonstrate *in vitro*.

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ORDINARY DIFFERENTIAL EQUATIONS WITH APPLICATIONS IN MOLECULAR BIOLOGY

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ORDINARY DIFFERENTIAL EQUATIONS WITH APPLICATIONS IN MOLECULAR BIOLOGY (Abstract): Differential equations are of basic importance in molecular biology mathematics because many biological laws and relations appear mathematically in the form of a differential equation . In this article we presented some applications of mathematical models represented by ordinary differential equations in molecular biology. **Material and method**s: The vast majority of quantitative models in cell and molecular biology are formulated in terms of ordinary differential equations for the time evolution of concentrations of molecular species. Assuming that the diffusion in the cell is high enough to make the spatial distribution of molecules homogenous, these equations describe systems with many participating molecules of each kind. **Results:**We propose an original mathematical model with small parameter for biological phospholipid pathway. All the equations system includes small parameter ε.The smallness of ε is relative to the size of the solution domain. If we reduce the size of the solution region the same small ε will result in a different condition number. It is clear that the solution for a smaller region is less difficult.We introduce the mathematical technique known as boundary function method for singular perturbation system. In this system, the small parameter is an asymptotic variable, different from the independent variable. In general, the solutions of such equations exhibit multiscale phenomena. Singularly perturbed problems form a special class of problems containing a small parameter which may tend to zero. **Conclusions:** Many molecular biology processes can be quantitatively characterized by ordinary differential equations. Mathematical cell biology is a very active and fast growing interdisciplinary area in which mathematical concepts, techniques, and models are applied to a variety of problems in developmental medicine and bioengineering. Among the different modeling approaches, ordinary differential equations (ODE) are particularly important and have led to significant advances. Ordinary differential equations are used to model biological processes on various levels ranging from DNA molecules or biosynthesis phospholipids on the cellular level. **Key words:** MOLECULAR BIOLOGY, DNA MOLECULE, PHOSPHOLIPID, DIFFERENTIAL EQUATIONS

Ordinary differential equations (ODEs), especially systems of (ODEs), have been applied in many felds such as molecular biology, medicine, bioengineering. Mathematical and theoretical biology is an interdisciplinary scientific research field with a range of applications in molecular biology. Finding and interpreting the solutions of

these differential equations is therefore a central part of applied mathematics, and a thorough understanding of differential equations is essential for any applied mathematician. In order to do this, precise mathematical models are required. By describing the systems in a quantitative manner, their behavior can be better simulated, and hence properties can be predicted that might not be evident to the experimenter. In order to get a quantitative understanding of a given molecular biology process,it is necessary to come up with a mathematical description of the processwe have in mind. If we want to solve an moleculaer biology problem, we first have to formulate the problem as a mathematical expression in terms of variables, functions, equations Such an expression is known as a mathematical model of the given problem. Give such a mathematical model, we have to analyze it in order to gain some understanding. The above rules are usually in terms of mathematics. They are called mathematical models. One important such models is the ordinary differential equations. It describes relations between variables and their derivatives. Such models appear everywhere [1].

Molecular set theory was introduced by Anthony Bartholomay, and its applications were developed in mathematical biology and especially in Mathematical Medicine. Molecular set theory (MST) is a mathematical formulation of the wide-sense chemical kinetics of biomolecular reactions in terms of sets of molecules and their chemical transformations represented by set-theoretical mappings between molecular sets. In a more general sense, (MST) is the theory of molecular categories defined as categories of molecular sets and their chemical transformations represented as set-theoretical mappings of molecular sets. However, it is usually difficult for people to choose a suitable model structure without sufficient domain details and human expertise, and the determination of parameters also requires the modeler have rich mathematical knowledge and professional skills.

MATERIALS AND METHODS

A remarkable development in molecular biology has been the recent upscaling to the genomic level of its experimental methods. These methods produce, on a routine basis, enormous amounts of data on different aspects of the cell. A large part of the experimental data available today concern genetic regulatory networks underlying the functioning and differentiation of cells. The miniaturization of techniques for the hybridization of labeled nucleic acids in solution to DNA molecules attached to a surface has given rise to DNA microarrays, tools for measuring the level of gene expression in a massively parallel way. The development of proteomic methods based on two-dimensional gel electrophoresis, mass spectrometry, and the double-hybrid system allows the identification of proteins and their interactions on a genomic scale [2]. We give the example of a simple genetic regulatory network, composed of two genes x and y, the proteins X and Y, and their regulatory interactions. An ordinary differential equation model of the network is:

$$(1)\quad \begin{cases} \dfrac{dx}{dt} = \alpha_1 f(y, \beta_2, \chi_2) - u_1 x \\ \dfrac{dy}{dt} = \alpha_2 f(x, \beta_1, \chi_1) - u_2 y \\ f(a, \beta, \chi) = \dfrac{\beta^\chi}{a^\chi + \beta^\chi} \end{cases}$$

where: the variables x and y correspond to the concentrations of proteins X and

Y, respectively parameters α_1 and α_2 to the synthesis rates of the proteins, parameters u_1 and u_2 to the degradation constants , parameters β_1 and β_2 to the threshold concentrations and parameters γ_1 and γ_2 to the degree of cooperativity of the interactions. All parameters are positive . The temporal derivative of x is the difference between the synthesis term $\alpha_1 f(y, \beta_2, \chi_2)$ and the degradation term $u_1 x$. The first term expresses that the rate of synthesis of protein X depends on the concentration of protein Y and is described by the function f. This so called Hill function is monotonically decreasing. The synthesis term $\alpha_1 f(y, \beta_2, \chi_2)$ thus means that, for low concentrations of protein Y, gene x is expressed at a rate close to its maximum rate α_1, whereas for high concentrations of Y, the expression of the gene is almost completely repressed. The second term of the differential equation, the degradation term, expresses that the degradation rate of protein X is proportional to its own concentration x, y being a degradation parameter ($u_1 > 0$). In other words, the degradation of the protein is not regulated in this example. The differential equation for y has an analogous interpretation.

Many mathematical models, including the ones arising is such important areas as cell biology, biochemistry, bioengineering have to include the concentrations of many "intermediate" reactants that are very hard to measure experimentally and many unknown constants (reaction rates) that can not be predicted theoretically with enough accuracy.

For example, the following system of nonlinear ODEs has been used to model the signal transduction and second messenger systems in cell biology. Second messengers are molecules that relay signals from receptors on the cell surface to target molecules inside the cell, in the cytoplasm or nucleus. They relay the signals of hormones like epinephrine (adrenalin), growth factors, and others, and cause some kind of change in the activity of the cell. There are several different secondary messenger systems, but they all are quite similar in overall mechanism, though the substances involved in those mechanisms and effects are different [3].

An ordinary differential equation model is:

$$(2)\quad \begin{cases} \dfrac{dx}{dt} = a(b_0 + b_1 u)(1-x) - \dfrac{\alpha_1 x^4}{\alpha_2^4 + x^4} \\ \dfrac{dy}{dt} = k_1 P y + k_{-1} z \\ \dfrac{dz}{dt} = -(k_{-1} + k_2 x) z + k_1 P y + k_{-2} u \\ \dfrac{du}{dt} = k_2 x z + k_{-3} w - (k_{-2} + k_3 x) u \\ \dfrac{dw}{dt} = k_3 x u - k_{-3} w \end{cases}$$

where: a, b_0, b_1, α_1, α_2, and P are various parameters, such as reaction rates. The variables x, y, z, u, w correspond to the concentrations of agonist activates membrane bound receptor, G-protein activated, G-protein produces effector, effector stimulates second messenger synthesis and second messenger activates intercellular process.

The main structural component of biological membranes are the phospholipids.

The biosynthesis phospholipids depends on the syntesis of diacylglycerol.We have conducted the experiment on the data of a metabolic network that consists of three substances. This target network is a part of the biological phospholipid pathway. Biological processes are often represented in form of networks such as protein-protein interaction networks and metabolic pathways [4]. Mathematical models of molecular biology systems are often formulated as a large set of differential equations with small parameter. When small singular perturbation parameters of the same order of magnitude are present in the dynamic model of a molecular system, the control problem is usually solved as a single parameter perturbation problem, such a system is called a singularly perturbed system.

RESULTS

We analyze an original singularly perturbed system of ordinary differential equations for the model discussed in Tomita [5]. We have conducted the experiment on the data of a metabolic network that consists of three substances. This target network is a part of the biological phospholipid pathway.This network can be approximated as:

$$(P_\varepsilon)\begin{cases}\varepsilon\dfrac{dx}{dt}=-xz\\ \varepsilon\dfrac{dy}{dz}=xz-\varepsilon k_2 y\\ \varepsilon\dfrac{dz}{dt}=-xz+\varepsilon k_2 y\end{cases}$$

with initial conditions: $$(3)\begin{cases}x(0)=x_0\\ y(0)=y_0\\ z(0)=z_0\end{cases}$$

We work on the assumption that $\dfrac{1}{\varepsilon}$, rate from reaction is long, where ε is the small parameter. Note that the parameter k_2 are known for the simulation experiment .The use of a small parameter here is simply for definiteness. For example, a problem depending on a large parameter θ can be rewritten as one depending on a small parameter: $\varepsilon=\dfrac{1}{\theta}$. Equation (P_ε) corresponds to the extended model. Let us now give some formal explications: the terms containing small parameter ε are called perturbations. The extended model (P_ε) is then called perturbed.

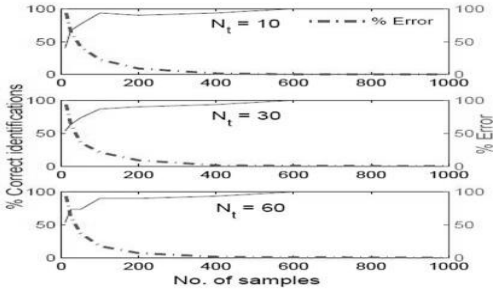

Fig. 1. Variation of correct identifications and identification errors with experimental samples and discretization. The variations are with respect to the number of experimental samples chosen and the number of discretizations, N_t. The "experimental" data are obtained by simulation using the synthetic network.

In this article, the model is formulated in terms of differential equations with small parameter multiplying the highest derivates.Simplified model to consider:

$$(4)(P_0)xz=0$$

Simplified model (P_0) is called unperturbed. Equation (P_0) might be a simplified model of this process. We started out with three differential equations, and ended up saying that one of the differential equa-

tions degenerates to an algebraic equation. A system of three differential equations is quite different from a single differential equation with an algebraic constraint. Unfortunately, in most cases when we change the parameters of the equations to move from one regime to another, the type of the equation changes as well and the solutions in different regimes are often dramatically different. On biological grounds, such as adimensionalisation , we expect that when this parameter reaches certain critical value (usually 0) the system would change its main characteristics which then would make it more amenable to a description at a different level. In this case ,when the coefficient of the highest derivative can take an arbitrary small value in $(0,1]$, the solution of (P_ε) may have limited smoothness. The derivative s of such solutions may increase without bound then the small parameter ε tends to zero.Singularly perturbed problems appear in any area of biology and can be approached by many techniques. Is classical asymptotic analysis based on expansion of the solution in series of powers of the parameter.As we see, three part of (P_ε) contains the small parameter multiplying the derivate. We use the notation $x(t,\varepsilon), y(t,\varepsilon)$ for the solution of (P_ε) and (3). The choice of a small parameter is connected with the order of the solution and the asymptotic sequence which describes the behaviour of the solution as $\varepsilon \to 0$. The overall procedure for work is [6]:

- Develop an appropriate scaling for the equations. Identify the small parameter.
- Writting regular series.
- Writting boundary function series.
- Match up the two solutions in an intermediate time range.

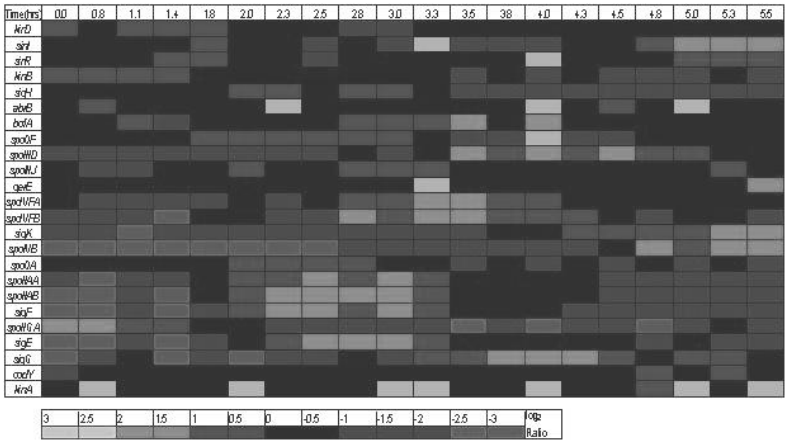

Fig. 2. The time-course (in hours) variation of the logarithm of expression ratios in color-coded format (green indicates up-regulation, red indicates down-regulation, grey indicates missing data and the intensity of the color indicates the level of regulation) of 24 important genes in the population cascade of *B. Anthracis.* An approximate measure of the intensity of the color to the magnitude of the $\log_2$ ratio (fold change) is also given. Note that the ratio refers to that of the actual expression value to the expression value at the reference state.

We will seek the asymptotic expansions for the solution of (P_ε) and (3) in a format that is quite typical for singularly perturbed problems:

$$(5)\quad \begin{cases} x(t,\varepsilon)=\sum_{i=0}^{2}(x^i+\pi_i x)\varepsilon^i+O(\varepsilon^3) \\ y(t,\varepsilon)=\sum_{i=0}^{2}(y^i+\pi_i y)\varepsilon^i+O(\varepsilon^3) \end{cases}$$

where : $\tau=\frac{t}{\varepsilon}$,deals with systems with clear separation of time-scale, $\sum_{i=0}^{2}x^i\varepsilon^i$ is regular series and $\sum_{i=0}^{2}\pi_i x\varepsilon^i$ is boundary function series. Recall that the functions $\pi_0 x,\pi_0 y,\pi_1 x,\pi_1 y$ should be boundary functions, they should approach zero as $\tau\to\infty$. The functions $\pi_0(\tau),\pi_1(\tau)$ decay exponentially as τ increases . Therefore they are important only in a small vicinity of the initial point $t=0$ For $t>>\theta$, where θ is an arbitrarily small, $\pi_k(\tau)\to 0$ as $\varepsilon\to 0$, faster any power of ε . Let is try to construct an asymptotic expansions of the solution of (P_ε), in the form of power series in the power of ε .Series (5) satisfies formally the differential system (P_ε) , but it does not in general satisfy the initial condition (3).

$$(6)\quad \frac{d\pi x}{dt}=\frac{d\pi x}{d\tau}\frac{d\tau}{dt}=\frac{1}{\varepsilon}\frac{d\pi x}{d\tau}$$

If we add the last two equations we obtain system:

$$(7)\begin{cases} \varepsilon\frac{dx}{dt}=-xz \\ \varepsilon\frac{d(y+z)}{dt}=0 \end{cases}\leftrightarrow\begin{cases} \varepsilon\frac{dx}{dt}=-xz \\ y+z=c, c\in\Re \end{cases}$$

CONCLUSIONS

Mathematical model is a representation of the essential aspects of an existing system which presents knowledge of that system in usable form. The use of mathematical models in molecular biology is at once both familiar and arcane. This kind of modeling is done by differential equations. Ordinary differential equations play an important role in the modeling of many real-world processes. What we have looked in this article is a collection of some of the ordinary differential equation models in molecular biology. Mechanistic models in molecular can be regarded as some form of dynamical system. A dynamical system describes the states of a molecular system and how these states change in time. Dynamical systems usually depend on parameters. The temporal dynamics are then described by ordinary differential equations. The classical modeling strategy in molecular biology the ordinary differential equation approach contains three initial phases, and starts with this kind of structural model, in which the reactions and effectors are known. The parameter values have highs impact on the accuracy of the model in representing real biological systems but these values are difficult to estimate experimentally.

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CERCETĂRI PRIVIND ÎMBUNĂTĂŢIREA CONDIŢIILOR DE ACILARE ÎN CAZUL OBŢINERII PENICILINELOR ANTISTAFILOCOCICE

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RESEARCHES REGARDING THE IMPROVEMENT OF ACYLATION CONDITIONS IN ANTISTAPHYLOCOCCAL PENICILLINS SYNTHESIS (Abstract): **Aim**: The 6-aminopenicillanic acid acylation with certain acyl chlorides was performed in order to obtain antistaphylococcal penicillins with bigger crystals, easy to filtrate (shorter filtration time), much pure, and an increased output. **Material and methods**: Oxacillin sodium salt was synthesized by acylating an aqueous solution of 6-aminopenicillanic acid sodium salt ($NaHCO_3$ not in excess) with an ethylacetate solution of 5-phenyl-3-methyl-isoxazolyl-4-carboxilic acid chloride. The crystallization was performed with a 40.5% sodium 2-ethyl hexanoate izopropanolic solution. All tests (IR spectrum, iodometric titration, and microbiological dosage) were performed according to the Xth Romanian Pharmacopoeia standards. **Results**: The amount of synthesized oxacillin was higher and the output of 88,21%. Oxacillin had a high chemical purity (98,72%), and a very good microbiological activity (95% of the standard activity). **Conclusions**: Oxacillin crystals were bigger, the filtration speed was increased, and process efficacy improved. The output of the process was also improved being higher than with classical acylation. **Key words:** ACYLATION, PENICILLINS, BETA-LACTAMS

Oxacilina sodică se produce la SC Antibiotice SA sub formă de capsule pentru administrare orală şi flacoane cu pulbere injectabilă pentru administrare parenterală.

Metoda actuală de obţinere a oxacilinei sodice presupune acilarea mol la mol a sării de sodiu a acidului 6-aminopenicilanic (6-AP) cu clorura acidului 3-fenil-5-metil-4-izoxazolilcarboxilic în prezenţa unui exces de bicarbonat de sodiu, în mediu hidro-acetonic (1, 2, 3). Ulterior, se supune cristalizării oxacilina ca acid din soluţie anhidră de acetat de butil cu o soluţie izopropanolică de 2-etilhexanoat de sodiu (2-EH Na). Se obţin astfel cristale mici de oxacilină sodică, ceea ce face ca procesul de filtrare al unei şarje de oxacilină să dureze foarte mult (aproximativ 8 ore). Pe parcursul acestui timp, oxacilina sodică se poate degrada parţial datorită expunerii la umiditatea atmosferică.

Pe lângă aceste două inconveniente ale

procesului industrial (timp mare de filtrare şi posibilitatea degradării parţiale a oxacilinei sodice), mai există unul şi anume, un randament total mediu de 74-80%.

Ţinând cont de aceste date, în acest studiu am încercat să îmbunătăţim procesul de obţinere în ceea ce priveşte mediul şi condiţiile de reacţie. În acest fel dorim să obţinem un randament superior şi cristale mai mari de oxacilină sodică, adică uşor filtrabile, pentru a avea un timp de filtrare mai scurt.

MATERIAL ŞI METODĂ

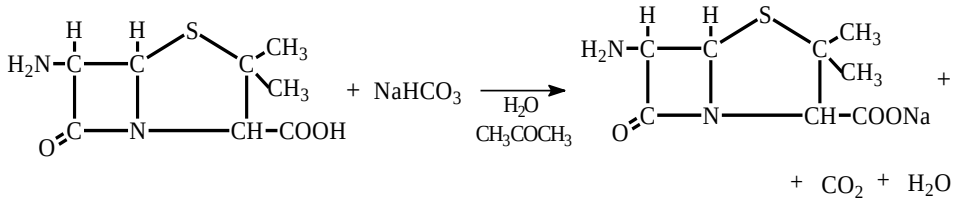
În acest studiu am încercat să obţinem oxacilina sodică sub formă de cristale uşor filtrabile realizând reacţia de acilare în solvenţi diferiţi, utilizând un mic exces de acid 6-AP (0,001 moli), iar bicarbonatul de sodiu fiind adăugat în acelaşi procent molar cu acidul 6-AP.

S-au realizat 3 variante:

1. solubilizarea acidului 6-AP într-un amestec de apă-acetonă, fără exces de bicarbonat şi adăugarea clorurii acide dizolvată în diclormetan (DCM);
2. solubilizarea acidului 6-AP în apă, fără exces de bicarbonat şi adăugarea clorurii acide dizolvată în DCM;
3. solubilizarea acidului 6-AP în apă, fără exces de bicarbonat şi adăugarea clorurii acide dizolvată în acetat de etil.

Chimismul reacţiilor, pentru toate cele 3 variante, este următorul:

1.



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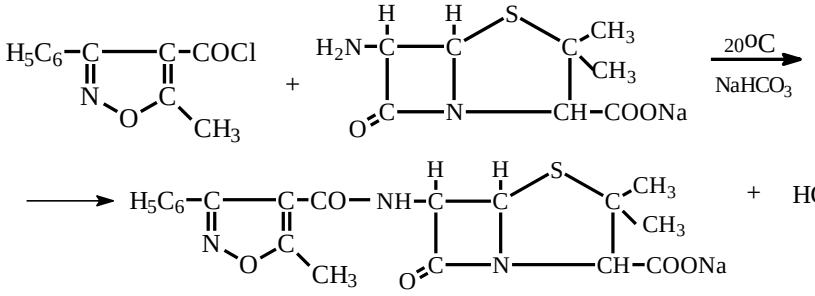

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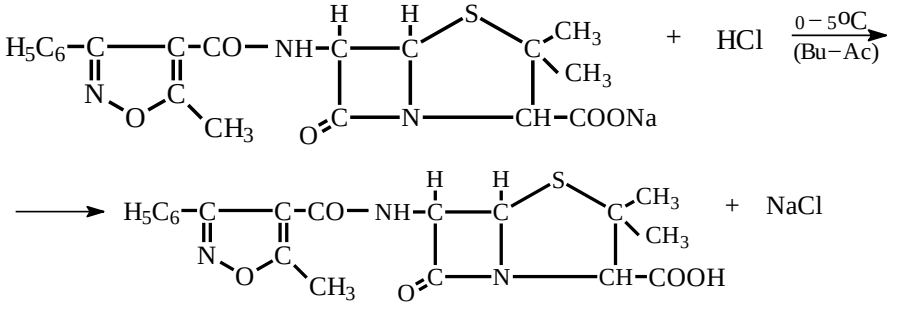

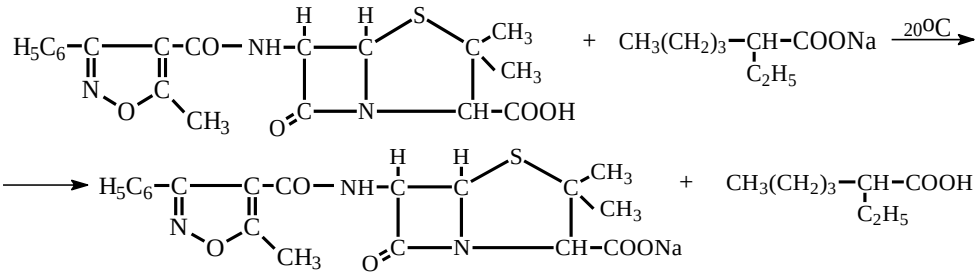

Modul de lucru este asemănător pentru toate cele 3 variante, diferind solvenţii, cantităţile şi condiţiile de lucru.

Se solubilizează 0,018 moli (4 g) de acid 6-AP cu 0,018 moli de $NaHCO_3$ într-un amestec de 60 ml apă:acetonă (1:1), respectiv 60 ml apă pentru variantele B şi C. Se agită continuu până la solubilizarea acidului 6-AP (aproximativ 0,5 ore). Soluţia obţinută, cu un pH=7, se răceşte pe baia de gheaţă la 0-5^{0}C. Sub agitare energică şi continuă se adaugă 0,017 moli (3,90 g) de clorură a acidului 3-fenil-5-metil-4-izoxazolilcarboxilic dizolvată în 45 ml de DCM, respectiv 45 ml de acetat de etil pentru varianta C. Adăugarea soluţiei organice a clorurii acide se realizează în aproximativ 45 min., astfel încât temperatura să nu depăşească 5^{0}C. Reacţia se perfectează 2,5-3 ore sub agitare energică şi continuă, la temperatura de 5-10^{0}C. Se lucrează folosind un mic exces de acid 6-AP (0,001 moli). Ulterior, se separă stratul hidro-acetonic superior în care se găseşte oxacilina ca sare de sodiu.

Soluţiei hidro-acetonice de oxacilină sodică i se adaugă 70 ml acetat de butil, se răceşte amestecul la 0-5^{0}C şi, sub agitare energică, se adaugă cât mai repede o soluţie de HCl 18% până la pH=1,8-2. Într-o pâlnie de separare se separă faza organică superioară ce conţine oxacilina ca acid. Faza organică se anhidrizează cu Na_2SO_4 anhidru şi se supune cristalizării cu o soluţie izopropanolică de 2-EH Na, de concentraţie 40,5% (2,98 g 2-EH Na, 0,018 moli, 7,7 ml), la temperatura camerei. Cristalizarea este greoaie, iar primele cristale apar după 1,5 ore. După perfectarea cristalizării (3 ore), se separă oxacilina sodică prin filtrare, se spală direct pe filtru cu butanol şi apoi cu acetonă şi se usucă sub vid. Cristalele sunt destul de mari, astfel încât filtrarea decurge uşor.

S-au realizat spectrele IR cu ajutorul unui spectrofotometru Specord 71 IR Carl Ziss-Jena, în domeniul 200 cm^{-1} - 4000 cm^{-1}. S-a comprimat prin pastilare un amestec ce conţine 1-3 mg oxacilină sodică şi 0,3 g bromură de potasiu (R) (4). Spectrele oxacilinei sodice, preparată prin cele trei variante, au fost comparate cu spectrul IR al oxacilinei standard de fabrică (S.C. Antibiotice S.A.). S-a realizat şi determinarea cantitativă a oxacilinei prin metoda volumetrică (titrare iodometrică) şi dozarea microbiologică prin metoda difuzimetrică în agar, conform prevederilor din Farmacopeea Română ediţia X (FR X) de la determinarea cantitativă a oxacilinei (5). S-a utilizat mediul V recomandat de FR X, microorganismul test utilizat a fost *Staphylococcus aureus* ATCC 6538P, iar ca diluant pentru prepararea soluţiilor de antibiotic s-a folosit soluţia de tampon fosfat pH=7. Ca standard s-a utilizat oxacilina sodică, standard de fabrică al S.C. Antibio-

tice S.A. Iaşi, cu activitatea microbiologică de 851,22 µg oxacilină/mg. Plăcile Petri se inoculează şi se incubează la termostat 18 ore la 35-37^{0}C, după care se măsoară şi se înegistrează zonele de inhibiţie, urmând să se calculeze media aritmetică a diametrelor zonelor de inhibiţie conform recomandărilor din FR X (5).

REZULTATE ŞI DISCUŢII

Pentru fiecare variantă s-au realizat 5 determinări. Cantităţile de oxacilină sodică şi media determinărilor sunt redate în tabelul I.

TABELUL I

Cantităţile de oxacilină sodică obţinute în cele trei variante de preparare

Metoda	Cantitatea obţinută (g)					
	1	2	3	4	5	Media determinărilor
Varianta A	5,86	6,06	5,99	5,98	6,24	6,02 g
Varianta B	6,86	6,79	6,59	6,76	6,54	6,70 g
Varianta C	7,18	7,15	7,25	7,21	7,20	7,04 g

Din datele obţinute se observă că cele mai mari cantităţi de oxacilină sodică s-au obţinut utilizând varianta C (acilarea în apă-acetat de etil, fără exces de bicarbonat).

S-au calculat randamentele pentru cele trei variante de preparare, rezultatele fiind trecute în tabelul II.

TABELUL II

Randamentele de obţinere a oxacilinei sodice în cele trei variante de preparare

Metoda	Randamentul (%)					
	1	2	3	4	5	Media determinărilor
Varianta A	71,83	74,26	73,40	73,28	76,47	73,84 %
Varianta B	84,06	83,21	80,75	82,84	80,14	82,20 %
Varianta C	87,99	87,62	88,84	88,35	88,23	88,21 %

Se observă că varianta C de obţinere a oxacilinei sodice are cele mai mari randamente (87-88 %). Se poate spune că prepararea oxacilinei sodice este mai avantajoasă folosind varianta C (acilarea în apă-acetat de etil, fără exces de bicarbonat) întrucât se obţin cantităţi mai mari de oxacilină sodică, respectiv randamente de producţie mai mari, iar filtrarea oxacilinei se realizează mult mai uşor.

Oxacilina sodică obţinută prin cele 3 variante se prezintă sub formă de pulbere cristalină albă, fără miros, cu gust slab amar, uşor solubilă în apă şi practic insolubilă în acetonă, eter şi cloroform.

Oxacilina sodică obţinută prin cele trei variante are spectre IR comparabile cu cel al standardului de fabrică. În cazul standardului se observă un maxim de absorbţie la 1760 cm^{-1} ce este caracteristic vibraţiilor legăturii carbonilice lactamice (=C=0) şi un maxim de absorbţie la 1580 cm^{-1} ce este caracteristic vibraţiilor legăturii amidice (-CO-NH-) din catena laterală (4). În cazul

celor trei probe de oxacilină sodică se observă că maximele de absorbţie corespunzătoare legăturii lactamice sunt la 1770 cm^{-1}, 1773 cm^{-1} şi respectiv, 1765 cm^{-1}. Maximele de absorbţie corespunzătoare legăturii amidice sunt la 1584 cm^{-1}, 1589 cm^{-1} şi respectiv, 1582 cm^{-1}. Datele sunt prezentate în tabelul III.

TABELUL III

Caracteristicile spectrale ale oxacilinei sodice obţinută prin cele trei variante de preparare şi ale standardului de fabrică

Oxacilina sodică	Frecvenţa caracteristică (cm^{-1})	
	(=C=O) lactamică	(-CO-NH-)din catena laterală
Varianta A	1770	1584
Varianta B	1773	1589
Varianta C	1765	1582
Standard de fabrică	1760	1580

Determinarea cantitativă s-a realizat prin titrare iodometrică, cele trei probe de oxacilină sodică având purităţi ce sunt redate în tab. IV. Pentru a corespunde prevederilor din FR X, oxacilina sodică trebuie să conţină cel puţin 90% şi cel mult 100,5% peniciline totale exprimate în $C_{19}H_{18}N_3NaO_5S \cdot H_2O$ (5). Se observă că oxacilina sodică obţinută prin varianta C are cea mai mare puritate (98,72%).

TABELUL IV

Concentraţia probelor de oxacilină sodică

Nr. crt.	Oxacilina sodică	Concentraţia (g%)
1.	Varianta A	90,23
2.	Varianta B	93,51
3.	Varianta C	98,72

În urma dozării microbiologice s-au obţinut rezultatele redate în tab. V. Proba este corespunzătoare dacă activitatea ei microbiologică reprezintă cel puţin 90,0% şi cel mult 110,0% din activitatea microbiologică a standardului utilizat (5). Se observă că din toate probele analizate, doar cea a oxacilinei sodice obţinută prin varianta C corespunde prevederilor, având o activitate microbiologică de 95,00% din activitatea standardului.

TABELUL V

Activitatea microbiologică a oxacilinei sodice

Nr. crt.	Oxacilina sodică	Activitatea microbiologică (µg/mg)	Activitatea microbiologică faţă de standard (%)
1.	Varianta A	751,6	88,29
2.	Varianta B	759,3	89,20
3.	Varianta C	808,7	95,00
4.	Standard de fabrică	851,22	-

CONCLUZII

Obţinerea oxacilinei sodice prin metoda aplicată în acest studiu (acilarea în apă-acetat de etil utilizând un mic exces de acid 6-AP şi adăugarea bicarbonatului de sodiu în acelaşi procent molar cu acidul 6-AP) este mai avantajoasă decât metoda clasică, întrucât din modul de lucru dispare o etapă de extracţie (luând un exces de acid 6-AP nu mai trebuie realizată extracţia pentru îndepărtarea clorurii acide nereacţionate).

La finalul sintezei se obţin cristale de oxacilină sodică mari, uşor filtrabile, a căror filtrare se realizează într-un timp mai scurt, dispărând posibilitatea degradării antibioticului pe parcursul filtrării. Această cristalizare este mai bună dacă oxacilina sodică se obţine prin varianta C.

Aceeaşi variantă C permite obţinerea unor cantităţi mai mari de oxacilină sodică (7,04 g), procesul decurgând cu randamente superioare (88,21%).

Analiza calitativă a evidenţiat că probele de oxacilină sodică obţinute corespund standardului, iar analiza cantitativă a relevat că oxacilina sodică obţinută prin varianta C are o puritate chimică mare (98,72%) şi o activitate microbiologică mare (95% din activitatea standardului).

În concluzie, oxacilina sodică se obţine cu randamente superioare şi timp de filtrare mult mai scurt, dacă sinteza se realizează prin acilarea acidului 6-AP, solubilizat ca sare de sodiu în apă, cu o soluţie în acetat de etil a clorurii acidului 3-fenil-5-metil-4-izoxazolilcarboxilic, acilare realizată la 0-5^{0}C timp de aproximativ 3 ore. Cristalizarea oxacilinei se realizează din soluţia organică anhidră a oxacilinei ca acid cu o soluţie izopropanolică de 2-etil hexanoat de sodiu.

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COMMUNITY PHARMACY – IDEAL LOCATION TO SCREEN AND MONITOR HYPERTENSIVE PATIENTS

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COMMUNITY PHARMACY – IDEAL LOCATION TO SCREEN AND MONITOR HYPERTENSIVE PATIENTS (Abstract): The goals of this study were (a) to create a patient electronic database by filling in the specially designed Patient Data Sheets (PDS) after interviewing hypertensive patients and (b) to evaluate pharmacists' opportunities for intervention in order to improve hypertensive patients' outcomes. **Material and method**: Pharmacists from two Romanian chain community pharmacies interviewed all hypertensive patients that entered the pharmacies during the study period (November – December 2008) and selected 106 subjects using several selection criteria. Pharmacists recorded in specially designed Patient Data Sheet relevant information such as: demographic data, additional diagnostics (if any), lifestyle behaviors and therapeutic data. The records were then transferred into an electronic database and interpreted by the research team. **Results**: Hypertension (HT) as a single disease was present in only 7 patients (6.6%), most of them being diagnosed with multiple illnesses (such as HT and cardiovascular disease (CVD) in 25 patients, HT and dyslipidemia (DL) in 20 patients). More than half of the subjects (61.3%) declared they have adopted at least one lifestyle modification component and almost 80% of the patients use two, three or more antihypertensive agents in their medication. **Conclusions**: The high incidence of complications among our hypertensive subjects justifies implementing blood pressure monitoring programs in community pharmacies and requires pharmacists' intervention for a better management of hypertension. **Key words**: HYPERTENSION, PATIENT DATA SHEET, LIFESTYLE MODIFICATIONS, MONITORING PROGRAMS

Hypertension (HT) is a major healthcare problem in Romania and one of the most frequent causes of cardiovascular morbidity and mortality. It affects people of all ages, social statuses and professions (1) and initially it causes no symptoms. In the case that some symptoms appear, these are of low intensity and non-specific (2).

HT is also the major risk factor for cardiovascular and cerebrovascular diseases, along with other contributing factors such as: dyslipidemia (DL), diabetes mellitus (DM), obesity, unhealthy diet or sedentary lifestyle (3). Major complications remain uncontrolled or poorly controlled for longer periods of time (4,5). Thus, an optimized control on blood pressure (BP) values is needed to reduce the prevalence of complications. BP values are considered "normal" as long as they are below 140/90 mmHg (in case of a patient with DM or chronic renal disease, the upper limit is 130/80 mmHg) (3,6,7) and "optimal" if they are below 120/80 mmHg (7). Based on evidences, it was found that the cardiovascular disease risk doubles with every increase of

20/10 mm Hg above 115/75 mmHg (1).

Although guidelines recommendations stress for early discovery and aggressive treatment of HT, many studies reported low compliance or poor adherence to therapy (8). Consequently, this determines poor control of BP values and high prevalence of complications. The management of HT in society became an important challenge for the healthcare system (5); identifying patients with high BP represents a major challenge for community pharmacists (5,9). Considered as the most accessible healthcare providers (9), community pharmacists are in a unique position to run screening programs (10). The beneficial of these programs is supported by large scale studies (4) because it was found to improve outcomes and compliance (8). Additional services like blood pressure monitoring and short counseling sessions are also proven to increase patient's compliance (1,11).

Some of the most effective non-pharmacological treatment measures consist in lifestyle changes (7). A healthy lifestyle, as pointed out by the Seventh Report of JNC (Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure), can lower BP values and decrease the progression of HT (6) preventing from complications or delaying them (5,6). Lifestyle changes are strongly recommended to hypertensive patients as well as to people having one or more risk factors (6). Community pharmacists have the opportunity to reinforce the importance of lifestyle changing each month a hypertensive patient refills her/his medication (5,12,13). They should also detect and solve potential drug related problems before they generate adverse reactions and reduce compliance to treatment (9).

Pharmacy based HT screening programs for identifying patients at risk for cardiovascular and cerebrovascular diseases have shown their effectiveness as an important percentage of interviewed patients were referred to physicians with high BP values (4). Equally important, monitoring programs for already diagnosed hypertensive patients contribute to a better management of HT, increase patient motivation and adherence to therapy (8,9,10).

This study was conducted according to law 46 and 667 (14,15).

MATERIAL AND METHOD

The rationale of this study was to pilot a methodology for gathering relevant patient information and to test its usefulness for future interventions in hypertensive patients. The goals were (a) to create a patient electronic database by filling in the specially designed Patient Data Sheets (PDS) after interviewing hypertensive patients by the time they refill their medication and (b) to evaluate pharmacists' opportunities for intervention (education, counseling, monitoring) in order to improve hypertensive patients' outcomes.

During the study period (November – December 2008), pharmacists from two chain community pharmacies have interviewed 106 hypertensive patients. Patients selection followed several criteria: (a) diagnosed with HT for more than 1 year, with no major cardiovascular or cerebrovascular event in the last 6 months; (b) they refill regularly their prescriptions for HT medication (all patients are well known to pharmacy staff for long time), therefore the compliance to treatment can be estimated as good; (c) the HT medication they take didn't changed recently.

Community pharmacists participating in the study followed a short training session where they were explained how to correctly fill in the PDS. They recorded in PDS relevant information such as: demographic data, additional diagnostics (if any), adopted lifestyle modifications (if the case) and therapeutic data.

The main outcome measures of this study

were: HT distribution vs. sex and age, prevalence of complications, lifestyle modification components adopted by patients, prescribed therapeutic classes.

Information recorded in PDS were transferred to an electronic database (Excel workbook) and interpreted by following criteria: distribution of diagnostics among interviewed hypertensive patients, patient behaviors and lifestyle, distribution of HT treatment schemes (how many anti-hypertensive drugs were chosen in patient's treatment) and of therapeutic classes.

RESULTS

The interviewed patients (n = 106, 44 men and 62 women) were previously diagnosed with: HT as single disease (7 patients), HT and diabetes mellitus (DM) (6 patients), HT and cardiovascular disease (CVD) (25 patients), HT and dyslipidemia (DL) (20 patients), HT and DL and CVD (35 patients), HT and other chronic diseases (13 patients). The frequency of diagnostics is presented in fig. 1.

Clinical evidences have been shown that, by adopting lifestyle modifications, hypertensive patients may notice significant decrease of blood pressure values and cardiovascular risk (3,6). The most important changes the patients should adopt would include weight loss (in case of overweight patients), reduction of sodium intake, physical activity, moderate alcohol consumption, smoke cessation (6, 7). When additional conditions occur, further measures might be needed: low fat diet for patients with

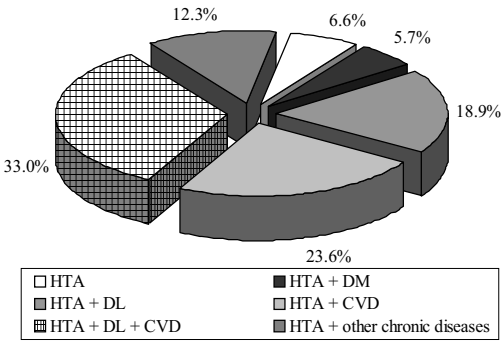

Fig. 1. Frequency of diagnostics at hypertensive patients

DL, low sugar intake for patients with DM.

Sixty-five patients (61.3%) declared they have adopted at least one lifestyle modification component while thirty eight chose to ignore the importance of these measures (fig. 2).

Lifestyle changes that patients in study have adopted are: lowering of sodium intake (57 patients), low fat diet (21 patients), moderation in alcohol consumption (12 patients), weight loss (6 patients), daily physical activity (5 patients) and low sugar diet (5 patients).

When lifestyle modifications cannot reach blood pressure goals, pharmacological treatment is needed. Published studies evidenced that most of the hypertensive patients are treated with more than one antihypertensive agent. The most commonly used medication classes cited by literature are diuretics, beta--blockers, ACE inhibitors, angiotensin II antagonists and calcium channel blockers (6).

Twenty two patients (20.8%) were treated with antihypertensive monotherapy, 45

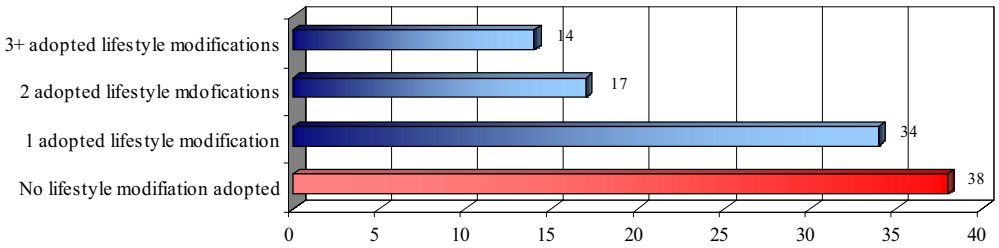

Fig. 2. Patients adopting lifestyle modifications

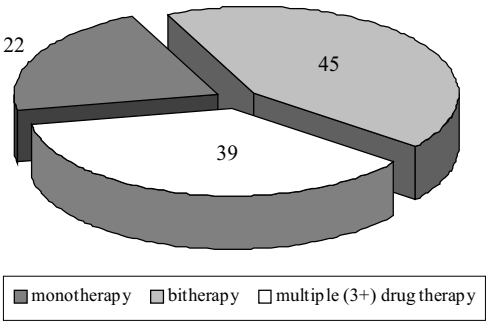

Fig. 3. Pharmacological therapy for hypertension

(42.4%) with bitherapy and 39 (36.8%) with three drugs or even more because of the complications of hypertension (fig. 3).

The most frequently prescribed therapeutic classes were: angiotensin converting enzyme inhibitors (67 patients), diuretics (65 patients) and beta-blockers (60 patients) (fig. 4).

DISCUSSIONS

The hypertensive subjects included in the study were also diagnosed with DM, DL and CVD therefore it should be more useful to have their BP values monitored (in a community pharmacy or by self-monitoring) for a better management of HT. We counted only 7 patients with HT as a single disease, the other 99 patients (93.4%) had additional diseases, most of them classified as risk factors for CVD. Early evaluation of risk factors followed by patient education on dietary regimen, lifestyle changes and pharmacological therapy, would prevent or delay the evolution of complications (7).

The high incidence of complications among our hypertensive subjects justifies implementing BP values monitoring programs in community pharmacies and requires pharmacists' intervention for a better management of HT. In addition, as many of our subjects (36.8%, more than a third) follow a multiple (3+) drug therapy, the probability of drug related problems (DRP) to occur is higher than in other patients. Thus, following programs should also address possible DRP, for the safety of the patients and for a higher, long-term, compliance to treatment.

Behavioral changes that have greater impact on BP values include diet modifications (richer in fruits, vegetables, low-fat products and lower sodium intake), more physical activity and weight loss (5). Although these changes are easy to understand and remember, hypertensive patients have difficulties in applying them over longer time. By involving community pharmacists in supporting patients to maintain a healthier lifestyle, a better control of HT might be achieved (13).

The database we created can be used for future programs where patients shall be closely monitored on how they stick to a healthier lifestyle and follow the treatment

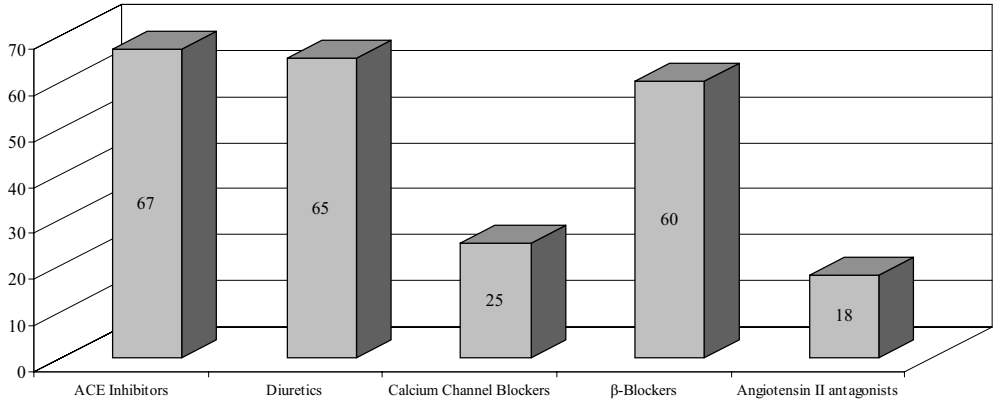

Fig. 4. Antihypertensive therapy – prescribed drug classes

as prescribed. By recording how often patients refill their medication (which medicines are regularly purchased) or if they adjust the dosage on higher/lower BP values (measured with self monitoring devices), pharmacists may evaluate both patient's compliance as well as BP values control. Looking forward, pharmacists may use a quiet space/area in the community pharmacy to measure patient's BP and to perform counseling sessions, as most of the subjects with uncontrolled HT would require closer attention.

The researchers did not aim to screen for uncontrolled BP values on hypertensive patients, therefore BP values were neither recorded nor measured in community pharmacies during this study. The methodology used to build the patient database may be improved by recording additional disease related or treatment related information such as risk factors for HT or CVD, OTC products or natural remedies the patients take on top of their chronic medication.

CONCLUSIONS

HT treatment in Romania follows the most important guidelines recommendations. However, the incidence of complications is extremely high leading to conclusion that HT is poorly controlled. The results of this study suggest that pharmacists can use various intervention strategies like : implementing more lifestyle modifications, BP values monitoring and making patients aware on severity of complications in order to achieve HT treatment goals.

Community pharmacies are some of the most accessible locations to develop pharmaceutical care programs for hypertensive patients due to the fact that community pharmacies are frequently visited by these patients and most of them accept the care and interest that pharmacists show for their medical condition.

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NOUTĂŢI

EFECTUL CONDIŢIONĂRII CU EDTA ÎN CAZUL UTILIZĂRII ADEZIVILOR CE CONŢIN ETANOL

Sistemele adezive cu gravaj acid presupun demineralizarea dentinei şi expunerea colagenului care va fi ulterior infiltrat de răşină. Prin demineralizare, aproape 50% din volumul mineral este înlocuit de apă. Pentru a substitui apa cu răşina hidrofugă, adezivii utilizează un solvent hidrofil. În cazul etanolului, timpul necesar pentru a disloca apa este destul de lung şi creşte cu cât demineralizarea este mai profundă. Pentru a scurta tipul operator autorii studiului propun realizarea unui strat hibrid mai subţire şi mai rezistent, prin demineralizarea dentinei cu EDTA. Rezistenţa adeziunii la tensiune, permeabilitatea interfeţei şi modul în care se produce desprinderea la nivelul ei, au fost evaluate pe secţiuni dentinare umede, condiţionate cu acid ortofosforic, respectiv EDTA, pe care s-au aplicat 5 amestecuri experimentale de monomeri şi etanol şi un adeziv comercial. In vitro, s-au efectuat testarea la microtensiune a secţiunilor longitudinale, examinarea interfeţei după fractură utilizând microscopul stereoscopic, microscopul electronic prin scanare şi microscopul electronic confocal prin scanare iar micropermeabilitatea a fost evaluată prin umplerea camerei pulpare cu o soluţie colorată de rodamină B. Cele mai hidrofobe răşini (similare răşinilor aplicate în etapa finală a sistemelor bi- şi tri-componente) au prezentat valorile cele mai mici ale adeziunii la dentina gravată acid iar probele la care aplicarea s-a făcut pe dentină tratată cu EDTA au cedat prematur la testul de rezistenţă la microtensiune. Una dintre răşinile hidrofile acide testate (similară adezivilor autogravanţi), a prezentat cele mai mari valori ale rezistenţei la microtensiune atât la dentina tratată cu EDTA cât şi la probele gravate acid, în timp ce cealaltă a prezentat o infiltrare redusă atunci când a fost aplicată pe dentina gravată acid. Adezivul comercial testat a prezentat valori mai mari atunci când a fost aplicat pe dentina gravată acid decât pe dentina condiţionată cu EDTA. Testul de micropermeabilitate a demonstrat valori mult mai mici pentru probele tratate cu EDTA pentru 4 din cei 6 adezivi testaţi. Este posibil ca EDTA-ul să denatureze într-o măsură mai redusă colagenul expus şi să determine o dentină mai susceptibilă la impregnare. Rezultatele au demonstrat că utilizarea EDTA-ului pentru condiţionare dentinară ar putea constitui o alternativă promiţătoare a gravajului acid în cadrul tehnicii de adeziune în mediu umed cu răşini dizolvate în etanol. Compoziţia chimică a amestecurilor de răşină reprezintă în continuare un factor determinant al capacităţii de adeziune atât la dentina tratată acid cât şi la dentina tratată cu EDTA. Studiile viitoare vor demonstra dacă acest tip de condiţionare determină o hibridizare dentinară mai rezistentă la solicitările din mediu oral, pe termen lung (Sauro S et al. Resin–dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. *Dent Mat* 2010 ; 26 (4) : 368-379).

Gianina Iovan

STUDIU COMPARATIV AL UNOR INDICATORI CLINICI ŞI DE LABORATOR LA UN LOT DE SUBIECŢI CARE FOLOSESC APA DE FÂNTÂNĂ CA SURSĂ DE APĂ POTABILĂ ŞI UN LOT MARTOR DE SUBIECŢI CARE AU SURSĂ DE APĂ POTABILĂ CONTROLATĂ SANITAR

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COMPARATIVE STUDY OF SOME CLINICAL AND LABORATORY INDICATORS IN A GROUP OF PATIENTS USING WELLS AS SOURCE OF DRINKING WATER AND A CONTROL GROUP USING SAFE WATER (Abstract): In time, well water, as a source of drinking and coking water, with physical-chemical, bacteriological, and biological indicators suggestive of alteration in water potability, determines complex, sometimes irreversible, metabolic disorders. **Material and methods**: Sixty individuals residing in a rural community were divided into 2 groups: study group -30 subjects using well water, and control group - 30 subjects using safe water. For the study group the selection criteria were: age, sex, use of well water as drinking and cooking water, history suggestive of chronic poisoning (pregnancy course, birth weight, susceptibility to infectious agents, and current chronic diseases). **Results**: In the study group, gestosis, prematurity, and altered body mass index are more frequent as compared to the subjects in the control group. The identified laboratory changes indicate moderate anemia, hepatic cytolysis, dyslipidemia, presence of nitrites in urine, and positive urine cultures. **Conclusions**: Long-term use of water with mineral constituents in excess, absent, or inadequate, the direct biological and chemical water pollution, or most frequently the indirect pollution through the soil determine, in time, complex, sometimes irreversible, metabolic disorders. **Key words**: WELL WATER GESTOSIS, ANEMIA, NITRITE POISONING

Apa este un element de mediu esenţial, indispensabil desfăşurării proceselor vitale, cu rol important în menţinerea stării de sănătate, dar şi o cauză posibilă – atunci când calităţile ei sunt afectate – de perturbare a proceselor metabolice.

Studii fiziologice, biochimice, clinice, şi epidemiologice efectuate asupra efectelor produse de poluarea factorilor de mediu, inclusiv a apei potabile, au demonstrat apariţia şi manifestarea mai pregnantă a efectelor nocive ale poluării mediului extern asupra organismelor tinere, care au o reactivitate generală scăzută împotriva acestor agresiuni, datorită stadiului de dezvoltare morfo-funcţional al organismului la vârsta copilăriei (1).

Apa de fântână, ca sursă de apă alimentară, cu indicatori fizici, chimici şi biologici ce indică afectarea potabilităţii apei, determină în timp tulburări metabolice complexe cu repercusiuni importante asupra dezvoltării psiho-somatice normale a organismului. Elementele minerale din apa potabilă (fier,

cupru, zinc, fluor, iod etc.), sunt componente structurale ale celulelor şi lichidelor interstiţiale cu rol important în cataliza enzimatică, asupra permeabilităţii membranare, a echilibrul hidro-electrolitic şi acido-bazic. Indiferent de cantităţile mici necesare zilnic organismului sunt esenţiale pentru organismul deoarece acesta nu le poate sintetiza sau înlocui. Carenţa sau excesul lor în organism, dacă persistă suficient timp, determină tulburări metabolice uneori ireversibile (2).

Îngrăşămintele chimice, biostimulatorii sau antidăunătorii cu biodegradare lentă, folosiţi în agricultură, formează în sol produşii de degradare ce persistă mult timp (pesticidele organo-clorurate, cu plumb, mercur au remanenţă în sol de peste 5 ani), iar potenţial toxic al intermediarilor de descompunere este uneori mai mare decât cel iniţial. Trecând din sol în plante, la animale sau în apa subterană ajung în organism prin consumul acestora (1).

În cazul intoxicaţiilor cu cantităţi mici în intervale lungi de timp, simptomatologia este mai puţin manifestă iar modificări umorale apar tardiv, bineînţeles cu variaţii de intensitate la diferiţii membrii ai colectivităţii expuse. Efectul nociv asupra organismului depinde de cantitatea de toxic ingerată, de structura şi proprietăţile sale fizico-chimice.

Pesticidele organoclorurate, datorită liposolubilităţii pe care o au se absorb digestiv uşor şi pot fi depozitate în ţesutul adipos, astfel încât îşi pot manifesta acţiunea nocivă atât după ingestie cât şi după mobilizarea din ţesuturi(3). Hepato- nefrotoxicitatea, caracterul mutagen şi cancerigen se datorează capacităţii pesticidelor organoclorurate de a fi inductori enzimatici prin care interferează căile metabolice normale; posibilitatea de a traversa membrana placentară şi de eliminare prin secreţia lactată afectează direct produsul de concepţie (3,4).

Nitroderivaţii, ei însăşi, nu prezintă un pericol prea mare pentru organism, dar în mediul înconjurător (apa, sol) sau la nivel intestinal, aceştia sunt transformaţi de unele bacterii în nitriţi. Nitriţii absorbiţi în sânge determină formarea methemoglobinei, cu hipoxie tisulară consecutivă şi afectare metabolică generală (5).

Surprinderea în fază incipientă a modificărilor umorale sugestive intoxicaţiei cronice consecutive consumului apei de fântână face obiectul studiului nostru.

MATERIAL ŞI METODĂ

Studiul a fost realizat pe un lot de 60 subiecţi, dintr-o colectivitate rurală, repartizaţi într-un lot de studiu de 30 subiecţi care folosesc apa de fântână ca sursă de apă potabilă şi un lot martor de 30 subiecţi care folosesc apă potabilă din sursă controlată sanitar.

Criteriile de selecţie a lotului investigat: vârstă, sex, sursa de apă potabilă, antecedente personale (evoluţia sarcinii, greutate la naştere, afecţiuni cronice sugestive pentru intoxicaţii cronice). Alţi factori investigaţi: obiceiurile alimentare, ocupaţia, sedentarismul, stresul, nivelul de trai, apartenenţa religioasă diferenţiază subiecţii investigaţi în grupe de risc.

Investigaţiile de laborator au vizat aprecierea profilului metabolic general la cele două loturi studiate: hemoleucogramă – metoda Coulter, VSH – metoda Westerngreen, glucoză plasmatică şi urinară – metoda Trinder GOD-POD, uree plasmatică şi urinară - metoda urează-GLDH, creatinină plasmatică şi urinară - metoda Jaffé, transaminaze GPT – metoda IFCC - LDH-NADH şi GOT – metoda IFCC - MDH-NADH, colesterol – metoda CHOD-POD, trigliceride - metoda GPO-POD, proteine totale – metoda biuret, fosfataza alcalină – metoda p-nitrofenil-fosfat, ASLO – metoda latex-aglutinare, sumar urină şi sediment urinar.

REZULTATE

Analiza rezultatelor obţinute s-a făcut după următoarele criterii:

1. Structura lotului investigat.

Lotul investigat cuprinde 60 de subiecţi din mediu rural aflaţi în evidenţa medicului de familie: 48,33% subiecţi de sex masculin – SM - (15% până în 20 ani, 23,33% între 21 şi 45 ani şi 10% peste 45 ani, media de vârstă este de 27 ani ± 15,75 ani cu limitele de vârstă 6 şi 47 ani) şi 51,66% subiecţi de sex feminin – SF - (16,66% până în 20 ani, 22% subiecţi între 21 şi 45 ani şi 13% peste 45 ani, media de vârstă este de 25,93 ani ± 15,42 ani, cu limitele de vârstă 6 şi 48 ani (tab. 1, fig. 1).

TABELUL I

Date anamnestice ale subiecţilor investigaţi

Chestionar/ Parametru	Lot studiu		Lot control	
	M* (%)	F* (%)	M (%)	F (%)
SARCINĂ, GREUTATE LA NAŞTERE				
Normală	-	83,87	-	96,78
Disgravidie	-	16,12	-	3,22
Prematuri	9,33	6,45	-	-
Normală	79,66	78,54	91,55	93,66
DATE MORFOMETRICE				
IMC**	26,65	23,65	9,99	3,66
ALIMENTAŢIE/SURSA DE APĂ POTABILĂ				
Puţin diversificată	29,33	26,66	24,66	21,66
Fântână	100	100	0	0
RECEPTIVITATEA CRESCUTĂ LA AGENŢI INFECŢIOŞI				
	19,66	14,33	9,33	6,33
AFECŢIUNI CRONICE				
	6,66	3,66	0	0

M – sex masculin, F – sex feminin, ** IMC – indice masă corporală

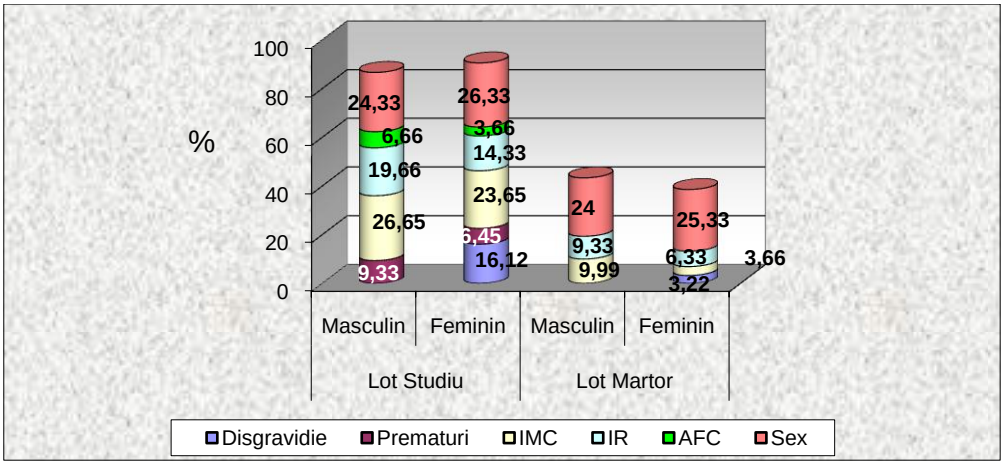

Fig. 1. Structura lotului investigat

(IMC – indice de masă corporală, IR – infecţii repetate, AFC – afecţiuni cronice).

În cadrul antecedentelor personale am constatat prezenţa disgravidiei la 16,12% subiecţi din lotul de studiu (LS) şi la 3,22% subiecţi din lotul martor (LM); prematuritatea la naştere este prezentă la 9,33% SM şi 6,45% SF din LS, iar greutatea normală la naştere au prezentat 79,66% SM şi 78,54% SF din LS şi 91,55% SM şi 93,66% SF din LM. Indicele de masă corporală (IMC) este afectat la 26,65% SM (scăzut la 16,66% până în 20 ani, 6,66% între 21 şi 44 ani şi crescut la 3,33% peste 45 ani) şi la 23,65% SF din LS (scăzut la 13,33% până în 20 ani, şi crescut la 6,66% între 21 şi 44 ani şi crescut la 3,66% peste 45 ani). IMC la subiecţii LM, este crescut la 9,99% SM (6,66% între 21 şi 44 ani şi 3, 33% peste 45 ani) şi la 3,66% SF între 21 şi 44 ani.

Infecţii repetate (amigdalite, otite, faringite, genito-urinare, stafilococii cutanate etc.) prezintă 19,66% SM şi 14,33% SF din LS comparativ cu 9,33% SM şi 6,33% SF din LM, iar afectări cronice hepatice şi renale (litiază renală, pielonefrită cronică) 6,66% SM şi 3,66% SF din LS.

2. Investigaţii de laborator.

Investigaţiile de laborator efectuate indică:

– Scăderea moderată a constantelor eritrocitare la 20,32% SM (12,33% până în 20 ani, 4,66% între 21 şi 44 ani şi 3,33% peste 45 ani) şi la 21,65% SF (10,66% până în 20 ani, 4,33% între 21 şi 44 ani şi 3,66% peste 45 ani) din LS.
– Nivelul glicemiei este crescut la 4,66% SM peste 45 ani şi la 3,66% SF (1,33% între 21 şi 44 ani şi 2,33% peste 45 ani) din LS şi la 1,33 SM din LM.
– Colesterolul seric este crescut la 10,99% SM (4,66% între 21 şi 44 ani şi 6,33% peste 45 ani) şi la 11,99% SF (4,33% între 21 şi 44 ani şi 7,66% peste 45 ani) din LS, iar din LM la 2,66% SM şi 2,33% SF peste 45 ani.
– Trigliceridele serice sunt crescute la 4,66% SM peste 45 ani şi la 6,33% SF peste 45 ani din LS şi la 2,33% SF peste 45 ani din LM.
– Testele de hepatocitoliză sunt pozitive la 8,32% SM (2,33% până în 20 ani, 1,66% între 21 şi 44 ani şi 4,33% peste 45 ani) şi la 7,65% SF (1,66% până în 20 ani, 2,33% între 21 şi 44 ani şi 3,66% peste 45 ani) din LS.
– ASLO este crescut la 16,99% SM (11,33% până în 20 ani, 5,66% între 21 şi 44 ani) şi la 15,99% SF (12,66% până în 20 ani, 3,66% între 21 şi 44 ani) din LS şi la 1,66% SM din LM (fig. 2).

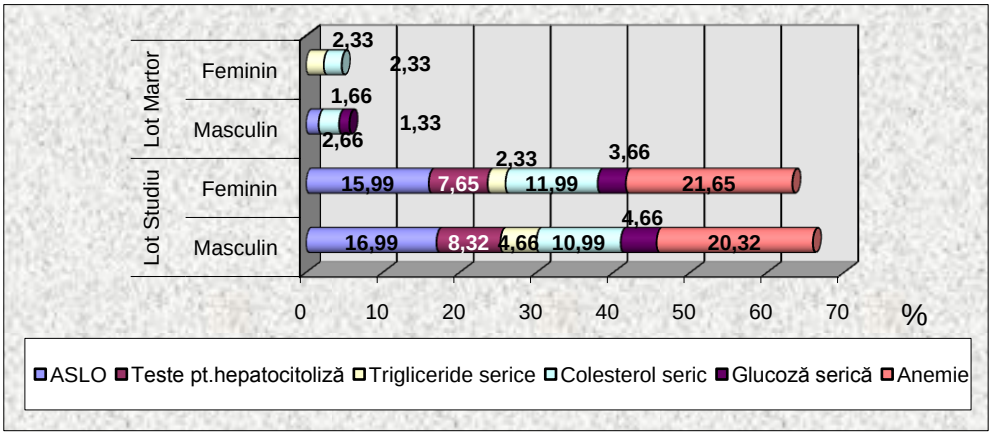

Fig. 2. Investigaţii de laborator sanguine

- Prezenţa nitriţilor în urină a fost identificată la 30,66% SM şi la 34,33% SF din LS.
- Uroculturi pozitive au prezentat 2,65% SM şi la 16,65% SF din LS.
- Cristalurie cu semnificaţie patologică a fost prezentă la 5,33% SM şi la 3,66% SF din LS (fig. 3).

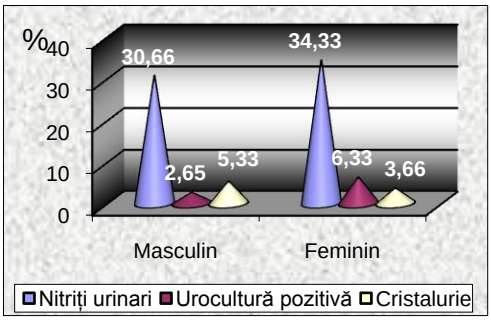

Fig. 3. Investigaţii de laborator în urină.

CONCLUZII

Apa, cu indicatorii fizici, chimici şi bacteriologici de evaluare în domeniul normal de potabilitate, atât sub aspect cantitativ cât şi sub aspect calitativ – al constituenţilor chimici conţinuţi – deţine un rol important în desfăşurarea normală a proceselor metabolice şi în consecinţă în menţinerea homeostaziei organismului.

Consumul îndelungat de apă cu constituenţii minerali prezenţi în exces, absenţi sau insuficienţi, poluarea biologică, chimică (reziduuri menajere, zootehnice, îngrăşăminte folosite în agricultură) directă a apei sau mai frecvent indirect prin intermediul solului, determină în timp dereglări metabolice complexe cu afectarea performanţelor morfo-funcţionale şi psihice ale individului. Receptivitatea la agenţii poluanţi externi este crescută la organismele tinere, în dezvoltare, iar consecinţa imediată şi ulterioară pentru organism este dezvoltarea psihosomatică deficitară, rezistenţă scăzută la factorii de mediu, consecinţe mutagene şi cancerigene.

Disgravidia, prematuritatea la naştere, afectarea IMC mai frecventă la subiecţii care au ca sursă de apă potabilă apa de fântână, comparativ cu subiecţii care folosesc apa din surse controlate sanitar, este o posibilă consecinţă a poluării apei de consum.

In cazul antecedentelor personale patologice se remarcă la subiecţii LS rezistenţă scăzută la agenţi infecţioşi datorită episoadelor infecţioase repetate pe care le prezintă şi a afectării cronice a ficatului şi a rinichilor – organe cu rol major în metabolizarea şi eliminarea noxelor din organism.

Rezultatele de laborator confirmă datele anamnestice constatate şi identifică aportul extern crescut de nitroderivaţi, cu probabilitatea ce mai mare prin apa ingerată.

Identificarea şi neutralizarea poluanţilor din sol şi apă, folosirea corectă a îngrăşămintelor agricole, îndepărtarea corespunzătoare a deşeurilor menajere, zootehnice şi nu în ultimul rând educaţia sanitară a colectivităţii sunt măsuri preventive de poluare a mediului extern, inclusiv a apei subterane şi de profilaxie pentru numeroase afecţiuni.

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CONSTRUCŢIA ŞI CARACTERIZAREA ELECTRODULUI MEMBRANĂ SELECTIV PENTRU DETERMINAREA TENOXICAMULUI

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CONSTRUCTION AND CHARACTERIZATION OF A SELECTIVE MEMBRANE ELECTRODE FOR TENOXICAM DETERMINATION (Abstract): This paper describes the construction and characterization of a selective membrane electrode which can be used for determination of tenoxicam. **Material and method**: The electroactive compound is a precipitate obtained in 2 N hydrocloric acid solution containing tenoxicam in which a solution of iodine is added. The membrane is made by mixing the electroactive compound with polyethylene using tetrahydrofurane as solvent. The solution is evaporated in order to obtain a thick membrane, which is attached at one end of a PVC tube and is fixed with the same polymeric solution. In this tube an internal Ag/AgCl reference electrode is inserted. The assembly is filled with an internal solution containing tenoxicam. The electrode was characterized (electrode slope, selectivity, optimal pH range, response time, life time). The developed method was validated. **Results**: The method showed a good liniarity in the range of 10^{-6} – 10^{-1} M (the correlation coefficient r = 0.9999). The detection limit (LD) was 7.347 x 10^{-7} M and the quantification limit (LQ) was 1.017 x 10^{-6} M. There were established the precision (RSD = 1.79%) and the accuracy (mean recovery is 100.17%) **Conclusions**: The experimental results demonstrated a good sensibility. **Key words:** TENOXICAM, MEMBRANE-SELECTIVE ELECTRODE, POTENTIOMETRY, VALIDATION

Tenoxicamul face parte din clasa antiinflamatoarelor nesteroidiene (AINS) din generaţia a II-a, fiind indicat în terapia simptomatică a afecţiunilor reumatice inflamatorii dureroase şi degenerative, în artrita reumatoidă, osteoartrită, sciatică, lombalgie, gonartroze etc.

Lucrarea de faţă prezintă construcţia şi caracterizarea unui electrod membrană selectiv, precum şi metoda de determinare potenţiometrică a tenoxicamului ce foloseşte acest electrod.

MATERIAL ŞI METODĂ

Reactivi şi aparatură:

- soluţie stoc tenoxicam 10^{-1} M în acid clorhidric 0,1 N;
- soluţii de lucru tenoxicam (10^{-2} M–10^{-8} M) obţinute prin diluţie cu apă din soluţia stoc;
- soluţie internă de referinţă – soluţie de tenoxicam 10^{-5} M în soluţie saturată de AgCl; (această soluţie se obţine prin diluţia unei soluţii de tenoxicam 10^{-1} M cu soluţie saturată de AgCl);
- soluţii tampon, diferite valori ale pH-ului (1,2-10) preparate după Clark şi Lubs (1);
- soluţii apoase de KNO_3 0,01 M, 0,1 M, 1 M, 2 M;
- soluţii apoase 10^{-3} M de KNO_3, NaCl, 2-aminopiridină;
- electrod de construcţie proprie utilizând

complexul tenoxicam-triiodură;
- electrod de Ag/AgCl;
- potenţiometru pH/mV Hanna 301.

Sinteza complexului tenoxicam – triiodură. La 100 mL soluţie de tenoxicam se adaugă 50 mL soluţie acid clorhidric 2N, apoi sub agitare continuă şi în porţiuni mici, 50 mL soluţie triiodură de potasiu; se formează un compus greu solubil în apă, de culoare brun închis. Precipitatul obţinut se separă prin creuzet filtrant G_4, se spală cu o soluţie saturată a complexului tenoxicam – triiodură, după care se usucă până la masă constantă la temperatura camerei, în exicatorul de vid.

Prepararea membranei selective. 0,02 g compus tenoxicam-triodură se dizolvă în 10 mL soluţie de polietilenă 5% şi se adaugă 0,2 mL dibutilftalat cu rol de plastifiant. Din această soluţie se introduc 1,5 mL în interiorul unui inel din inox cu diametrul interior de 20 mm, aşezat pe o placă din sticlă şi lipit în exterior cu soluţie de polietilenă în tetrahidrofuran (fig. 1). Deasupra inelului se aşează o hârtie de filtru perforată pentru a proteja membrana de diverse impurităţi în timpul evaporării solventului, iar deasupra hârtiei de filtru se aşează un alt inel cu rol de greutate.

După evaporarea solventului, în interiorul inelului rămâne o membrană polimerică ce are înglobat materialul electroactiv (2).

Construcţia electrodului membrană selectiv. Pentru corpul electrodului s-a folosit o eprubetă din polietilenă. La capătul rotund al acesteia s-a realizat o perforaţie pe axul cilindrului (în care se introduce electrodul intern de referinţă) şi o perforaţie pe partea laterală care serveşte la umplerea electrodului construit cu soluţia internă de referinţă (o soluţie de tenoxicam 10^{-5} M realizată într-o soluţie saturată de clorură de argint).

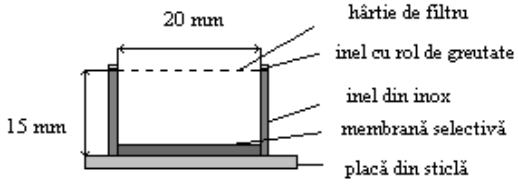

Fig. 1. Prepararea membranei selective

La celălalt capăt al eprubetei din polietilenă s-a fixat membrana preparată, aşa cum a fost descris, prin lipire cu ajutorul unui adeziv obţinut prin dizolvarea polietilenei în tetrahidrofuran, soluţie 5%.

Ca electrod intern de referinţă se utilizează un electrod de Ag/AgCl obţinut dintr-un fir de argint care se fixează într-un ansamblu din material plastic şi se conectează la un cablu ecranat. După realizarea conexiunii, firul de argint se curăţă la suprafaţă prin spălare cu o soluţie de acid azotic după care se imersează într-o soluţie diluată de acid clorhidric timp de 24 ore. Pe suprafaţa firului de argint se depune un strat subţire de AgCl (1).

Caracteristicile funcţionale ale electrozilor membrană selectivi. Celula electrochimică este formată din electrodul membrană ion – selectiv de construcţie proprie ca electrod indicator şi electrodul de Ag/AgCl cu rol de electrod de referinţă extern. Ambii electrozi sunt imersaţi în soluţia probei de analizat care conţine tenoxicam şi sunt conectaţi la un milivoltmetru electronic cu rezistenţă internă mare.

Au fost studiate:
- *funcţia electrodică:* s-au efectuat măsurători de potenţial pe o serie de soluţii de tenoxicam de concentraţie 10^{-8} – 10^{-1} M. S-a trasat curba de calibrare a electrodului reprezentând potenţialul în funcţie de valoarea pC = – log [tenoxicam]. Se determină domeniul de răspuns liniar, panta electrodului şi potenţialul standard condiţional al electrodului (3).
- *influenţa tăriei ionice*: se efectuează determinări pe seturi de soluţii de tenoxicam, folosind soluţii de KNO_3 de diferite concentraţii pentru a ajusta tăria ionică. Se compară valorile pantelor dreptelor de calibrare obţinute prin metoda regresiei liniare şi se lucrează în conti-

nuare la o tărie ionică constantă pentru care panta a avut cea mai mare valoare (3,4).

- *efectul pH-ului* : se determină prin măsurarea potenţialului la trei nivele de concentraţie 10^{-2}, 10^{-4}, 10^{-6} M tenoxicam. Modificarea pH-ului se realizează prin adăugare de soluţii tampon (1).
- *timpul de răspuns şi timpul de viaţă* : s-au înregistrat valorile potenţialelor în funcţie de timp.
- *selectivitatea electrozilor :* au fost testaţi din punctul de vedere al interferenţei ionilor K^+, NH_4^+, Cl^- şi NO_3^- deoarece aceştia sunt introduşi în soluţiile probelor de măsură în cazul ajustării pH-ului şi a tăriei ionice, dar şi al 2-aminopiridinei deoarece reprezintă principala impuritate care poate apărea în urma procesului de sinteză. Selectivitatea este apreciată prin coeficientul de selectivitate K (5).

Mod de lucru. Din soluţia stoc de tenoxicam 10^{-1}M se prepară prin diluţii succesive cu apă distilată soluţiile de lucru, de concentraţii în intervalul 10^{-2} – 10^{-6} M. Din fiecare soluţie de tenoxicam se măsoară un volum de 20 mL şi se aduc în pahare Berzelius. La soluţia de tenoxicam se adaugă un volum de 2,5 mL soluţie tampon pH = 1,2 şi 2,5 mL KNO_3 1 M. Se citesc valorile potenţialului folosind electrodul de construcţie proprie şi electrodul de referinţă de Ag/AgCl.

Validarea metodei

Determinarea liniarităţii : se prepară prin diluţii succesive cu apă distilată soluţiile de lucru de concentraţii cuprinse în intervalul 10^{-2} – 10^{-6} M. Se citesc valorile potenţialului folosind aceeaşi celulă electrochimică. Datele obţinute s-au prelucrat prin regresie matematică şi s-a trasat curba de calibrare. Metoda se consideră liniară dacă coeficientul de corelaţie este mai mare decât 0,998.

Precizia şi exactitatea : au fost analizate probe pentru următoarele concentraţii : 10^{-3} M, 10^{-4} M şi 10^{-5} M. În cazul preciziei se efectuează 2 seturi de probe pentru a evalua repetabilitatea metodei şi precizia intermediară. Pentru fiecare valoare a concentraţiei au fost efectuate trei determinări. Precizia şi exactitatea se exprimă prin deviaţia standard relativă (RSD) a valorilor obţinute care trebuie să fie mai mică de 2%.

Limita de detecţie şi cuantificare s-a calculat utilizând metoda grafică (6,7,8).

REZULTATE ŞI DISCUŢII

Funcţia electrodică. În fig. 2 este reprezentată grafic funcţia electrodică a electrodului tenoxicam selectiv.

Panta electrodului pentru domeniul liniar este P = –29,90 mV/decadă de concentraţie, iar potenţialul standard condiţional al electrodului determinat de pe curba prezentată în fig. 2 este E_0' = + 372,1 mV.

Influenţa tăriei ionice. Valorile poten-

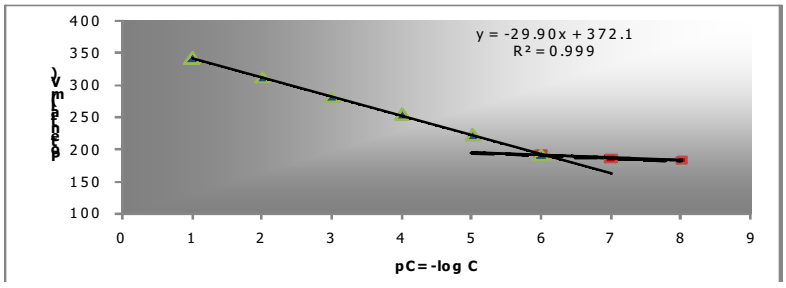

Fig. 2. Funcţia electrodică a electrodului tenoxicam – selectiv

TABELUL I

Valorile potenţialului de electrod pentru valori diferite ale tăriei ionice

pC=logC	Soluţie KNO_3 0,01M	Soluţie KNO_3 0,1M	Soluţie KNO_3 1M	Soluţie KNO_3 2M
1	312,5	321,9	341,6	340,2
2	289,4	310,3	310,6	311,2
3	275,3	291,5	279,9	280,4
4	263,1	269,8	250,4	251,6
5	246,2	252,4	221,7	222,3
6	234,1	231,1	191,4	190,1
Panta	**15,251**	**18,554**	**29,920**	**29,886**

TABELUL II

Valorile coeficienţilor de selectivitate calculate

Nr. crt.	Interferent	E(I)	E(II)	P	Coeficient de selectivitate, K
1	K^+	281,3	104,2	29,799	$1,140 \times 10^{-6}$
2	NH_4^+		108,4		$1,577 \times 10^{-6}$
3	Cl^-		115,5		$2,729 \times 10^{-6}$
5	NO^{3-}		102,8		$1,023 \times 10^{-6}$
6	2-aminopiridină		142,1		$2,132 \times 10^{-5}$

TABELUL III

Liniaritatea metodei

Tenoxicam pC = –log C	Potenţial (mV)				
	Seria I	Seria II	Seria III	Seria IV	Media
1	342,1	341,9	341,6	342,7	342,07
2	311,2	312,1	311,9	311,8	311,75
3	280,7	281,5	280,3	281,5	281,00
4	252,3	251,9	250,1	250,7	251,25
5	221,7	222,1	221,7	222,6	222,02
6	191,5	192,1	191,9	192,1	191,90
7	185,2	184,1	186,3	185,4	185,25
8	184,1	183,5	183,6	183,4	183,65
Coeficienţii de corelaţie şi regresie **r = 0,9999, r^2 = 0,9999** Eroare standard = **0,493** Intercept = **371,6** Panta = **-29,994**					

ţialului de electrod înregistrate la diferite valori ale tăriei ionice sunt prezentate în tab. I. Tăria ionică (constantă) pentru care panta a avut cea mai mare valoare se realizează prin adăugare de soluţie KNO_3 1 M.

Efectul pH-ului. Domeniul de pH optim este cuprins între 1,2 şi 2,0. Măsurătorile de potenţial pentru curba de calibrare şi determinarea potenţiometrică a tenoxicamului au fost efectuate după ajustarea pH-ului la valoarea 1,2 prin adăugare de soluţie tampon pH = 1,2.

Timpul de răspuns şi durata de întrebuinţare a electrodului. Timpul de răspuns este de aproximativ 15 secunde. Stabilitatea valorilor potenţialului de electrod este bună pentru o perioadă de aproximativ 2 luni, după care valoarea pantei electrodului începe să se modifice fapt ce indică degradarea membranei selective.

Selectivitatea electrodului. Valorile coeficienţilor de selectivitate sunt redate în tab. II.

Valorile coeficientului de selectivitate, K, sunt foarte mici deci, prezenţa ionilor

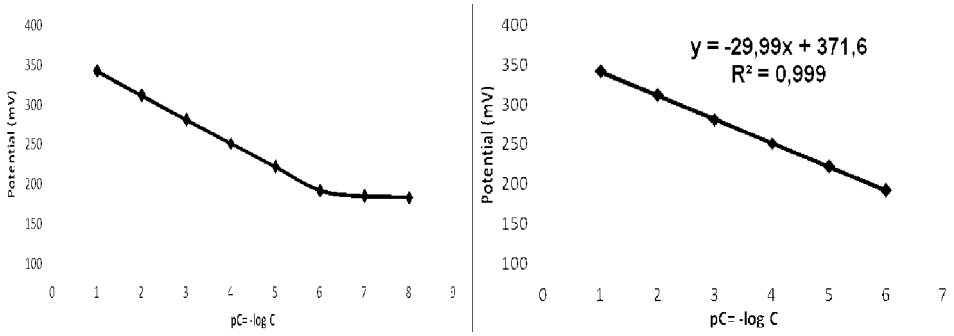

Fig. 3. Determinarea liniarității metodei

TABELUL IV

Precizia şi exactitatea metodei

Conc. (mol/L)	Repetabilitatea metodei		Precizia intermediară		Exactitatea metodei	
	E (mV)	Regăsire %	E (mV)	Regăsire %	E (mV)	Regăsire %
10^{-3}	281,9	101,83	281,6	99,51	281,8	101,05
	282,1	103,41	281,7	100,28	281,4	98,00
	281,5	98,75	281,9	101,83	281,9	101,83
10^{-4}	251,6	99,47	251,9	101,79	251,7	100,23
	251,7	100,23	252,1	103,36	252,1	103,36
	252,1	103,36	251,4	97,95	251,3	97,20
10^{-5}	221,9	101,74	221,6	99,42	221,5	98,66
	221,5	98,66	221,9	101.74	221,7	100,19
	221,6	99,42	222,0	102.52	221,8	100,96
Date statistice	**Medie** = 100,76% **RSD**=1,85%		**Medie** = 100,73% **RSD**=1,73%		**Medie** = 100,17% **RSD**=1,93%	

testaţi în probe nu influenţează calitatea determinărilor.

Validarea metodei

Determinarea liniarităţii. S-a studiat liniaritatea pentru domeniul de concentraţie 10^{-8} - 10^{-1} M. Datele obţinute sunt evaluate statistic (tab. III), iar prelucrarea acestora conduce la obţinerea dreptei de calibrare (fig. 3).

Domeniul de concentraţii în care electrodul prezintă un răspuns liniar este cuprins între 10^{-6} M şi 10^{-1} M. Dreapta de calibrare calculată prin regresie matematică este :

$$E = -29{,}994 \times pC + 371{,}65;$$

$$r^2 = 0{,}9999$$

Limita de detecţie şi limita de cuantificare

Limita de detecţie are valoarea pC = 6,134 $(LD = 7{,}347 \times 10^{-7} M)$.

Limita de cuantificare are valoarea pC = 5,993 $(LC = 1{,}017 \times 10^{-6} M)$.

Precizia metodei. Se constată că atât pentru fiecare set de date separat, cât şi pentru ambele seturi de date luate împreună deviaţia standard relativă are o valoare mai mică de 2% (RSD = 1,79%), deci metoda de analiză este precisă (tab. IV).

Exactitatea. Din valorile experimentale (tab. IV) se constată că, pe domeniul de concentraţie studiat, regăsirea medie este de 100,17% (minim 97,20 % şi maxim 103,36 %), iar deviatia relativă standard (RSD= 1,93) este mai mică de 2% deci metoda de analiză propusă este exactă.

CONCLUZII

A fost construit şi caracterizat un electrod

membrană selectiv ce utilizează ca material electroactiv complexul tenoxicam-triiodură.

Electrodul membrană a fost caracterizat stabilindu-se funcţia electrodică, efectul pH-ului, influenţa tăriei ionice, timpul de răspuns şi timpul de întrebuinţare, precum şi selectivitatea electrodului.

Electrodul membrană selectiv a fost utilizat pentru determinarea potenţiometrică directă a tenoxicamului din soluţii apoase.

Metoda de analiză a fost validată, stabilindu-se: liniaritatea metodei (pe domeniul 10^{-1} M - 10^{-6} M), coeficientul de corelaţie (r = 0,9999), limita de detecţie (7,34 x 10^{-7} M), limita de cuantificare (1,01 x 10^{-6} M), precizia metodei (RSD = 1,79%) şi exactitatea metodei (regăsire medie = 100,17%).

În concluzie, metoda de analiză propusă este simplă, uşor de executat şi sensibilă. De asemenea, metoda este liniară, precisă şi exactă.

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NOUTĂŢI

FUMATUL CRONIC ALTEREAZĂ COMPOZIŢIA ŞI PROPRIETĂŢILE LIPIDELOR MEMBRANARE ERITROCITARE

Fumatul este un obicei major în viaţa cotidiană a multor oameni. Studiind modificările induse de fumat în compoziţia lipidică a membranei eritrocitare, fluiditatea membranară şi rolul oxidului nitric (NO), pe un lot de 30 de fumători (12 +/- 2 tigarete /zi, vârsta 35 +/- 8 ani), comparativ cu un lot de 30 de nefumători, s-au observat, în cazul fumătorilor, următoarele modificări: nivel plasmatic crescut de nitriţi/nitraţi, nivel ridicat al hemolizei, o creştere a gradului de peroxidare a lipidelor şi a raportului colesterol/proteine (C/P), precum şi o scădere a activităţii Na^+/K^+-ATPazei şi a grupărilor sulfhidril. Nivelul de NO, în cazul celulelor lizate, a fost corelat pozitiv cu raportul C/P (r = 0,56) şi cu valoarea anizotropiei de fluorescenţă. În concluzie, fumatul induce generarea de specii reactive oxigen/nitrogen care pot altera proprietăţile fizico-chimice ale membranei eritrocitare, cu implicaţii majore fiziopatologice (Padmavathi P, Reddy VD, Kavitha G, Paramahamsa M, Varadacharyulu N. Chronic cigarette smoking alters erythrocyte membrane lipid composition and properties in male human volunteers. *Nitric Oxide* 2010; 23 (3): 181-186).

M. Guţu

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AN ALTERNATIVE METHOD FOR THE REMOVAL OF THE OCULAR ARTIFACT FROM THE EEG SIGNAL

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AN ALTERNATIVE METHOD FOR THE REMOVAL OF THE OCULAR ARTIFACT FROM THE EEG SIGNAL (Abstract): The aim of this paper is to develop a technique of rejection or minimization of ocular artifacts from the electroencephalographic (EEG) recordings. **Material and method**: The method presented is based on mathematical morphology. The algorithm and the individual structuring element are presented along with the particularities concerning the parameters that characterize the structuring element. **Results**: The obtained results are shown in a comprehensive form by means of an illustrating example that evidences the efficiency of the method. **Conclusions**: The proposed method simplyfies the task of removing the ocular artifacts by means of nonlinear filtering and introduces a new structural element for the job. The results are validated by means of Fourier analysis and this clearly shows its effectiveness. **Key words**: BRAIN-COMPUTER-INTERFACE, EEG ARTIFACT, MATHEMATICAL MORPHOLOGY, STRUCTURING ELEMENT

When dealing with brain-computer interfaces (BCI), the electro-encephalographic (EEG) signal is the one that facilitates the communication between the human being and the machine. Some features of the EEG signal that were previously chosen to represent the information carrier are usually processed in order to detect the subject's response to a stimulus which was typically computer generated. To do so, only the real EEG signal must be considered; it is a well known fact that eye movement, heartbeats and muscle artifacts are unwanted signals that interfere with the actual EEG signal. Due to the various artifacts that are present in BCI systems, not all of them can be rejected. Most authors do not show if they took into account the artifacts in their analysis (1). The goal of an artifact-removal method is to put into practice the possibility of removing the artifacts, while keeping the EEG signal intact. The main methods for the reduction of artifacts from the EEG signals are: linear filtering (2), regression (3), principal component analysis (PCA) (4), independent component analysis (ICA) (5), nonlinear adaptive filtering (6) and wavelet transform (7). Any new method may be a good candidate to replace the existing ones if it is easy to implement, the artifact rejection is satisfactory and, at least, as effective as the actual methods.

We intend to show the way in which mathematical morphology may be an answer to the EEG artifact removal. The shape description based on mathematical morphology was used, from its introduction by Matheron and Serrat (8,9,10), in image analysis or in biological research like the one in (11). Works of Maragos and Schafer (12,13)

allowed additional fields of interest like nonlinear filtering of signals. Our application is based on the last application and, as far as we know, wasn't applied for artifact removal from the EEG signal until now.

MATERIALS AND METHOD

We have used the EEG datasets from the SIESTA project (14). Several types of artifacts such as ocular, cardiac and muscle artifacts were recorded along with the sleep EEG. Seven EEG channels of 90 minutes each, from 15 subjects were available. For our purpose, we have chosen an EEG segment contaminated with ocular artifacts. Besides the EEG recordings there were also recorded vertical (VEOG) and horizontal EOG (HEOG) signals. The sampling rate of the signal was 256 Hz.

By means of a simple visual inspection, it was found that the effect of ocular artifacts (slow eye movements) is dominant in the frontal channels FP1 and FP2 recordings (slow wave EEG artifacts).

Mathematical morphology deals in our case with two time functions: the first one is the EEG recording itself and the second is the structuring element. Based on the shape of the structuring element and of two basic operators which will be defined below, it is possible to "extract" the artifacts. Usually, the structuring element is a circle when dealing with images. For signal processing purposes, more complicated shapes are needed, because there are several parameters that must be "adapted" to the signal to perform a high-quality artifact removal. The circle has just one parameter, the radius, but nevertheless, this may be cured transforming it into an ellipse which has two parameters, the lengths of its axes; unfortunately this doesn't guarantee the success of the operation.

Our work showed that for the structuring element the best suited function is a second degree one, given by the following formula:

$$se(t) = -\alpha t^2 + \beta \qquad \textbf{(1)}$$

Parameters á and â are in fact the values of two statistical indicators, the standard deviation and the mean value:

$$\alpha = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(v_i - \beta)^2}, \quad \beta = \frac{1}{N}\sum_{i=1}^{N} v_i \qquad \textbf{(2)}$$

where v_i are the samples of the EEG signal.

The two basic operators used by the mathematical morphology are *erosion* and *dilation*. For a comprehensive cover of this topic, reference (15) may be a good choice. Shortly, *erosion* performs a search for a minimum between the values of the function and those of the structuring element in a given interval, while *dilation* does the same for the maximum. This is done for each argument of the function representing the signal.

Based on these two operators, one may define mixtures between them, bearing in mind the fact that the order in which they are applied is essential. In this way, other two functions are defined, *closing* and *opening*, based on the initial signal, *v* and the structuring element, *se*:

$$closing(v, se) = erosion(dilation(v, se)) \qquad \textbf{(3)}$$

$$opening(v, se) = dilation(erosion(v, se)) \qquad \textbf{(4)}$$

There is also the possibility of applying *closing* and *opening* operators one after the other to get the desired result. In our study, to remove the ocular artifacts we used the mean value obtained from the *close-opening* and *open-closing* functions:

$$close_opening(v, se) = \\ = closing(opening(v, se)) \qquad \textbf{(5)}$$

$$open_closing(v, se) = \\ = opening(closing(v, se)) \qquad \textbf{(6)}$$

We used a small structure element, about 0.1% from the whole length of the EEG recording whose function is given by formula (1); it proved to be the best suited for the job.

RESULTS

As acknowledged above, the FP1 and FP2 EEG recordings were contaminated with ocular artifacts. The recording of the FP1 channel presents an artifact between samples 900 and 1100 (a slow eye movement resulting in slow-wave EEG artifact). Our purpose is to eliminate it using nonlinear filtering tools based on mathematical morphology.

First we applied the two basic operators, *erosion* and *dilation*; the result is presented in fig. 1 and fig. 2.

Next, *closing* and *opening* operators were applied and finally, the *close-opening* and *open-closing* operations were performed (figs. 3 and 4).

Finally, the artifact-free signal is presented in fig. 5. It was obtained by substracting the mean value of signals given by formulas (5) and (6) from the initial EEG recording.

In order to be able to compare the signals represented in fig. 5, we have represented their amplitude spectra (fig. 6 and fig. 7).

From the two figures above, it is worth noticing that the low frequency components (up to 7 Hz) are substantially reduced, while the high frequency components are preserved. So, this is a certain proof that the

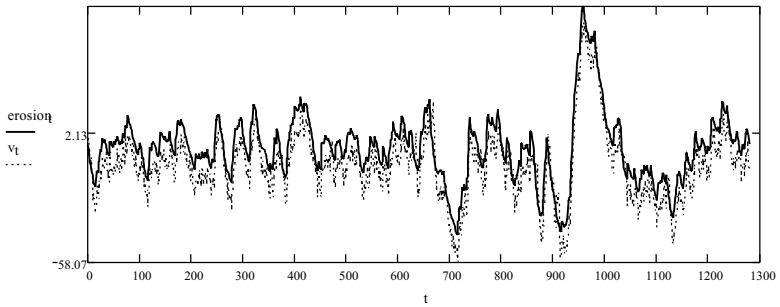

Fig. 1. Erosion (continuous line) vs. initial EEG signal (dotted line)

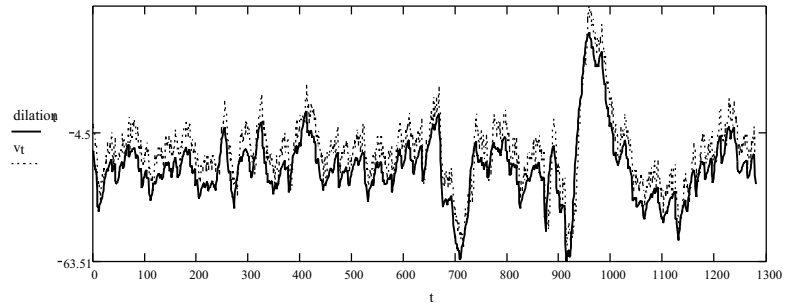

Fig. 2. Dilation (continuous line) vs. initial EEG signal (dotted line)

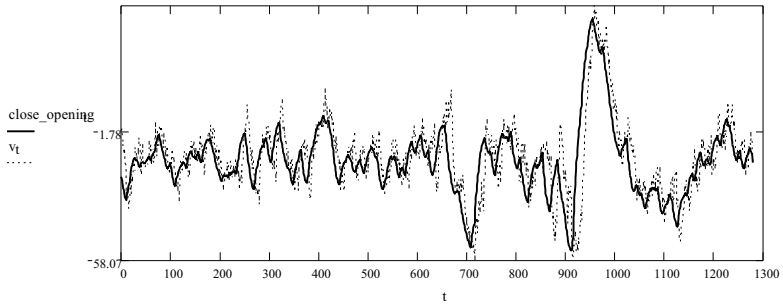

Fig. 3. Close-opening (continuous line) vs. initial EEG signal (dotted line)

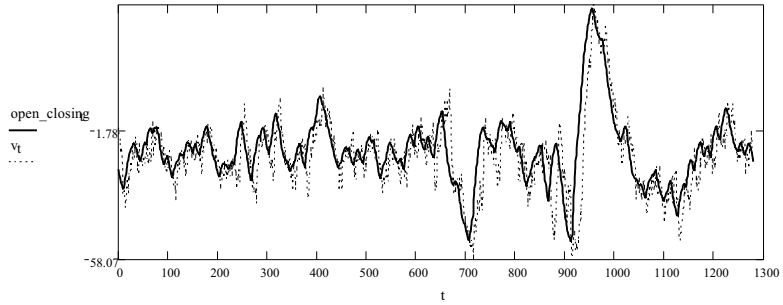

Fig. 4. Open-closing (continuous line) vs. initial EEG signal (dotted line)

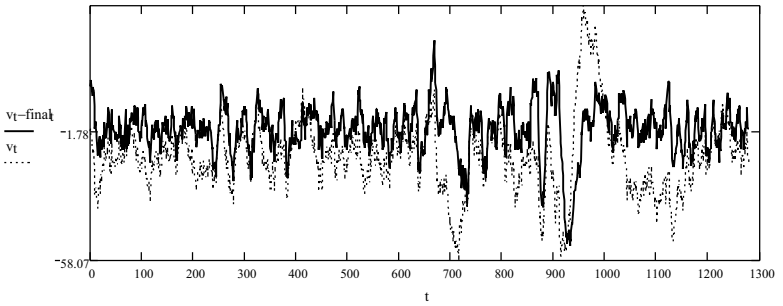

Fig. 5. Artifact-free signal (continous line) vs. initial EEG signal (dotted line)

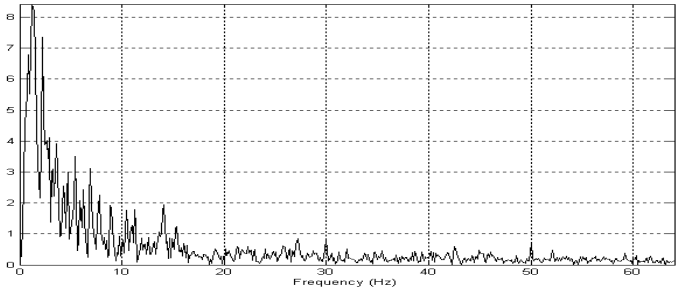

Fig. 6. Single-sided amplitude spectrum of the initial EEG signal

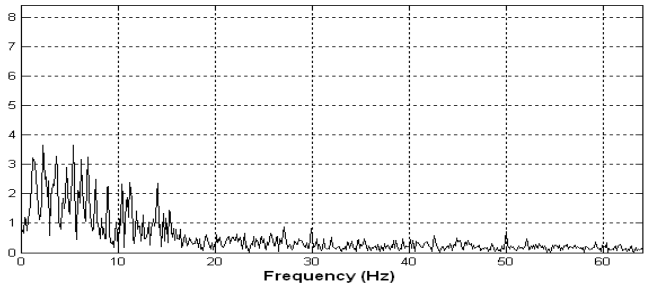

Fig. 7. Single-sided amplitude spectrum of artifact-free EEG

applied algorithm removed only the ocular artifacts (which are low frequency) without modifying the EEG signal. Similar results were obtained for the FP2 channel.

DISCUSSION

The use of mathematical morphology for artifact removal from the EEG signal may

be an efficient method involved in brain--computer interfaces. The method was priorly used for epileptic spikes detection, (16), in a somewhat different manner : the link between the signal and the parameters of the structuring element was optimised by means of dedicated software and the morphological operators involved different structure elements for *closing* and *opening*.

CONCLUSION

The method proposed in this paper is suitable for eye movement artifact removal from the EEG signal. Its simplicity lies in using the same structuring element for both *opening* and *closing* and in determining the parameters of the structuring element by means of the well known statistical indicators : the mean value and the standard deviation of the EEG signal.

ACKNOWLEDGEMENTS

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SEPARAREA SELECTIVĂ A VITAMINEI C DIN AMESTEC CU ACIDUL 2-CETOGLUCONIC PRIN PERTRACŢIE FACILITATĂ

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SELECTIVE SEPARATION OF VITAMIN C AND 2-KETOGLUCONIC ACID BY FACILITATED PERTRACTION (Abstract) : The removal of vitamin C from a mixture with 2-ketogluconic acid by using a bulk liquid membrane (BLM) has been investigated. The studies on facilitated pertraction of vitamin C with Amberlite LA-2 indicated the major parameters that affect the separation efficiency : pH gradient between the two aqueous phases, carrier concentration in the liquid membrane and phase mixing intensity. The overall results obtained in this work showed that liquid membrane system can effectively be used to selectively separate vitamin C from its mixture with the fermentation by-product, 2-ketogluconic acid. **Key words :** VITAMIN C, 2-KETOGLUCONIC ACID, AMBERLITE LA-2, CARRIER, PERTRACTION

Vitamina C (acid ascorbic) este un nutrient esenţial vieţii şi poate fi sintetizată de către majoritatea speciilor animale şi vegetale, excepţie făcând primatele şi cobaiul. Este o vitamină hidrosolubilă implicată în metabolismul glucozei, al colagenului, al acidului folic şi al anumitor acizi aminaţi, în neutralizarea radicalilor liberi şi a nitrozaminelor, în reacţii imunologice care facilitează absorbţia fierului de către tubul digestiv, constituind un cofactor pentru o serie de enzime. În organismul uman, vitamina C deţine un rol deosebit de important, datorită caracterului său antioxidant, proprietate care justifică şi numeroasele utilizări în medicină şi alimentaţie (1,2). În cosmetică, vitamina C constituie un ingredient valoros al multor loţiuni şi creme, datorită acţiunii sale antioxidante şi protectoare a pielii împotriva radiaţiilor UV (3,4).

Acidul 2-cetogluconic este principalul produs secundar rezultat la obţinerea vitaminei C prin biosinteză, dar poate fi obţinut şi individual prin fermentaţie pornind de la sorboză, având utilizări în industria de sinteză (obţinerea xilitolului, fie prin decarboxilare, fie prin reducere chimică sau enzimatică) (3).

Vitamina C poate fi obţinută la nivel industrial prin două procedee, şi anume biosinteza cuplată cu transformarea chimică (procedeul Reichstein) şi fermentaţia în două stadii cu ajutorul unor microorganisme modificate genetic (1,2,5,6). Indiferent de procedeul utilizat, un intermediar inevitabil în sinteza sau biosinteza vitaminei C îl reprezintă acidul 2-cetogluconic. Diferenţele structurale între aceşti doi compuşi sunt datorate doar structurii lactonice (fig. 1), astfel încât caracteristicile fizico-chimice ale acizilor sunt destul de asemănătoare.

Ambii compuşi au caracteristici similare în ceea ce priveşte solubilitatea : sunt puternic solubili în solvenţi polari, în special apă, şi slab solubili în solvenţi organici

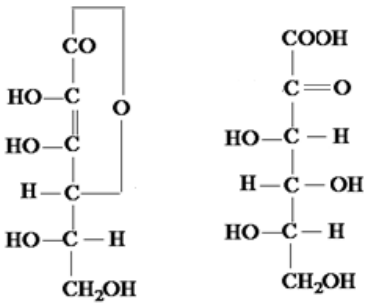

Fig. 1. Structurile chimice ale vitaminei C şi acidului 2-cetogluconic

(2). În consecinţă, separarea prin extracţie lichid-lichid din amestecul rezultat la fermentaţie este dificilă din punct de vedere economic. Bazându-se pe rezultatele anterioare privind mecanismul separării şi influenţa factorilor determinanţi în extracţia reactivă a vitaminei C şi a acidului 2-cetogluconic, studiile experimentale prezentate în continuare urmăresc stabilirea condiţiilor optime pentru separarea selectivă a celor doi componenţi prin pertracţie facilitată cu Amberlite LA-2 (9,10).

MATERIAL ŞI METODĂ

Instalaţia de pertracţie originală utilizată în vederea realizării experimentelor a fost prezentată în detaliu în lucrările anterioare (7,8). Aceasta utilizează o membrană lichidă liberă (cele două faze apoase miscibile sunt separate de stratul de solvent organic nemiscibil cu acestea care, însă, permite transportul masic al celor doi componenţi). Experimentele au fost realizate în sistem pseudostaţionar, soluţiile apoase fiind alimentate separat cu un debit volumic de 1,9 L/h.

Solventul organic care a constituit membrana lichidă a fost diclormetanul în care a fost dizolvat agentul purtător Amberlite LA-2 în concentraţii cuprinse între 0 şi 100 g/L). Faza apoasă iniţială a constat dintr-un amestec de 1 g/L acid 2-cetogluconic şi 7 g/L vitamina C, iar faza apoasă finală a constat din soluţii de hidroxid de sodiu, cu diferite valori ale pH-ului. Modificarea pH-ului celor două faze apoase la valorile prestabilite de programul experimental (intervalul 1 - 6 pentru soluţia iniţială şi 7 - 12 pentru cea finală) s-a efectuat cu soluţii 5% H_2SO_4, respectiv 5% NaOH, utilizând un pH-metru digitalic de tip HI 213 (Hanna Instruments).

Pentru a urmări desfăşurarea procesului s-au urmărit fluxurile masice iniţiale, n_i, şi finale, n_f, ale acizilor , factorul de permeabilitate prin membrana lichidă, P şi factorul de selectivitate (S) (7,8). Fluxurile masice s-au calculat prin dozarea concentraţiei ambilor compuşi din soluţia iniţială şi din rafinat şi efectuarea bilanţului de masă în sistemul de pertracţie. Factorul de permeabilitate prin membrana lichidă, P, este definit ca raportul între fluxul masic final şi iniţial al celor doi compuşi, iar factorul de selectivitate ca raportul între factorii de permeabilitate. Dozarea s-a realizat prin metoda HPLC, utilizând o coloană de tip Hamilton PRP-X300 (diametru 4,1 mm, lungime 150 mm), la temperatura de 25°C. Eluentul folosit a fost o soluţie apoasă 4 mM de acid sulfuric, cu un debit de 0,5 mL/min. Detecţia s-a realizat în domeniul UV la 210 nm.

REZULTATE ŞI DISCUŢII

În scopul optimizării condiţiilor de operare pentru separarea selectivă a vitaminei C, s-au analizat efectul gradientului de pH dintre fazele apoase, al concentraţiei agentului purtător dizolvat în membrana lichidă şi intensitatea amestecării fazelor, principalii parametri care controlează procesul.

În studiul influenţei pH-ului fazelor apoase, trebuie ţinut cont de faptul că atât procesul de extracţie al celor doi compuşi, cât şi cel de reextracţie se bazează pe dependenţa coeficientului de distribuţie de pH-ul soluţiilor iniţială şi finală. Separarea vitaminei C şi viteza transferului de masă al ambilor compuşi din amestec sunt controlate de gradientul de pH dintre aceste

soluţii. Astfel, s-au observat diferenţe majore între variaţia fluxurilor masice în funcţie de pH între cei doi compuşi (fig. 2). Odată cu creşterea pH-ului soluţiei din care se realizează extracţia, pH_i, are loc scăderea continuă a fluxurilor pentru vitamina C, în timp ce pentru acidul 2-cetogluconic graficul înregistrează un maxim corespunzător valorii de pH = 3.

În cazul vitaminei C, reducerea pH-ului soluţiei apoase iniţiale determină intensificarea fluxului masic dinspre soluţia apoasă iniţială spre membrana lichidă, datorită mecanismului de realizare a extracţiei reactive. Vitamina C reacţionează cu Amberlite LA-2, dizolvat în membrana lichidă, la interfaţa de separare dintre faza apoasă iniţială şi membrană formând complexul solut-agent purtător puternic hidrofob. Creşterea pH-ul fazei apoase iniţiale controlează transformarea vitaminei C în acid dehidroascorbic, incapabil să reacţioneze cu Amberlite LA-2 (determinând scăderea fluxului masic iniţial) (1). Datorită capacităţii acidului 2-cetogluconic de a forma dimeri la pH puternic acid, acesta este extras cu eficienţă ridicată doar la un pH al fazei apoase mai mare decât 2 (10). În acelaşi timp, depaşirea valorii de pH corespunzătoare pK_a-ului acidului (pK_a = 2,66 la 25°C (2)) determină disocierea grupării –COOH, împiedicând reacţia cu agentul purtător la interfaţa dintre faza apoasă iniţială şi membrana lichidă (9). Fluxurile masice superioare ale acidului 2-cetogluconic în domeniul de pH = 3-5 se datorează acidităţii superioare a acestuia comparativ cu vitamina C, caracterul acid fiind cel care controlează viteza reacţiilor interfaciale dintre solut şi agentul purtător. Din acelaşi motiv, reducerea fluxului masic iniţial este mai accentuată pentru acest acid odată cu apropierea de domeniul neutru de pH (fig. 2).

Scăderea pH-ului fazei apoase iniţiale induce creşterea concentraţiei vitaminei C în membrana lichidă, determinând creşterea

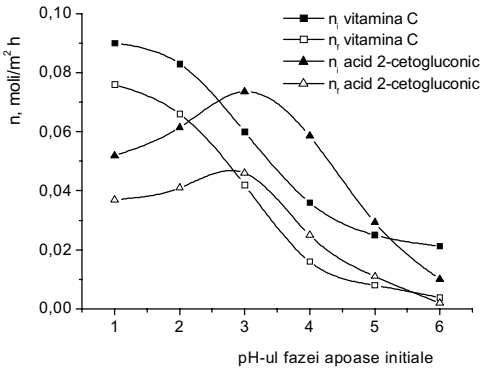

Fig. 2. Influenţa pH-ului fazei apoase iniţiale asupra fluxurilor masice ale vitaminei C şi acidului 2-cetogluconic (pH_f = 11, concentraţia Amberlite LA-2 = 40 g/L, turaţia = 500 rpm)

fluxului masic final, deoarece fluxurilor masice finale ale celor doi compuşi sunt direct dependente de concentraţia acestora în membrana lichidă. În cazul acidului 2-cetogluconic, fluxul masic dinspre membrana lichidă înspre faza apoasă finală are o alură similară celui iniţial. Acesta creşte până la o valoare a pH_i-ului de 3, apoi se reduce, datorită unei eficienţe scăzute a extracţiei reactive, care induce o concentraţie mai mică a acidului şi în membrana lichidă.

Influenţa pH-ului fazei apoase finale asupra fluxurilor masice iniţiale şi finale ale celor doi compuşi este reprezentată în figura 3. Se observă intensificarea acestora atât pentru vitamina C, cât şi pentru acidul 2-cetogluconic, datorită creşterii vitezei de transformare în sarea de sodiu, şi, respectiv, a vitezei de reextracţie determinată de mărirea concentraţiei agentului de reextracţie (hidroxid de sodiu) şi, implicit, a gradientului de pH dintre cele două faze apoase (fig. 3).

Factorii de permeabilitate pentru ambii componenţi ai amestecului scad cu creşterea pH-ului fazei apoase iniţiale, după cum se poate observa din figura 4. Limitările induse de difuzia dinspre membrana lichidă către faza apoasă finală, mai accentuate în cazul vitaminei C datorită structurii ciclice

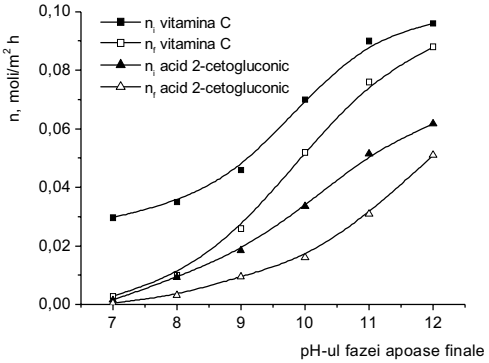

Fig. 3. Influenţa pH-ului fazei apoase finale asupra fluxurilor masice ale vitaminei C şi acidului 2-cetogluconic (pH_i = 1, concentraţia Amberlite LA-2 = 40 g/L, turaţia = 500 rpm)

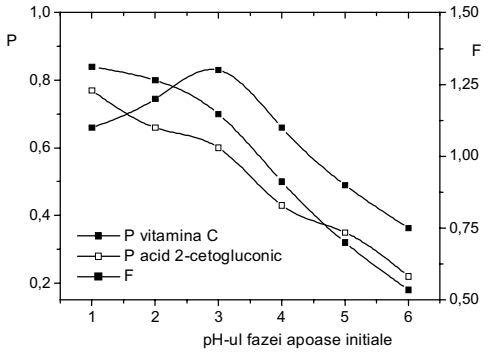

Fig. 4. Influenţa pH-ului fazei apoase iniţiale asupra factorilor de permeabilitate a vitaminei C şi acidului 2-cetogluconic şi a factorului de selectivitate (pH_f = 11, concentraţia Amberlite LA-2 = 40 g/L, turaţia = 500 rpm)

mai voluminoase, cumulat cu faptul că fluxul masic iniţial al acidului 2-cetogluconic este mult mai mic pentru $pH_i > 5$, explică valorile superioare ale factorului de permeabilitate al acidului comparativ cu cele corespunzătoare vitaminei C pentru pH > 4 al fazei apoase iniţiale.

Factorul de selectivitate, F, calculat ca raportul între factorii de permeabilitate a vitaminei C şi acidului 2-cetogluconic, înregistrează o valoare maximă la pH = 3 pentru faza apoasă iniţială, care poate fi justificată prin diferenţa mare între concentraţiile din membrana lichidă a celor doi compuşi, la această valoare a pH-ului. Pentru acidul 2-cetogluconic, viteza de extracţie este superioară celei înregistrate pentru vitamina C, astfel încât cantitatea de acid extrasă în stratul de solvent este mult mai ridicată decât cea reextrasă, fluxul masic final neputându-l compensa semnificativ pe cel iniţial (fig. 4).

Creşterea valorii pH-ului fazei apoase finale induce mărirea factorilor de permeabilitate, ceea ce evidenţiază faptul că pe măsura apropierii de domeniul puternic acid, viteza de reextracţie a celor doi compuşi devine mai importantă decât viteza extracţiei reactive. Creşterea este mai accentuată pentru vitamina C, ca rezultat al vitezei superioare de extracţie la pH_i = 1 comparativ cu acidul 2-cetogluconic. Odată cu creşterea pH-ului acestei faze apoase, viteza de reextracţie creşte semnificativ, pentru pH_f = 12 fluxurile masice iniţiale fiind echilibrate de cele finale (P = 0,96), aproape întreaga cantitate de vitamină fiind reextrasă din membrană.

Factorul de selectivitate creşte cu creşterea pH-ului fazei apoase finale, observându-se atingerea unor valori maxime ale factorului de selectivitate pentru diferenţe minime de pH dintre soluţiile apoase (fig. 5).

Un alt parametru care influenţează eficienţa transportului prin membrane lichide îl reprezintă intensitatea amestecării celor două faze apoase. Mărirea turaţiei agitatoarelor care realizează amestecarea acestora s-a realizat până la 600 rpm, rotaţie care a permis menţinerea plană a interfeţei de contact dintre cele trei soluţii. Şi în cazul pertracţiei, creşterea continuă a fluxurilor masice cu turaţia corespunde unui proces limitat de difuzia componenţilor sistemului, în timp ce atingerea unui nivel constant de variaţie al fluxurilor indică trecerea în domeniul cinetic de desfăşurare a separării.

Din figura 6 se poate observa că intensificarea amestecării induce creşterea fluxurilor masice iniţiale şi finale atât pentru

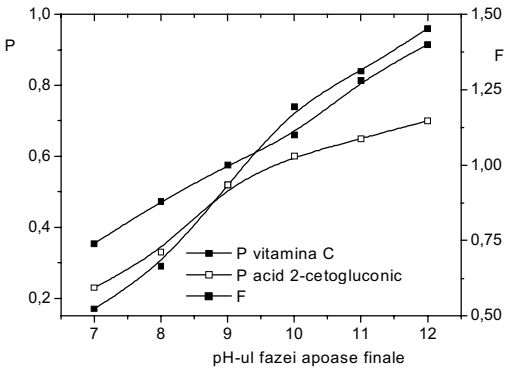

Fig. 5. Influenţa pH-ului fazei apoase finale asupra factorilor de permeabilitate a vitaminei C şi acidului 2-cetogluconic şi a factorului de selectivitate (pH_i = 1, concentraţia Amberlite LA-2 = 40 g/L, turaţia = 500 rpm)

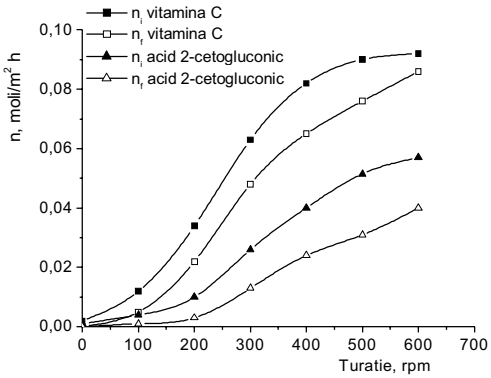

Fig. 6. Influenţa intensităţii amestecării fazelor apoase asupra fluxurilor masice ale vitaminei C şi acidului 2-cetogluconic (pH_i = 1, pH_f = 11, concentraţia Amberlite LA-2 = 40 g/L)

acidul 2-cetogluconic, cât şi pentru vitamina C, ca efect al reducerii rezistenţei datorate difuziei prin filmele de lichid din vecinătatea celor două interfeţe de separare. Fluxurile masice sunt mai mici pentru acidul 2-cetogluconic, evidenţiind un efect mai important al amestecării în cazul vitaminei C, datorită rezistenţei mai mari la difuzia prin straturile limită (fig. 6).

Fluxul masic final al acidului este mult mai mic comparativ cu cel iniţial, datorită stabilităţii superioare a complexului (acid 2-cetogluconic - Amberlite LA-2) în reacţia de reextracţie, respectiv formarea 2-cetogluconatului de sodiu.

Creşterea factorilor de permeabilitate cu turaţia, reprezentată în figura 7, sugerează faptul că difuzia ascorbatului de sodiu, respectiv a 2-cetogluconatului de sodiu, în faza apoasă finală creează o rezistenţă superioară celei opuse de difuzia din faza apoasă iniţială, datorită structurii chimice mai voluminoase a sărurilor. Factorul de selectivitate scade cu creşterea turaţiei, înregistrând un maxim atunci când fazele apoase sunt în repaus, caz în care, datorită diferenţelor între gradele de extracţie reactivă, fluxurile masice ale vitaminei C sunt mai mari decât cele ale acidului 2-cetogluconic (fig. 7).

Concentraţia agentului purtător, Amberlite LA-2, manifestă, de asemenea, o influenţă semnificativă asupra pertracţiei selective a vitaminei C, influenţând atât capacitatea de transport a membranei (difuzia complexului prin membrană), cât şi cinetica reacţiilor interfaciale. Conform rezultatelor experimentale reprezentate grafic în figura 8, se constată intensificarea fluxurilor masice iniţiale şi finale ale soluţiilor odată cu mărirea concentraţiei agentului purtător în membrana lichidă (fig. 8).

Această evoluţie a fluxurilor masice este

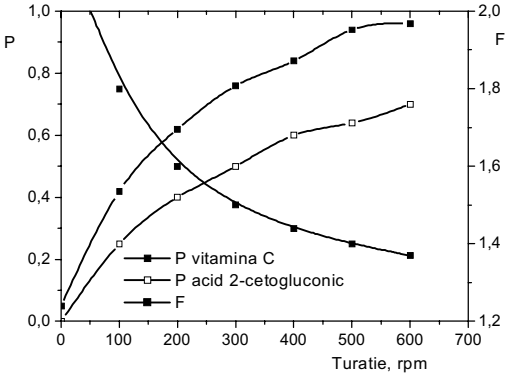

Fig. 7. Influenţa intensităţii amestecării fazelor apoase asupra factorilor de permeabilitate ale vitaminei C şi acidului 2-cetogluconic şi a factorului de selectivitate (pH_i = 1, pH_f = 11, concentraţia Amberlite LA-2 = 40 g/L)

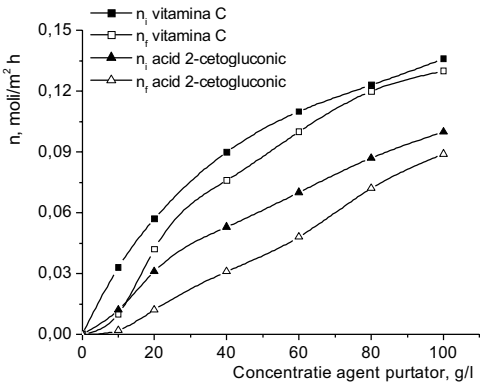

Fig. 8. Influenţa concentraţiei agentului purtător asupra fluxurilor masice ale vitaminei C şi acidului 2-cetogluconic (pH_i = 1, pH_f = 11, turaţia = 500 rpm)

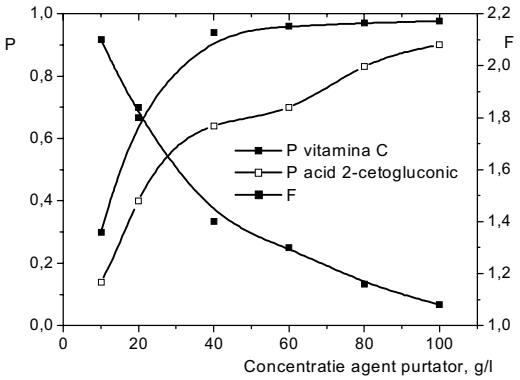

Fig. 9. Influenţa concentraţiei agentului purtător asupra factorilor de permeabilitate a vitaminei C şi acidului 2-cetogluconic şi a factorului de selectivitate (pH_i = 1, pH_f = 11, turaţia = 500 rpm)

consecinţa creşterii concentraţiei unuia dintre participanţii la reacţia chimică care se desfaşoară la interfaţa de separare dintre faza apoasă iniţială şi solvent. Creşterea concentraţiei Amberlite LA-2 în membrana lichidă manifestă un efect pozitiv mai accentuat asupra fluxurilor masice ale vitaminei C, datorită solvatării complexului format de către moleculele de extractant.

Aceeaşi evoluţie crescătoare o înregistrează şi factorii de permeabilitate, fapt evidenţiat de rezultatele expuse în figura 9, datorită rezistenţei de natură cinetică superioară care afectează procesul global de separare. În cazul vitaminei C, factorul de permeabilitate creşte până la o concentraţie de Amberlite LA-2 de 40 g/L, peste această valoare efectul acestui parametru devenind neglijabil.

Aplatizarea curbei este rezultatul unor eficienţe apropiate între procesele de extracţie şi reextracţie la cele două interfeţe ale membranei lichide. Deoarece la pH_i = 1, acidul 2-cetogluconic se găseşte sub formă de dimeri, factorul de permeabilitate este inferior celui corespunzător vitaminei C (fig. 9).

Factorul de selectivitate scade puternic cu creşterea concentraţiei agentului purtător, valoarea maximă (F = 2,1) fiind atinsă pentru concentraţia aminei de 10 g/L. La această valoare a concentraţiei, Amberlite LA-2 reacţionează preferenţial cu vitamina C, însă cu creşterea concentraţiei agentului purtător creşte probabilitatea reacţiei şi cu acidul 2-cetogluconic.

CONCLUZII

Rezultatele obţinute au demonstrat faptul că vitamina C poate fi extrasă selectiv din amestecul cu acidul 2-cetogluconic, produs secundar rezultat din procesul de fermentaţie. Parametrul cu importanţa decisivă asupra eficienţei pertracţiei a fost pH-ul soluţiei apoase. Astfel, pentru a separa selectiv vitamina C este necesară menţinerea pH-ului fazei apoase iniţiale la valoarea 1, corespunzătoare unor fluxuri masice superioare datorate mecanismului de realizare a extracţiei reactive. Agitarea fazelor iniţială şi finală are un efect pozitiv, însă nu similar, asupra vitezelor de extracţie şi, respectiv, de reextracţie, sugerând faptul că, în timp ce procesul de extracţie este controlat atât difuzional, cât şi cinetic, cel de reextracţie este controlat predominant de difuzie.

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NOUTĂŢI

EFECTELE ANTIDEPRESIVE ALE UNEI FRACŢIUNI POLIZAHARIDICE ACIDE DIN GINSENG

Rădăcinile speciei *Panax ginseng* C. A. Meyer (*Araliaceae*) sunt utilizate pentru proprietăţile tonic generale. Profilul farmacologic al extractelor de Ginseng este foarte complex ; extractele de Ginseng modulează axa hipotalamo-pituitaro-adrenergică şi activitatea sistemelor monoaminice. În contextul noilor teorii referitoare la etiopatogenia depresiei, extractele de Ginseng acţionează neuroprotector şi, în acelaşi timp, cresc rezistenţa celulelor la diferiţi agenţi agresori. Ginseng-ul conţine peste 15% polizaharide, neutre şi acide, solubile în apă, considerate a fi răspunzătoare pentru multe dintre efectele benefice ale extractelor. Fracţiunea polizaharidică neutră conţine, în principal, glucani şi cantităţi mici de arabinogalactani. Fracţiunea polizaharidică acidă este un amestec complex de pectine bogate în unităţi de tip arabinogalactan, ramnogalacturonan şi homogalacturonan. Această fracţiune a fost administrată animalelor de experienţă (şoareci masculi ICR) pe cale orală, în doze de 100 mg/kg şi 200 mg/kg. Administrarea s-a făcut zilnic, timp de o săptămână înainte de efectuarea experimentelor şi în timpul desfăşurării experimentelor. Au fost evaluate spontaneitatea şi abilităţile de socializare ale animalelor de experienţă, precum şi comportamentul acestora în testul labirintului şi testul înotului forţat. Fracţiunea polizaharidică acidă nu a influenţat spontaneitatea animalelor şi comportamentul acestora în testul labirintului. În doză de 100 mg/kg, fracţiunea polizaharidică acidă a redus semnificativ imobilitatea animalelor în testul înotului forţat ; în doze de 100 mg/kg şi 200 mg/kg, această fracţiune a diminuat agresivitatea şi a îmbunătăţit abilităţile de socializare ale animalelor (Wang J, Flaisher-Grinberg S, Li S et al. Antidepressant-like effects of the active acidic polysaccharide portion of ginseng in mice. *J Ethnopharmacol* 2010 ; 132 : 65-69).

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CONTRIBUŢII LA STUDIUL PROPRIETĂŢILOR BIOELECTRICE A ZONELOR DE ACUPUNCTURĂ

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CONTRIBUTION TO BIOELETRICAL PROPERTIES RESEARCH OF AREAS WITH ACUPUNCTURE (Abstract) : The research has as a goal the investigation of the bioelectrical behaviour of the acupunctural areas in order to obtain a scientific substantiation of the correspondence between the extreme – eastern medical tradition and the academical medicine. It is obvious that the Chinese traditional medicine knows a certain mechanism of ensurance of homeostasis of the organisms, a mechanism represented, according to the Chinese medical tradition, by the net of acupuncture points and meridians. The experimental data presented in this paper lead to the discovery of the fact that this net works as the fourth system of integration, the electrical – regulator one. The maintenance of the internal homeostasis of the superior organisms and their adaptation to the environment is ensured by the neuro – endocrine and circulatory systems through a mechanism described by Selye under the name of the Uncertain General Syndrome of Adaptation represented by the hypothalamo – hypophyso – corticoadrenal axis. The evolution of these systems and of their mechanisms can be supervised on the basis of the cortisole concentration. The research emphasized the direct proportionality between the cortisone levels and the variation of the electrical values of the acupuncture points, a fact that demonstrates the involvement of the net of acupuncture points and meridians in the maintenance processes of the homeostasis, control, adjustment and adaptation of the living organisms. Additionally, the analysis of the bioelectrical behavior of the acupuncture areas led to the conclusion that the morpho – functional substrate of this net is the conjunctive tissue. The physical – chemical properties of the interstitial connective tissue assures its working as an electro – regulator system on one side and as a harmonization agent of the neuro – endocrine and circulatory systems on the other side, this aspect being to be developed in a future paper.
Key words: POINTS AND MERIDIANS OF ACUPUNCTURE, ELECTRICAL CONDUCTIBILITY, QI ENERGY, ENERGY EXCESS OR DEFICIT, ELECTRICAL – REGULATOR SYSTEM

Identificarea unei structuri anatomice a punctelor şi meridianelor de acupunctură a eşuat în timp, câştigând în schimb tot mai mult teren teoria substratului bioelectric, teorie care are la bază ideea unei rezistenţe bioelectrice mai mică în punctele de acupunctură decât în zonele tegumentare înconjurătoare (1). Experimentele ulterioare au confirmat existenţa acestor gradiente de rezistenţă electrică şi pentru meridiane (2,3).

Astfel, s-au descoperit modificări ale configuraţiei bioelectrice a punctului, precum şi variaţii ale rezistenţei sale în caz de boală, s-a putut urmări o evoluţie ritmică a comportamentului conductibilităţii electrice cutanate şi dependenţa sa de factori ciclici exteriori (4,5). Mulţi cercetători au văzut în aceste manifestări electrice soluţia problemei determinării suportului ştiinţific al acupuncturii şi s-au imaginat corespondenţe între teoria tradiţională şi medicina modernă, făcându-se echivalenţa între "energia Qi" şi sarcina electrică, iar "deficitul sau excesul energetic" este interpretat ca rezistenţă electrică mai mare sau mai mică (6). Cercetarea noastră îşi propune ca scop investigarea experimentală a comportamentului bioelectric al zonelor acupunctice, recoltând o cantitate semnificativă de date necesare formulării unor concluzii statistice, necesare unei fundamentări ştiinţifice a corespondenţei dintre tradiţia medicală extrem-orientală şi medicina academică.

Urmare a prelucrării datelor bioelectrice recoltate s-a putut constata şi faptul că modelul de biosistem al MTC este unul realist, verificabil şi obiectivabil cu ajutorul metodelor moderne.

Este evident că medicina tradiţională chineză se bazează pe stăpânirea unui mecanism de reglare ce pare încă necunoscut medicinii moderne. Şcoala românească de acupunctură are meritul de a se situa printre pionierii acestei direcţii de cercetare şi astfel, Constantin D. (1977) explică efectele acupuncturii ca pe o acţiune de electro-reglare, de restabilire a echilibrului fiziologic şi refacere a homeostaziei electrice şi biochimice a organismului (7,8).

Urmând ideea prof. D. Constantin privitoare la acţiunea de electro-reglare a acupuncturii colectivul nostru şi-a propus să dezvolte cercetările în două direcţii principale :

- studiul teoretic şi experimental asupra mecanismului de electro-reglare a organismului uman ;
- evidenţierea parametrilor măsurabili cu valenţe diagnostice şi terapeutice specifici modelului fizic propus.

Prigogine (9. arată că pentru orice sistem termodinamic deschis (aşa cum este şi organismul viu) starea momentană de echilibru se caracterizează printr-un set de mărimi fizice măsurabile numit parametri de stare. Starea momentană de sănătate sau boală poate fi exprimată printr-o submulţime a mulţimii parametrilor de stare. Parametrii de stare ai acestei submulţimi se împart în mai multe categorii :

a. parametrii standardizaţi, recunoscuţi oficial, care dau informaţii asupra stării de sănătate şi sunt utilizaţi în practica curentă medicală pentru stabilirea diagnosticului ;
b. parametrii nestandardizaţi a căror valoare diagnostică nu este utilizată sau cunoscută ;
c. parametri necunoscuţi încă.

Există o teoremă în termodinamică care spune că alegerea sistemului complet de parametri de stare (un număr de parametri care caracterizează fără echivoc starea momentană a sistemului termodinamic) nu este unică. Cu alte cuvinte se poate găsi oricând un alt sistem de parametri de stare, format din alţi parametri şi care să caracterizeze» la fel de bine» starea momentană a sistemului termodinamic ca şi sistemul anterior de parametri.

Aceste consideraţii fizice ne permit să afirmăm că în cazul organismelor vii se poate găsi o altă serie de investigaţii paraclinice, care să ofere rezultate echivalente seriei oficiale de determinări.

Pentru a putea alege şi măsura parametrii semnificativi nestandardizaţi sau necunoscuţi este necesară alcătuirea unui mecanism de reglare specific acţiunii acupuncturii, respectiv a măsurării valorilor de conductibilitate electrică.

Existenţa acestui sistem reglator cu acţiune interferată cu cea a sistemelor nervos, endocrin şi circulator prin intermediul reţelei

de zone acupunctice este postulată de şcoala românească de acupunctură astfel : «având o rezistenţă electrică mică şi deci o conductibilitate electrică mare, aceste puncte pot fi considerate ca zone de conducţie care asigură stabilitatea structurii electrice a organismului. Sub raport electric, boala înseamnă o scădere a rezistenţei electrice şi o creştere a potenţialului electric în punctele cutanate corespunzătoare organului aflat în suferinţă. Acţiunea asupra lor are ca rezultat refacerea acestor parametri la valorile corespunzătoare stării de sănătate.» (10).

Aceste consideraţii fizice ne permit să afirmăm că în cazul organismelor vii se poate găsi o altă serie de investigaţii paraclinice, care să ofere rezultate echivalente seriei actuale de determinări.

Pornind de la acest adevăr am investigat experimental parametri electrici care pot fi puşi în directă legătură cu diagnosticul energetic din cadrul MTC. Am optat pentru măsurarea conductibilităţii meridianelor de acupunctură utilizând o tehnică derivată din tehnica de măsurare Ryodoraku.

Pornind de la testarea electropermeabilităţii pielii la un pacient cu edeme puternice datorate nefrozei cronice, Nakatani (11) descoperă pe toracele acestuia puncte cu o permeabilitate electrică mai mare (Ryodoten) decât cea medie, punctele fiind dispuse după meridianul rinichiului. Prin unirea acestor puncte cu o mai bună electropermeabilitate s-a obţinut o serie de curbe, numite Ryodoraku, identice cu tradiţionalele meridiane de acupunctură. Prin măsurarea electropermeabilităţii anumitor puncte de la încheietura mâinilor şi picioarelor pacientului, cercetătorul a constatat că se pot determina stările de excitaţie sau inhibiţie energetică a tuturor celor 12 meridiane.

Aceste puncte reactive electropermeabile se suprapun cu o bună aproximaţie punctelor P9, VS 7, C7, IS5, TF 4, IG 5, SP 3, F 4, R 4, VU 65, VB 40, şi S 42 bilateral.

Testarea se face cu o sursă de curent continuu de 12V şi 200 μA. Interpretarea datelor de măsură se face potrivit metodei Ryodoraku (12) prin dispunerea valorilor obţinute pe o diagramă numită *neurogramă*. În urma medierii valorilor se obţin trei regiuni : regiunea normală, regiunea meridianelor aflate în exces energetic şi regiunea meridianelor aflate în deficit energetic.

MATERIAL ŞI METODĂ

În cazul cercetării de faţă s-a preferat determinarea rezistenţei electrice şi nu a diferenţelor de potenţial datorită faptului că ţesuturile prezintă calitatea de *volum-conductor* (mediu bun conducător de electricitate). În consecinţă, activitatea unui organ intern, cum ar fi inima, creierul etc., are efectul unui dipol generat într-un ţesut situat în profunzimea organismului şi care influenţează întreg mediul bun conducător dimprejur, având răsunet până la piele (13).

Pentru realizarea măsurătorilor bioelectrice, respectiv a rezistenţei electrice cutanate atât în punctele de acupunctură cât şi la nivelul non-punctelor, s-a folosit un aparat de măsură a rezistenţei opusă de piele la trecerea unui curent electric test, de concepţie proprie, alcătuit după standardele tehnice specifice acestei destinaţii şi conform normelor de protecţie a subiectului investigat (fig. 1).

Toate tipurile de dispozitive pentru determinarea rezistenţei cutanate se bazează pe acelaşi mod de funcţionare, piesa principală fiind constituită dintr-un ohmmetru care produce un curent de 200 μA curent continuu, la 12 volţi.

P1 – potenţiometru carbon, se etalonează instrumentul de măsură prin scurtcircuitarea S1 cu S2. (se obţine indicaţia maximă pe scală – are acces permanent la panou).

P2 – potenţiometru semireglabil pentru reglarea sensibilitaţii microampermetrului. (se reglează de către constructor).

Ic1 – circuit integrat stabilizator de tensiune ce asigură o tensiune stabilizată de

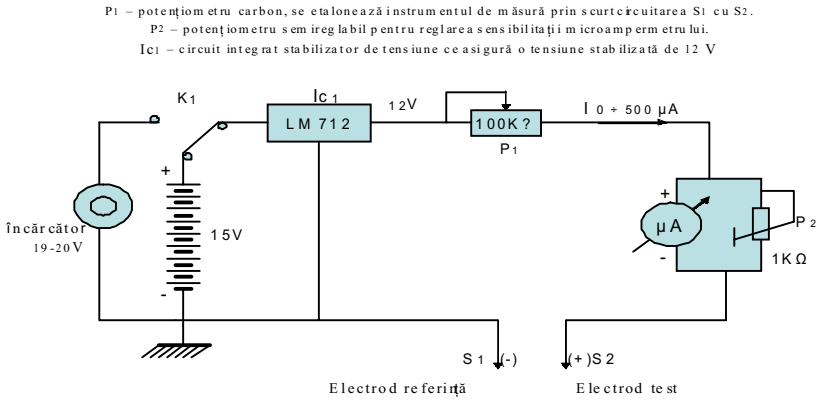

Fig. 1. Schema aparatului de măsură a rezistenţei electrice pe punctele de acupunctură

12 V, LM 712.

În cursul procesului de măsurare a rezistenţei punctelor de acupunctură se respectă legea lui Ohm :

$I = U/R$, în care $R = Rp1 + RA$

De unde :

$I = U/RP1 + RA$

Rezultă că :

$RA = R\,\mu\,A \,.\, RP2/P\mu A + RP2$

Calculul rezistenţei măsurate la electrozii aparatului de măsură (fig. 2).

R p – rezistenţa electrică a pielii,

Rint – rezistenţa electrică a circuitului în interiorul organismului explorat,

RX – rezistenţa electrică măsurată,

S1, S2 – electrozii de măsură,

Aparatul de măsură determină valoarea **Rx**, care, la rândul ei este formată din rezistenţa electrică a pielii (**Rp**) împreună cu rezistenţa ţesuturilor din interiorul organismului (**R int**).

Curentul explorator care pătrunde în ţesuturile subcutanate va alege traseul cu rezistenţa electrică cea mai mică. Acest traseu, conform cercetărilor, este reprezentat de ţesuturile ce constituie substratul meridianului de acupunctură.

Dacă rezistenţa electrică medie a pielii **Rp** este considerată constantă :

Rp ~ Constant,

Atunci, calculul lui **RX** rezultă din relaţiile :

1/RX = 1/Rp + 1/Rint,

de unde :

Rx = Rp . Rint/Rp + R int

Deoarece rezistenţa electrică a pielii este considerată constantă, rezultă că mărimea varibilă este rezistenţa **R int**, care este dată de circuitul din interiorul organismului.

Această mărime variază la nivelul punctului de acupunctură în funcţie de meridianul căruia îi este alocat punctul. De exemplu, electrodul de referinţă este plasat în mâna stângă, electrodul explorator măsoară rezistenţa electrică la un punct de pe încheietura mâinii dreapte, caz în care circuitul electric măsurat trece prin cele două membre superioare dar şi prin interiorul toraco-abdomenului şi invers.

Luând **Rp** constantă, rezultă că singura rezistenţă care variază este dată de cea a meridianelor parcurse de curentul explorator **R int**.

În consecinţă, măsurătoarea nu determină rezistenţa cutanată pe punctele de acupunctură, ci pe aceea reprezentată de traseul intern parcurs de curent de la un electrod la celălalt.

S-a verificat acest aspect şi de către noi, constatându-se că acest lucru este în general corect, dar valorile astfel obţinute sunt semnificative în cazul măsurării impedanţei, care implică şi procesele capacitive ale ţesuturilor, şi mai puţin semnificative în măsurătorile

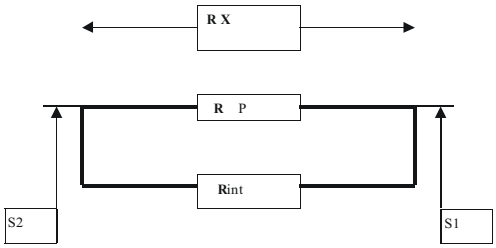

Fig. 2. Calculul rezistenţei măsurate la electrozii aparatului de măsură

de rezistenţă.

Ceea ce trebuie reţinut în acest context al procesului de măsurare este şi faptul că indicaţia la panoul aparatului este dată în valori de curent. În speţă, se măsoară valoarea de curent care reuşeşte să treacă prin circuitul explorat. În acest sens, ţesutul parcurs opune o anumită rezistenţă la trecerea curentului electric test, acest curent ce trece de opoziţia reprezentată de rezistenţă este măsurat în realitate, rezistenţa ţesutului fiind dedusă din relaţia care descrie legea lui Ohm : $R = U/I$.

Un alt aspect care rezultă din procesul de măsurare este legat tocmai de faptul că măsurătoarea nu se reduce la determinarea unei mărimi într-un punct, ci de-a lungul unui conductor. Acest tip de conductor nu este unul omogen, ci unul de tip anizotrop, format dintr-un hidroelectrolit specific ţesuturilor conjunctive ale organismelor vii.

Măsurătorile realizate pe conductori electrici clasici, metalici, oferă valori ale căror variaţii sunt liniare.

În cazul măsurătorilor pe structuri de tip hidroelectrolitic, aceste valori nu manifestă un comportament liniar.

În aceste cazuri se măsoară o rezistenţă compusă din mai multe variabile : lungimea traseului (l), secţiunea acestuia (s) şi rezistivitatea (ρ) acestuia.

$R = \rho \times l/s$

Deoarece secţiunea **s** este reprezentată de masa ţesutului cu cea mai mică rezistenţă, iar lungimea este constantă, rezultă că singura variabilă, cu cea mai reprezentativă semnificaţie, este dată de valoarea ρ a rezistivităţii electrice a ţesuturilor.

Alegerea tensiunii de 12 V şi a intensităţii de 200 μA decurge din faptul că aceste valori nu antrenează manifestări supărătoare (senzaţie de electrocutare, contracţii musculare), sau dăunătoare (arsuri, ca urmare a încălzirii locale a ţesuturilor, aşa numitele puncte fierbinţi).

Punctele de acupunctură prezintă caracteristici electrice particulare, care au fost studiate amănunţit în ultimii 50 de ani. Opinia generală, bazată pe rezultate experimentale, relevă că la nivelul acupunctelor se constată o scădere foarte importantă a rezistenţei sau a impedanţei electrice cutanate, până la 10 : 1, această caracteristică a permis construirea a numeroase detectoare de puncte, al căror principiu de bază rămâne măsurarea unei diferenţe de rezistenţă electrică faţă de rezistenţa medie a tegumentului (14,15,16,17). Întrucât măsurarea se face în curent continuu, noţiunea de impedanţă electrică se reduce la cea de rezistenţă, dispărând factorii dinamici de reactanţă (componenta capacitivă), deoarece curentul continuu este invariabil ca mărime şi sens (este staţionar) (18).

Cercetarea s-a efectuat pe trei loturi de subiecţi.

Primul lot, format din 486 persoane, 412 bărbaţi şi 74 femei, cu vârstele cuprinse între 18 şi 55 ani, a fost constituit din angajaţii de la CFS Săvineşti, Secţia Lactama 3, în cadrul controlului medical periodic preventiv, la care s-au prelevat în paralel parametrii bioelectrici şi analizele standard de laborator pentru noxele specifice locului lor de muncă conform normelor în vigoare. Cercetarea s-a derulat între iunie 1987- decembrie 1989.

Al doilea lot a fost constituit din 520 de voluntari, în cadrul Staţiunii Bălţăteşti, 210 bărbaţi şi 310 femei, cu vârstele între 16 şi 82 ani, în perioada 1986-1992, persoane neexpuse noxelor industriale, lot cu ajuto-

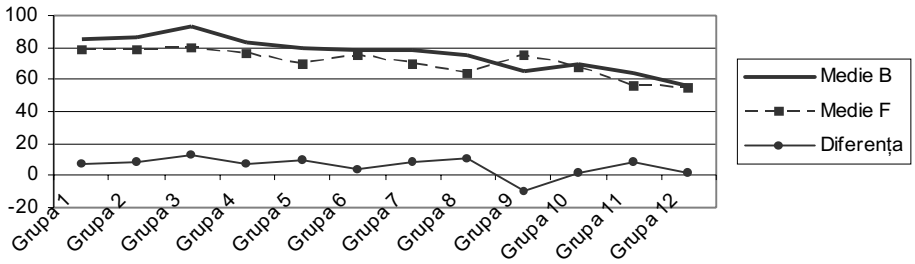

Fig. 3. Variaţia conductibilităţii cu vârsta şi sexul

rul căruia s-au verificat prin comparaţie variaţiile specifice unei activităţi desfăşurate în condiţii de viaţă modernă.

Al treilea lot a fost alcătuit din 106 angajaţi ai firmei Kober, Piatra Neamţ, dintre care 83 bărbaţi şi 23 femei, cu vârstele între 18 şi 55 ani, determinările fiind efectuate de-a lungul anului 1995, lunile ianuarie, martie, aprilie, iulie şi august.

REZULTATE

Evaluările datelor de conductibilitate electrică de la nivelul acupunctelor investigate conform metodei pe lotul de control format din muncitori agricoli neexpuşi noxelor industriale (lotul Bălţăteşti) au permis formularea următoarelor observaţii :

1. conductibilitatea electrică în acupuncte scade o dată cu creşterea vârstei subiecţilor (fig. 3).

2. la bărbaţi se constată o mai bună conductibilitate electrică decât la femei (fig. 3).

3. valorile culese în cursul primei părţi a zilei arată o conductibilitate electrică mai ridicată decât cele prelevate după amiaza (fig. 4).

Rezultate similare au fost obţinute şi de Dumitrescu (7) care a constat de asemenea că o creştere progresivă a potenţialelor electrice în punctele electrodermice situate pe craniu în primele ore ale dimineţii (8-11) şi o diminuare sau o reducere a lor spre orele de seara (19-22).

4. valorile electrice provenite de la determinările conform sezoanelor, se dispun de-a lungul unei curbe sinusoidale care indică creşterea conductibilităţii electrice începând cu luna februarie cu un maximum în luna mai, după care începe să scadă până ajunge la un minimum în luna decembrie (fig. 5).

5. comparând lotul de control cu loturile 1 Săvineşti şi 3 Kober, formate din lucrători în mediu cu noxe industriale chimice (benzen şi solvenţi organici şi lucrul în trei schimburi) s-a constat că aliura graficelor ce reprezintă derularea valorilor de conductibilitate nu respectă diagramele obţinute pe lotul de control.

Aceste constatări au atras atenţia asupra faptului că oscilaţia valorilor de impedanţă electrică din punctele de acupunctură ar putea indica de fapt probleme de adaptare a organismului la ritmul şi eforturile specifice mediului industrial.

Totodată a fost uşor de sesizat că există o relaţie directă între modul în care variază

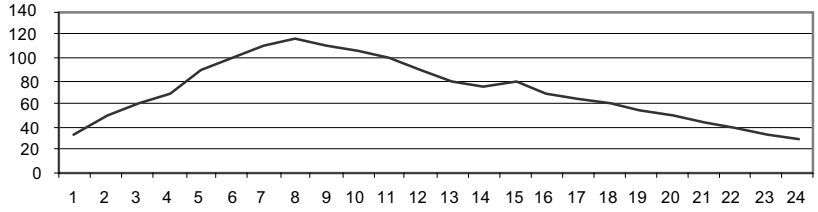

Fig. 4. Variaţia conductibilităţii de-a lungul zilei

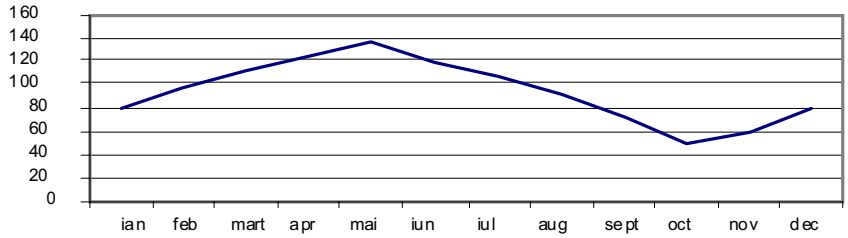

Fig. 5. Variaţia conductibilităţii dupa sezon

comportamentul electric în acupuncte şi curba nictemerală a cortizolului, hormon cu importanţă cardinală în acţiunile de adaptare.

S-a trecut la investigarea în paralel a valorilor cortizolului cu cea a valorilor bioelectrice constatându-se relaţia directă dintre cele două variabile.

Întrucât metoda de măsurare este o metodă invazivă, am constatat că testul de conductibilitate nu poate fi repetat cu rezultate mulţumitoare decât după circa 30-60 minute după prima testare. Aceasta dovedeşte că organismul sesizează curentul de test ca pe un factor perturbator extern şi ca atare, reacţionează prin modificarea parametrilor electrici ai însuşi sistemului de electroreglare testat.

Condiţiile stresante duc de asemenea la alterarea neurogramei ca urmare a aceluiaşi mecanism de reglare. Ingerarea alcoolului, a hranei sau a substanţelor medicamentoase duce la modificări notabile ale aliurii neurogramei cu alterări majore ale preciziei diagnosticului.

De aceea, este necesar ca prelevarea neurogramei să se realizeze în condiţii de repaus, pe nemâncate, în absenţa consumului de alcool, a stresului şi a tratamentului medicamentos.

Şi acest aspect demonstrează că oscilaţiile valorilor impedanţei electrice în zonele acupunctice sunt efectul perturbării induse de diferiţi agenţi stresori externi.

Important este că măsurătorile se referă la caracteristicile de profunzime ale ţesuturilor dependente de starea de sănătate a organismului şi nu la caracteristici de suprafaţă cum ar fi umiditatea tegumentului. Astfel, testând subiecţii imediat după baia salină, administrată ca tratament balnear la Băltăţeşti şi repetând testul după circa o oră, adică după uscarea tegumentelor şi reechilibrarea electrică, s-a constatat o aliură a neurogramei aproape identică cu aceleaşi valenţe diagnostice.

Rezultatele experimentale obţinute de autori au permis formularea unui model biofizic numit în literatură sistem electro-regulator.

DISCUŢII

Măsurătorile electrice efectuate de autori au confirmat existenţa şi coincidenţa unor zone cutanate cu rezistenţă electrică redusă cu cea a punctelor descrise de tradiţia medicală chineză.

Prin aceasta se demonstrează că proprietăţile funcţionale ale pielii se modifică dependent de activitatea substructurilor, activităţile lor circumscriindu-se unor zone foarte mici ca dimensiune pe piele (1-4 mm^2).

Această realitate ne obligă să apreciem că în timp ce potenţialul de activitate electrică este distribuit pe toată suprafaţa corpului, punctul, activat prin variaţia impedanţei, este conectat cu însuşi organul intern.

Noi am avansat deja ipoteza că această legătură se realizează prin intermediul ţesutului conjunctiv, schimbările fiziopatologice producându-se prin intermediul lichidelor extra-vasculare, care prin intermediul traveelor conjunctive din hipoderm sunt legate

direct de conductibilitatea pielii.

Căile prin care circulă acest tip de lichide sunt cele formate din ţesut conjunctiv lax, teoretic fiind coincidente cu meridianele Ryodoraku şi, de asemenea, cu clasicele meridiane (16,19).

Determinările obţinute de cercetarea de faţă corespund într-un mod semnificativ cu cele găsite şi de alte colective de cercetare.

Cercetările lui Târgovişte (1972) (8) au găsit pentru punctul de acupunctură valori ale rezistenţelor de ordinul 914+/-299 Ω la o profunzime a acului de 0,5 cm, iar la 2 cm: 502+/-85 Ω. Între punctele IG4 - IG15 a obţinut 1375 +/-525 Ω, iar pentru IG10 - IG11 : 1016+/-317 Ω.

Menţinerea homeostaziei interne a organismelor superioare este asigurată de sistemele neuro-endocrin şi circulator. Mecanismul prin intermediul căruia acţionează cele două sisteme este descris de Selye (20) sub numele de Sindrom genertal nespecific de adaptare, identificat în axa hipotalamo-hipofizo-corticosuprarenală. Măsura activităţii acestui ax este cel mai bine urmărită pe baza concentraţiei cortizolului (dozabil şi în sânge şi urină).

Dependenţa direct proporţională a variaţiei valorilor rezistenţei electrice cutanate din acupuncte şi meridiane cu variaţia concentraţiei cortizolului în stări atât fiziologice cât şi de stres, ne permite să concluzionăm că pe lângă cele trei sisteme de integrare cunoscute mai există un al patrulea, numit sistem electro-reglator.

Modelul teoretic al sistemului electro-reglator (presupus şi de alţi autori) este confirmat experimental de rezultatele cercetării prezente.

În plus, modul în care este demonstrat şi înţeles comportamentul biolectric în zonele de acupunctură ne-a permis să identificăm substratul morfo-funcţional al acestor zone ca fiind ţesutul conjunctiv interstiţial, acest aspect urmând a fi dezvoltat într-o lucrare ulterioară.

CONCLUZII

1. Modelul de biosistem descris de MTC nu contrazice cu nimic ceea ce ştiinţa medicală modernă a statuat legat de comportamentul acestuia pe baza verificărilor şi a rezultatelor experimentale, doar că a folosit un limbaj specific culturii şi vremurilor când acest tip de medicină a apărut.
2. Faţă de modul de gândire occidental, cel extrem-oriental intuieşte şi descrie sistemele vii în dinamică, prezentând toate fluxurile care străbat un organism: fluxurile de sânge, limfă, aer şi chiar pe cele reprezentate de biocurenţii electrici şi de schimburile termice, schimburi ce asigură corelarea şi adaptarea permanentă la mediul extern, constituind prin aceasta primul model de medicină holistică.
3. Aceste constatări au atras atenţia asupra faptului că oscilaţia valorilor de impedanţă electrică din punctele de acupunctură ar putea indica, de fapt, problemele de adaptare a organismului la ritmurile mediului extern natural, pe de o parte, dar şi eforturile de adaptare la modul de viaţă specific lumii moderne şi mediului industrial.

   Acest lucru este dovedit de existenţa relaţiei directe dintre comportamentul electric în acupuncte şi curba nictemerală, sezonieră şi de stres a cortizolului, hormon a cărui importanţă în acţiunile de adaptare este bine cunoscută.
4. Dependenţa direct proporţională a variaţiei valorilor rezistenţei electrice cutanate din acupuncte şi meridiane cu variaţia concentraţiei cortizolului în stări atât fiziologice cât şi de stres, ne permite să concluzionăm că pe lângă cele trei sisteme de integrare cunoscute, sistemul circulator şi neuroendocrin, mai există un al patrulea, numit sistem electro-reglator.
5. Deşi teoretic acest model a fost intuit şi

de alţi cercetători, prima dovadă experimentală în acest sens este adusă de autorii prezentului material.

6. Modul în care este explicat comportamentul bioelectric din zonele de acupunctură a permis identificarea şi justificarea ştiinţifică a faptului că substratul morfo-funcţional al acestora este reprezentat de ţesutul conjunctiv interstiţial, ca spaţiul al doilea extracelular.

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DIFFERENTIAL EQUATIONS WITH SMALL PARAMETER WITH APPLICATIONS IN RADIOIMMUNOTHERAPY

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DIFFERENTIAL EQUATIONS WITH SMALL PARAMETER WITH APPLICATIONS IN RADIOIMMUNOTHERAPY (Abstract): The Akzo Nobel research laboratories formulated this problem in their study of the penetration of radio-labeled antibodies into a tissue that has been infected by a tumor. This study was carried out for diagnostic as well as therapeutic purposes. The treatment of malignant diseases, after its primary treatment by surgery, is either by external beam radiotherapy, which is effective but local, or by chemoteraphy, which is effective but not selective. The radiotherapy labelled antibody has to be able to penetrate the whole of the tumour, whereas for imaging only uptake on the surface of the tumour is needed. Factors such as dose, rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the major dose-limiting toxicities. In this article, we introduced a system of differential equations with small parameter with applications in radioimmunotherapy. The problem consists of two partial differential equations. Both the equation of this system includes small parameter ε. We introduce the mathematical technique known as boundary function method for singular perturbation system. In this system, the small parameter is an asymptotic variable, different from the independent variable. We write solution of this system in a small parameter, and investigation of asymptotic solution for system. Using the program Matlab and numerical method Runge-Kutta, I did various simulations for different values of biological parameters presented in the model studied. **Key words**: RADIATION THERAPY, ANTIBODY, MALIGNANT, CANCER

Radioimmunotherapy is a newly approved cancer treatment that combines two types of therapies radiation therapy and immune therapy using monoclonal antibodies. Radioimmunotherapy (RIT) is use of radio-labeled antibodies in the therapy of cancer. The concept of localizing the cytotoxic radionuclide to the cancer cell is an important supplement to conventional forms of radiotherapy. In theory the intimate contract between a radioactive antibody conjugate and a target cell enables the absorbed radiation dose to be concentrated at the site of abnormality with minimal injury to the normal surrounding cells and tissues (1). A variety of approaches and combinations of this strategy are now being pursued. (RIT) has been more successful in the radiosensitive hematologic malignancies, for example lymphomas and leukemias as compared with small solid tumors (2). Obstacles to successful (RIT) have been recognized and strategies to overcome these hurdles and to improve efficacy are continuously being developed resulting in encouraging outcome particularly with locoregional routes

of administration in solid tumors. The results in solid tumors are somewhat less favorable but new strategies for patients with minimal residual disease using adjuvant and locoregional treatment are evolving (3). When your body detects something that does not belong, such as bacteria (pathogen), one way it eliminates the threat is to produce antibodies that bind to the protein shapes that are specific to the pathogen. (RIT) agents are man-made antibodies with different radiation components attached and is considered a targeted therapy, because the antibodies that deliver the radiation are specific to one type of cell. The ability of the antibody to bind to a tumor-associated antigen (a molecule that can stimulate an immune response) ensures that the tumor gets a high dose of radiation, which can kill the targeted cancer cells and nearby cancer cells, while normal tissuee only a minimal dose. This minimizes toxicity to normal tissues. Radioimmunotherapy is being studied in several cancers, but has shown the most promise in the treatment of blood cancers, such as leukemia and lymphoma (4).

MATHERIALS AND METHODS

Let us consider an original reaction diffusion system in one spatial dimension:

$$(P_\varepsilon)\begin{cases}\varepsilon\dfrac{\partial u}{\partial t}=\varepsilon\dfrac{\partial^2 u}{\partial x^2}-uv\\ \varepsilon\dfrac{\partial v}{\partial t}=-uv\end{cases}$$

which originates from the chemical reaction: $U+V\xrightarrow{\frac{1}{\varepsilon}}W$. Here U, the radio-labeled antibody, reacts with substrate V, the tissue with the tumor, and $\frac{1}{\varepsilon}$ denotes the rate constant (5). The concentrations of U and V are denoted by υ and ν, respectively. In the derivation of the equations (P_ε) it was assumed that the reaction is governed by mass action kinetics and in addition that the chemical U is mobile while V is immobile.

To model this penetration, the equations (P_ε) are considered in the strip:

$A_{N,T}=\{(x,t):0<x<M,0<t<T\}$

for some constants M, T, along with the following initial and boundary conditions:

$$\begin{cases}u(x,0)=0\\ v(x,0)=v_0\end{cases},\ \text{for}\ x>0$$

where v_0 is a constant, and :

$$\begin{cases}u(0,t)=\theta(t),0<t<T\\ \dfrac{\partial u}{\partial x}(1,t)=0,0<t<T\end{cases}$$

Let us now give some formal explications: the terms containing small parameter $0<\varepsilon<1$ are called perturbations. The extended model (P_ε) is then called perturbed, and the simplified model is called unperturbed. In this article, the model is formulated in terms of differential equations with small parameter multiplying the highest derivates.The use of a small parameter here is simply for definiteness. For example, a problem depending on a large parameter φ ccan be rewritten as one depending on a small parameter: $\varepsilon=\frac{1}{\varphi}$. Equation (P_ε) corresponds to the extended model. Sometimes small but not small enought to be ignored,and sometimes small but in a non-uniform way such that they are important locally. We work on the assumption that the chemical reaction rate constant is very high.

RESULTS

As we see, both part of (P_ε) contains the small parameter multiplying the derivate. The choice of a small parameter is connected with the order of the solution and the asymptotic sequence which describes the behaviour of the solution as $\varepsilon\to 0$.

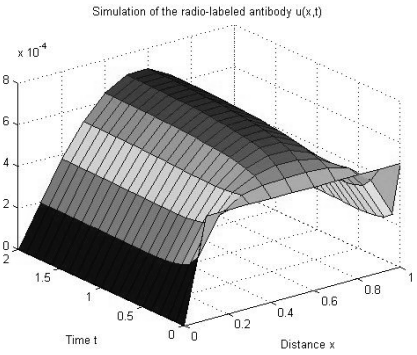

Fig. 1. Simulation of concentration radio-labelled antibody for parameter value $\varepsilon = 0.1$.

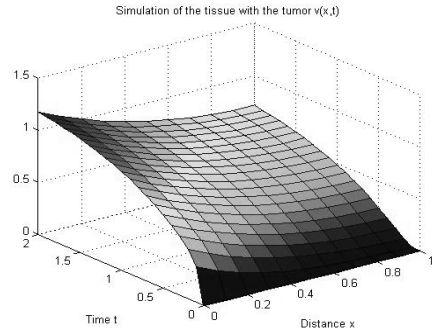

Fig. 2. Simulation of concentration the tissue with tumour for parameter value $\varepsilon = 0.1$.

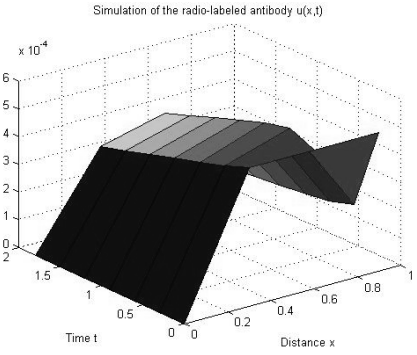

Fig. 3. Simulation of concentration radio-labelled antibody for for parameter value $\varepsilon = 0.1$. With increasing reaction rate by one tenth ,one can notice a more pronounced dynamic concentration radio-labelled antibody.

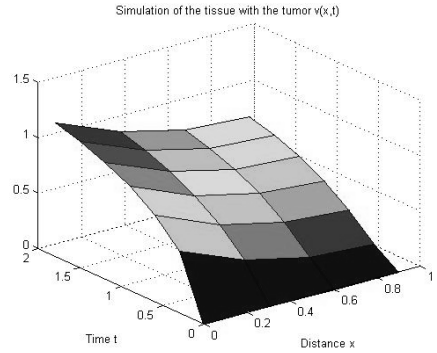

Fig. 4. Simulation of concentration the tissue with tumour for parameter value $\varepsilon = 0.01$

The fact that a small parameter ε multiplies a derivative in (P_ε) indicates that it is a singular perturbation problem.One class of such problems is immediately recognised if,on setting $\varepsilon = 0$, the order of the system of differential equations is reduced,such a reduced system cannot in general satisfy all the initial condition and boundary condition.Setting $\varepsilon = 0$ in (P_ε), we obtain :

$$(P_0)\begin{cases} \breve{u}(x,t) = 0 \\ \breve{v}(x,t) = F(x,t,\varepsilon) \end{cases} or \begin{cases} \breve{u}(x,t) = C(x,t,\varepsilon) \\ \breve{v}(x,t) - 0 \end{cases}$$

Equation (P_0) might be a simplified model of this process. We started out with two differential equations, and ended up saying that one of the differential equations degenerates to an algebraic equation. A system of two differential equations is quite different from a single differential equation with an algebraic constraint (6). The system (P_0) his is called the degenerate system. In the case when the boundary of the domain $A_{M,T}$ is no longer smooth, but contains corner points, the structure of the asymptotic solutions becomes more complicated in the vicinity of these points. We need to introduce a new type of boundary

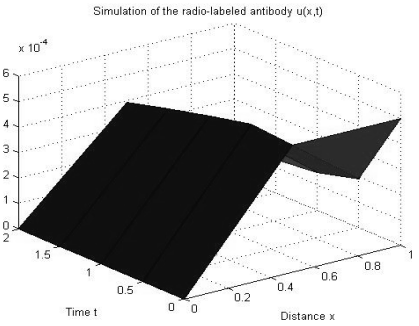

Fig. 5. Simulation of concentration radio-labelled antibody for parameter value $\varepsilon = 0.01$

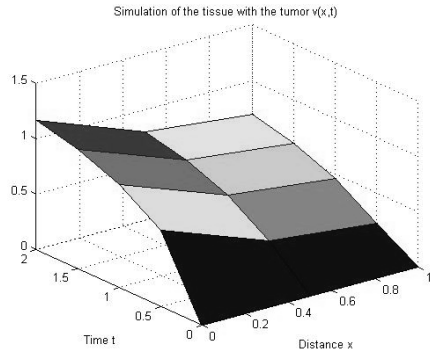

Fig. 6. Simulation of concentration the tissue with tumour for parameter value $\varepsilon = 0.01$

function, corner boundary functions, in the vicinity of the corner points. The corner points its :

$$O(0,0), A(M,0), B(M,T), C(0,T).$$

Usually the boundary functions are defined as solutions of partial differential equations, where the stretched distance along the normal to the boundary. We will seek the asymptotic expansions of the solutions of perturbed problem, initial and boundary conditions in the form :

$$\begin{cases} u = \bar{u} + \pi u + \omega u \\ v = \bar{v} + \pi v + \omega v \end{cases}$$

Here $\bar{u}, \bar{v}$ is the regular part of the asymptotic solution. The function $\pi u, \pi v$ is used for the boundary functions playing an important role near the sides of the rectangle, and the function $\omega u, \omega v$ is used for the corner boundary functions important in the vicinities of the vertices of the rectangle. Corresponding to the sides of the rectangle, π can be represented :

$$\begin{cases} \pi = \sum_{i=0}^{m} \varepsilon^i (\pi_i^{(1)}(x,\theta) + \pi_i^{(2)}(\mu,t) + \pi_i^{(3)}(x,\theta_1) + \pi_i^{(4)}(\mu_1,t) \\ \theta = \frac{t}{\varepsilon}, \theta_1 = \frac{T-t}{\varepsilon} \\ \mu = \frac{x}{\varepsilon}, \theta_2 = \frac{M-x}{\varepsilon} \end{cases}$$

The functions $\pi_i^{(1)}(x,\theta)$ representing the boundary layer near the side $t = 0$. The $\pi^{(1)}$ functions compensate for the discrepancy by the regular part of the asymptotic expansions to the boundary condition on the side $t = 0$. The boundary functions $\pi_i^{(1)}(x,\theta)$ compensating for the discrepancy in the boundary condition on the side $t = 0$, introduce additional discrepancies in the boundary conditions on the sides $x = 0$ and $= M$. These discrepancies are essential in the vicinities of the corner points $O(0,0)$ and $A(M,0)$, they decay exponentially as θ increases. Similar discrepancies are introduced by the functions $\pi_i^{(2)}(\mu,t)$ in the conditions on $t = 0$ and $t = T$, by the functions $\pi_i^{(3)}(x,\theta_1)$ in the conditions on $x = 0$ and $x = M$, and by functions $\pi_i^{(4)}(\mu_1,t)$ in the conditions on $t = 0$ and $t = T$. To compensate for these discrepancies we introduce corner boundary functions :

$$\omega = \sum_{i=0}^{m} \varepsilon^i (\omega_i^{(1)}(\mu,\theta) + \pi_i^{(2)}(\mu,\theta_1) + \pi_i^{(3)}(\mu_1,\theta_1) + \pi_i^{(4)}(\mu_1,\theta))$$

The functions $\omega_i^{(1)}$ are needed to compensate for the errors introduced by $\pi_i^{(1)}$ functions in the boundary condition along $x = 0$ and by $\pi_i^{(2)}$ functions in the boundary condition on $t = 0$. In initial-value approach of the perturbed ODE system (P_ε), initial guesses for equation's para-

meters are chosen and the dynamical equations are solved numerically. As a matter of fact, our concept of the perturbation search method was inspired by the prospect of basic perturbation theory.The principle of perturbation theory is to study dynamical systems that are small perturbation of integrable perturbed system . For this system,solutions can change on a time scale that is very short compared to the interval of integration, but the solution of interes changes on a much longer time scale (7).

DISCUSSION

The simulation of a single set of equation can be performed on a single workstation because the numerical integration of a system (P_ε) not very time consuming. The parameter values have highs impact on the accuracy of the model in representing real biological systems but these values are difficult to estimate experimentally (8) . The complexity of this biological process is revealed every time a mathematical simulation of the processes is carried out. The parameter ε estimation process usually implies time-consuming computations due to algorithms of linear regression and stochastic methods. Parameter estimation adjusts the model to reproduce the experimental results in the best possible way for a set of experimental data. When using an implicit Runge-Kutta method for solving a differential system, (P_ε) the order of the error can be reduced compared to what the classical theory predicts. Runge-Kutta methods have to advantage of being one-step methods, possibly having high order and good stability properties. The Runge-Kutta method for solving differential equations is fairly difficult to understand and rather involved algebraically to derive.

CONCLUSIONS

Radioimmunotherapy is usually delivered over a shorter period of time than chemotherapy. Although the length of treatment varies, most patients receive chemotherapy over the course of several months or occasionally longer. In contrast, radioimmunotherapy is usually delivered over a shorter period of treatment. Radioimmunotherapy is designed to target specific cancer cells. Chemotherapy, on the other hand, destroys any rapidly dividing cell, which often include non-cancer cells as well as cancer cells. Because the cells of the immune system, bone marrow, lining cells of the intestines, skin and hair follicles cells tend to be especially sensitive to the effects of chemotherapy, they are often damaged or destroyed along with the cancer cells. Regional administration of radio-labeled antibody can lead to inceased tumour binding if the antibody binds rapidly to the appropiate target antigen while still in the region compartment. System of differential equations studied in this article, contains small parameter ε Setting this parameter to zero $(\varepsilon = 0)$ leads to an- ofter easier- problem, the unperturbed or reduced problem.The solution of the unperturbed problem might be used as an approximation of the perturbed problem $(\varepsilon \neq 0)$. In this article suppose that we are interested in improving on this model by adding some previously ignored aspects or effects. So it seems that the relation between solution and model studied in this article is not continuous in the problem parameters ε. Whatever small ε we take, from a transparent and exact solution of the simple model at $\varepsilon = 0$,we abruptly face a far more complicated solution of a model that is just a little bit better. The approximation constructed in this article is almost always as asymptotic approximation, i.e. where the error reduces with the small parameter.

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NOUTĂŢI

PARODONTITA MARGINALĂ CRONICĂ PROFUNDĂ – POSIBILĂ CAUZĂ DE ANEMIE SEVERĂ

Numeroase studii au demonstrat deja asocierea infecţiilor parodontale cu boli sistemice grave de tipul diabetului, bolilor cardio-vasculare, accidentului vascular, pneumoniei bacteriene şi cu naşterea prematură. Există însă foarte puţine cazuri raportate în literatura de specialitate care să demonstreze relaţia cauzală directă dintre parodontita marginală şi anemie deşi pacienţii cu inflamaţii parodontale prezintă concentraţii serice crescute ale markerilor inflamaţiei şi reacţii imune care pot supresa eritropoieza. Anemia asociată maladiilor cronice însoţeşte de obicei infecţiile cronice, bolile autoimune, cancerul şi bolile renale cronice dar şi parodontitele marginale severe pot constiui o cauză a acestei patologii. Pe de o parte, medicii dentişti au tendinţa să ignore semnele clinice şi paraclinice ale anemiei. Pe de altă parte, medicii de familie rareori corelează anemia cu leziunile parodontale grave şi în absenţa unei patologii sistemice grave, diagnostichează greşit afecţiunea ca fiind anemie feriprivă datorită nivelului scăzut de fier seric. Cazul prezentat subliniază şi demonstrează această relaţie cauzală. Pacienta, în vârstă de 50 ani, prezenta o anemie severă şi parodontită marginală complexă cu hiperplazie şi inflamaţie gingivală, pungi infraosoase active, mobilitate dentară şi depozite mari de tartru. Patologia orală împiedica o masticaţie adecvată şi implicit o alimentaţie echilibrată. Eliminarea celorlalte cauze posibile şi biopsia medulară care a arătat eritropoeză crescută ca răspuns la anemia severă, au condus în final la suspectarea parodontitei drept cauză posibilă a bolii. Tratamentul etiologic al parodontitei care a urmărit în primul rând controlul infecţiei şi reducerea inflamaţiei, urmat de extracţia dinţilor mobili şi protezare a determinat vindecarea completă a anemiei fără nici o altă medicaţie. În decurs de 4 luni, examenele hematologice au revenit la normal, simptomele clinice au dispărut şi pacienta a început să câştige în greutate (Lu S-Y, Eng H-L. Dramatic recovery from severe anemia by resolution of severe periodontitis. *J Dent Sci* 2010 ; 5 (1) : 41-46).

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ISTORIA MEDICINEI

CORPUL CA SURSĂ DE SEMNIFICAŢIE. PERSPECTIVE INTERDISCIPLINARE

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THE BODY AS SOURCE OF SIGNIFICANCE. INTERDISCIPLINARY PERSPETIVES (Abstract): This paper concerns a general theoretical aspect, followed by different examples which discuses the thesis in relation to major connected domains of research: psychology and philosophy. The thesis which we are arguing for is that the body represents a source of significance in the definitions of he self used as theoretical background in moral problems (philosophical aspect) and the explanations of the way the image of the self is consituted (psychological aspect). The philosophical "conclusion " is that the body, in its materiality, cannot be judged by the metaphysical dualism scheme, which assigns it a secondary role in the hierarchy of categories. From a psychological point of view, this paper tends to show that the body does not represent an accident of personality or a "prison of the soul" as Plato refers to it, but rather an element through which personality is built on and develops – and similarly, the mental and personality structures are those which allow the forming of corporeality and then the person's reference to it. **Key words**: BODY IMAGE, PERSONALITY DEVELOPMENT, MORALITY

G. Ryle numeşte dualismul cartezian „dogma of the ghost in the machine" şi îşi constituie demersul din The Concept of Mind ca o discuţie critică a acesteia. Doctrina dualismului se dovedeşte a fi un mit filosofic, organizat în jurul unei erori categoriale, prin care „the differences between the physical and the mental were represented as differences inside the common framework of the categories of thing, stuff, atribute, state, process (…). Minds are things, but different sort of things than bodies (…)" (1). Formularea dualismului, specifică filosofiei minţii, poate fi reţinută, deşi discuţia dualismului îşi găseşte aplicaţii diferite în psihologie, medicină sau chiar în discipline, speculative, ce pot fi trecute în contul, generos, al „filosofiei", precum antropologie sau, cum vom vedea, estetică. Una dintre condiţiile de posibilitate ale bioeticii stă, de asemenea, în precizarea statutului corporalităţii în raport cu persoana umană, în integralitatea ei. Altfel spus, în măsura în care corporalitatea poate pune o problemă etică, ea nu poate sta sub spectrul unui dualism ireductibil, care îi atribuie o cauzalitate strict mecanică. „Solidaritatea genetică", puternic documentată în cadrul discuţiilor contemporane, problemele legate de „dreptul de proprietate" asupra corpului propriu sau, mai evident, problema utilizării ADN-ului în scopuri medicale sau în scopul reproducerii presupun, în fundal, un răspuns la întrebarea Ce este corpul? şi care este relaţia sa cu celelalte instanţe ale subiectivităţii? În fond, problema dualismului se reiterează, cu o intenţie diferită faţă de contextul ei primar. Dualismul, înţeles în datele lui generale, presupune situarea responsabilităţii la nivelul unei instanţe care transcende corpul şi care este, în mod tradiţional, „adecvat" categoriilor morale: sufle-

tul. În măsura în care, însă, dualismul este pus sub semnul întrebării, corporalitatea poate şi ea să fie văzută, precum sufletul, ca sursă a imperativului etic. Definiţia agentului etic este, în mod esenţial, cea a unei instanţe „întrupate", iar nu a unui subiect „metafizic", lipsit de determinări mundane. Mai mult, încercări de a găsi eticii un fundament non-religios, non-metafizic şi totuşi universal în virtutea unor consideraţii istorico-antropologice vine în prelungirea aceleiaşi idei de a „integra" corporalitatea în constituţia subiectului moral. La fel, într-o problemă care trimite în mod strict la istoria filosofiei, încercarea lui J. Zammito de a citi sistemul critic kantian nu în sine, ci plecând de la cursurile sale, care l-au făcut celebru în epocă, de antropologie (2). Textul de faţă are ca intenţie asumarea aceleiaşi probleme a corporalităţii dintr-o perspectivă interdisciplinară. Pe de o parte, un demers care ţine de hermeneutica faptului cultural, pe de altă parte unul care ţine de sfera psihologiei vin să discute modalitatea în care corporalitatea capătă relevanţă din punct de vedere etic. Teza generală a textului ar putea fi aceea că atât determinări istorice ale codurilor etice, cât şi comportamente etice individuale depind de corporalitate, ca sursă de semnificaţie pentru ierarhiile de valori morale.

DE LA CORPUL ASCETIC LA CORPUL VIRTUAL

Un prim moment al discuţiei este reprezentat de monahismul egiptean al secolului IV. Unul dintre fenomenele paradoxale ale creştinismului, prin caracterul său paradigmatic dar, în acelaşi timp, inimitabil, monahismul pune în joc o anumită relaţie între corp şi suflet, care poate fi doar superficial înţeleasă ca negaţie a primului. Există, desigur, apoftegme ale Patericului care transmit amărăciunea nevoitorilor Pustiei la vederea celor trupeşti. Pe de altă parte, însă, modelul vieţii duhovniceşti a Părinţilor este chiar cel al lui Hristos Întrupat, iar dogma Sinodului ecumenic de la Niceea este apărată – şi transpusă în faptă şi viaţă - prin însăşi esenţa exerciţiului monahicesc. M. Henry, în Întrupare. O filosofie a trupului discută încă de la primele pagini despre teza originală a creştinismului, implicată în concepţia ioaneică a Logosului (3). Trupul capătă valoare în Creştinism tocmai datorită faptului că este, de fapt, receptacol al Oaspetelui de dincolo de vremi. Odată cu Întruparea nu doar Revelaţia îşi stabileşte modul său lumesc de a fi – anume, cel trupesc – ci, pe de altă parte, trupul însuşi îşi află originea cerească: „În lutul pământului nu există decât corpuri, şi nici un trup. Un lucru cum este un trup nu poate veni şi nu ne vine decât de la Cuvântul" (3). Una dintre distincţiile clasice care se ivesc cu acest prilej este cea dintre trup şi corp: primul, definit de fapt ca Viaţă, care simte fericirea şi suferinţa, care este indivizibil prin faptul că asumă şi pune la încercare un Sine; cel de-al doilea, divizibil, material, loc al senzaţiilor disparate şi al impersonalului biologic: „A fi viu înseamnă pentru cel viu puterea de a se încerca pe sine însuşi. Esenţa vieţii constă în purul fapt de a se încerca pe sine însăşi, de care e, din contră, lipsit tot ceea ce ţine de materie şi, în general, de lume" (3). Reabilitarea corporalităţii se face, pentru creştin, atâta vreme cât se poate concepe posibilitatea unui trup, al vieţii în concretul ei şi în unitatea desăvârşită pe care o presupune. Altfel spus, atâta vreme cât se poate vorbi despre transformarea corpului în trup, despre învierea acestuia sau, în continuarea consideraţiilor lui M. Henry, despre asumarea, la nivelul corpului, a unui Sine care se pune la încercare. Ascetismul vizează tocmai acest moment, al transformării corpului în trup. Avva Macarie Orăşeanul vorbeşte despre tensiunea „cărnii" – sarx – şi despre drumul care duce dincolo de ea. Chemat de fraţi să vină să mănânce, marele nevoitor le spune „Copii, voi trebuie să mâncaţi, pentru că încă sunteţi carne. Eu nu mai mănânc acum" (4). O complexă prezentare a fenomenului ascetic în formele sale multiple, nu neapărat creştine şi, mai ales, nu neapărat religioase poate fi întâlnită în lucrarea lui

G. Flood, The Ascetic Self. Subjectivity, Memory and Tradition (5). Două elemente pot fi schiţate, plecând de la această lucrare. Pe de o parte, modul în care ascetismul este o practică ce vizează, prin excelenţă, corpul. În al doilea rând, modul cum practica ascetică îşi înscrie semnele pe corpul pe care îl vizează. Corpul ascetic devine un corp „semnificativ", o sursă de semnificaţii tocmai datorită faptului că el se înscrie astfel într-o tradiţie, se dezindividualizează şi ajunge să poarte înscrise semnele transcendenţei. Ascetismul este cel care „dă Viaţă" corpului, îl transformă în trup şi astfel reduce diferenţa care, pentru grec, dar şi pentru modern, de pildă, îl separă de interioritate. La o primă vedere – fenomenologic vorbind, de la această primă vedere şi trebuie pornit – ascetismul este „the reversal of the flow of the body, which is also an attempt to revears the flow of time" (5). Ascetismul supune corpul unui referenţial format din mai mulţi termeni. Pe de o parte, este vorba de transcendenţă. Experienţa ascetică are la scop transformarea sinelui în sensul unei „donări" a acestuia către transcendenţă. Pe de altă parte, cosmosul, lumea, intervine prin aceea că ascetismul are ca efect dobândirea unei stări arhetipale a creaţiei, neatinsă de efectele corosive ale istoriei. În al treilea rând, tradiţia este cea care oferă ascetului un ritm şi o justificare printr-o naraţiune mai largă decât aceea a sinelui propriu. Astfel, ascetic este, într-un sens special, public : el trimite la o formă de intersubiectivitate ce are ca axa relaţia Avva-frate sau maestru-discipol. În acelaşi timp, un al treilea vine să fundamenteze şi să ofere un sens acestei relaţii : Divinul – oricare ar fi el –, cel către care sinele scetic se îndreaptă prin tehnici corporale. Ambiguitatea sinelui ascetic, cum numeşte G. Flood fenomenul, constă în următoarea situaţie : ascetul doreşte să îşi supună voinţa voinţei divine ; acest lucru se înfăptuieşte, paradoxal, prin asumarea unei intenţii ascetice, altfel spus prin supunerea voluntară a corpului unor tehnici impuse de tradiţie si prin care carnea (sarx) este în-viată şi donată sursei ultime a Vieţii. Evagrie sau Sf. Maxim Mărturisitorul descriu scenariul despre care vorbeam, acela în care soteriologia se deschide spre cosmologie. Corpul nu reprezintă, în acest joc, doar un efect secundar, nici doar un instrument. Trimiterea teologică, aceea că Hristos nu se întrupează într-un corp aparent, cum afirmau dochetiştii, ci real este valabilă şi în motivaţia ce stă în spatele asumării experienţei ascetice. Corpul este cel care, transfigurat prin tehnicile tradiţiei, conferă realitate unui Sine, unei interiorităţi care, în mod esenţial, este al întâlnirii cu celălalt : fie că este vorba de învăţător, a cărui taină o duce mai departe, fie că este vorba de cosmosul pe care îl limpezeşte, fie de Celălalt pe care îl lasă să vorbească prin propria voinţă. Corporalitatea capătă, pentru ascet, o realitate care este în acelaşi timp o miză. Subiectul ascetic este un subiect concret, istoric iar corpul îi constituie facticitatea din interiorul căreia experienţa monahală poate porni. În plus, corpul ascetului capătă, tocmai prin aceasta, o funcţie pedagogică importantă. Avva Arsenie capătă, în rugăciune, trup de foc ; avva Achilas îndepărtează cuvintele de la sine scuipându-le sub formă de sânge ; în cazul cel mai celebru, avvei Sisoe faţa i se luminează, devine ca soarele, apoi ca un fulger în clipa morţii ; avva Ioan cel Pitic, dar nu numai, se roagă să scape de patimi, iar apoi, mai fierbinte, sa le primească înapoi întrucât doar prin luptă sufletul se desăvârşeşte (4). Urmele trupului sunt astfel constitutive călugărului. Odată cu trupul, sufletul va învia, iar nevoirea pe pământ este una a unui suflet în trup, nu despărţit de acesta. Limitaţia pe care o poartă în sine carnea precum şi scopul ascezei, acela de a transforma corpul – plin de patimi –, în trup de foc fac din corpul ascetic o referinţă şi un pol de semnificaţie : condiţia trupească dă sens ascetismului, ea defineşte locul, contextul din care creştinul învaţă să vorbească despre sine şi despre Ceruri, ceea ce face ca monahismul să fie un continuu „urcuş duhovnicesc" iar nu un popas. O revalo-

rizare intensă a corpului intră, la sfârşitul modernităţii, în atenţia unor autori precum Foucault sau Deleuze. Pentru Foucault, „prin exerciţiile de abstinenţă şi de stăpânire de sine ce constituie necesara askesis, locul făcut cunoaşterii de sine devine mai important: obligaţia de a se pune la încercare, de a se examina, de a se controla printr-o serie de exerciţii bine stabilite, aşează problema adevărului – ce eşti, ce faci, ce eşti în stare să faci – în centrul constituirii subiectului moral"(6). Referindu-se cu precădere la spaţiul antic păgân, autorul francez subliniază legătura dintre asceză şi tehnicile corporale, cele legate de sexualitate şi problema adevărului. Corporalitatea intervine în mod esenţial în problema constituirii sinelui, întrucât existenţa raţională este de neconceput în absenţa unui set de reguli prin care cotidianul, mediul înconjurător sunt supuse unor tehnici „de optimizare" iar corpul devine parte a unei experienţe „unde raportarea la sine ia forma, nu numai a unei dominări, ci şi a unei desfătări lipsită de dorinţă şi frământare" (6). Nu este vorba de supunere la rigorile unei legi, nici de principii imuabile ale binelui şi răului. Mai degrabă, rigorile unei vieţi în acord cu sine şi în vederea unui sine îmbunătăţit sunt cele care determină scopul şi miza ascezei. Această situaţie se va schimba în modernitate. Prima secţiune din lucrarea închinată istoriei regimurilor de detenţie (7) poartă titlul Corpul condamnaţilor (Le corps des condamnes). Corpul devine important, arată Foucault, în măsura în care sistemele punitive sunt privite din perspectiva unor „tehnologii politice ale corpului" şi a unor metode de „subiectivizare" prin care ia naştere omul ca obiect – mai apoi, ca obiect de studiu ştiinţific. Ca şi în cazul spitalului modern, al secolului XVIII, regimul penitenciar modern conturează, prin tehnici ale controlului, ale organizării cotidiene, ale „reeducării" un joc de adevăr în care subiectul intră în calitate de obiect. Controlul, sistematizarea stau, în aceeaşi măsură, la originea exercitării puterii, a vindecării sau a discursului ştiinţific. Toate aceste zone ale modernităţii au ca origine legătura dintre adevăr şi putere: adevărul cu privire la subiect poate fi obţinut în măsura în care acesta se transformă de fapt in obiect experimentabil, catalogabil, inscriptibil în procedee metodice şi legităţi. Corpul joacă, în acest loc, un rol fundamental. În regimul penitenciar, ca şi în cel spitalicesc, puterea se exercită prin intermediul corpului. Bolnavul sau condamnatul nu mai reprezintă o prezenţă ambiguă, indefinită. Corpul este cel care îi transformă într-o prezenţă concretă, care se confruntă cu procedee uneori difuze, alteori instituţionale sau ideologice dar de fiecare dată materiale, tehnice (7). Modernitatea aduce aşadar corpul în prim-plan pe un traseu diferit faţă de cel discutat anterior în cazul monahismului, dar nu mai puţin relevant. El devine constitutiv subiectului, văzut acum ca miză a relaţiilor de forţe şi tocmai în acest mod capătă relevanţă. Putem vorbi, în înţeles modern, despre subiect deoarece corpul este cel care îl face vizibil din punctul de vedere al puterii, cea care ajunge vorbească despre adevăr. „Eliberarea corpului" ca fenomen al postmodernităţii nu face altceva decât să scoată corpul din aria de exercitare a puterii, condiţie specifică modernităţii. Estetizarea corpului, virtualizarea lui sunt fenomene care determină această nouă resemnificare. Din punct de vedere cultural, tatoo-art sau, mai larg, Body-art în ansamblul ei exploatează resursele semnificante ale corpului şi simbolistica pe care o poate sugera. Explorarea vizuală a corporalităţii în cinematografia contemporană în detrimentul unui joc de semnificaţii poate fi, de asemenea, amintită (8). Asumarea unor sincretisme orientale, de exemplu, ca alternativă la mentalul modern, european considerat unul al jocurilor de putere şi al dominaţiei violente oferă resurse pentru reevaluarea postmodernă a corpului, dintr-un punct de vedere al unei estetizări a acestuia, iar nu a unei „tehnologii politice". Mai mult decât enumerarea acestor fenomene, interesează rândurile de faţă răspunsul la întrebarea: cum

devine corpul în postmodernitate o sursă de semnificaţie? Ce stă la baza resemnificării sale postmoderne? Un răspuns poate fi sugerat plecând de la lucrările lui Mircea Eliade. Sacrul, arată savantul român, nu poate fi redus la dimensiunea sa istorică. El reprezintă de fapt un element constitutiv al conştiinţei umane ca atare, ţinând de nevoia esenţială a omului de a discerne între ceva semnificativ, adevărat, Real şi un alt domeniu care există în virtutea acestia, îi suportă determinările, întemeierea şi sancţiunile. De aici ideea supravieţuirii camuflate a miturilor în perioada contemporană, a mersului ireductibil şi coroziv al istoriei. Mitul supravieţuieşte, pentru că fără el, lumea devine de netrăit; şi, odată cu mitul, structurile sale de semnificaţie. În Sacrul şi profanul, Eliade discută despre semnificaţia cosmică a trupului: corpul reproduce, „la scară", cosmosul, în măsura în care coloana vertebrală devine asimilabilă Stâlpului cosmic, inima Centrului lumii sau respiraţia vântului (9). Cântarea cântărilor este, din acest punct de vedere şi dincolo de alegorismul ei, prototipul slăvirii corpului-cosmos şi înţelegerii nobleţii sale. Perioada contemporană, aşadar, recuperează aceste semnificaţii cosmice ale corpului, iar asimilarea postmodernă, deşi superficială, a textelor şi doctrinelor arhaice nu poate decât să înlesnească acest proces. Tatuajul, de pildă, îşi pierde funcţia originară, apotropaică; păstrează însă funcţia estetică şi devine semnificativ prin aceasta. În plus, „nudismul sau mişcările pentru libertatea sexuală absolută se bazează de pildă pe ideologii în care se pot desluşi urmele nostalgiei Paradisului, (…) când nu exista păcat şi nici ruptură între plăcerile cărnii şi conştiinţă" (9). O concluzie poate fi trasă privind această primă parte a studiului. Anume, este vorba de faptul că, din punct de vedere al rolului său cultural, corpul dispune de resurse majore de semnificaţie. Se poate gândi plecând de la corp, acesta nu este doar un efect, o iluzie sau o aparenţă. Corpul, în materialitatea sa, nu poate fi judecat după schema dualismului metafizic, care îi atribuie un rol subordonat în ierarhia categoriilor. Ascetismul, dominaţia tehnico-politică sau afirmarea identităţii postmoderne sunt gesturi pentru care corporalitatea conferă consistenţă gestului şi impune acestuia o anumită desfăşurare. Subminarea platonismului – sau, mai degrabă, a variantei sale vulgare – conduce la eliberarea reflecţiei despre exterioritate şi despre suprafaţă, despre efectele nebănuite ale corporalităţii. Foucault, comentându-l la un moment dat pe Deleuze, remarcă o posibilă ieşire din umbră, împotriva lui Platon, a epicureicilor, cei care ştiu să vorbească despre „emisii care vin din adâncul corpului şi care se înalţă asemeni unor dâre de ceaţă – fantome ale interiorului repede reabsorbite de miros, de gură, de poftă; pelicule absolut mărunte ce se desprind de pe suprafaţa obiectelor şi fixează în adâncul ochilor noştri culori şi profiluri (epiderme zburătoare, idoli ai privirii); fantasme ale spaimei şi dorinţei (zei de nori pe cer, frumos chip adorat, mizerabilă dorinţă dusă de vânt)"(10).

CORPUL ŞI CONCEPTUL DE SINE

Conştiinţa de sine se constituie firesc în devenirea umană având la bază corpul ca instanţă obiectivă stabilă pe de o parte şi reprezentările sale mentale, dinamice şi în continuă formare pe de altă parte. Interacţiunea suflet-corp, în felul în care ea intră în atenţia disciplinelor aflate în sfera psihologiei, face obiectul secţiunii de faţă. Corpul intervine astfel, voi încerca să arăt, în constituirea imaginii de sine şi, ulterior, în adoptarea unei atitudini morale şi în fundamentarea unor comportamente mentale dezvoltate, precum formularea unor opţiuni estetice. Invers, de asemenea, corporalitatea poate fi văzută nu ca un dat, ci ca o construcţie, aflată în schimbare şi care depinde de reprezentările sociale ale persoanei, de tipul de interacţiune avut cu cei din jur sau de tipul de personalitate. Această interacţine, în care ambii membri (corpul şi sufletul) joacă un rol la fel de important în definirea psihică a unei personae, va face obiectul precizărilor şi exemplificărilor ulte-

rioare. În perspectiva psihodinamică iniţiată de Freud « sinele corporal » se referă la experienţa subiectivă a senzaţiilor fizice, a funcţionării şi a imaginii corporale, aceasta din urmă devenind componenta activă a imaginii de sine (11). Corpul conţine şi exprimă trăirile afective individuale, fiind în acelaşi timp concept si fapt. Alfred Korzybski afirmă că „Harta nu este teritoriul" (12), ceea ce ilustrează oportun felul în care imaginea corporală şi imaginea de sine sunt doar reprezentări subiective având o înţelegere limitată a teritoriului. Corpul intermediază experienţa vieţii, o orientează şi o cenzurează, facilitând cunoaşterea lumii prin estomparea propriilor funcţii, transformate în reflexe. O reprezentare de sine incomplet construită sau greşit asumată, poate întârzia dezvoltarea afectivă iar individul va avea dificultăţi în a înţelege şi a integra propriile emoţii în diversitatea lor. În astfel de cazuri corpul devine din nou mijlocitor pentru exteriorizarea a ceea ce nu a fost înţeles şi nu poate fi exprimat prin cuvânt sau acceptat conştient. Corpul este purtător de simptome, martor indiscret al trecerii timpului şi al emoţiilor trăite de individ. Din punct de vedere biologic, dualismul corp – minte îşi pierde sensul. Corpul este generator al vieţii psihice şi al minţii prin sistemul nervos central. Neuroştiinţele au încă largi mistere de clarificat in ceea ce priveşte funcţionarea complexă a mecanismelor mentale, dar rezultatele actuale reuşesc să combată satisfăcător teza separaţiei categorice între minte si corp. La Peyronie (13), a fost printre primii care în Franţa secolului al 18-lea a presupus în urma cercetărilor sale, că mintea nu dispune de o distribuţie uniformă în creier. Secolele ce au urmat au înlesnit observaţiile neurologice prin apariţia tehnologiei de imagistică funcţională cerebrală cu ajutorul căreia relaţia dintre minte şi corp a putut fi analizată în condiţii neuropsihologice strict controlate. Brent A. Vogt şi Orrin Devinsky susţin (14) că mintea este adesea definită ca reprezentând „conştiinţa de sine", operaţionalizare pe care ei o demonstrează a fi reducţionistă. Copiii născuţi prematur şi izolaţi nu se dezvoltă normal din punct de vedere psiho-biologic iar experimentele de stabilizare senzorială realizate în încăperi special concepute provocau reducerea performanţei intelectuale, alterarea percepţiei, halucinaţii şi schimbări afective. Prin urmare teoria conform căreia sinele poate exista independent de evenimentele interne şi externe, este o concluzie extremă ce are la baza dualismul minte / corp ce nu poate fi integrat în discursul neuroştiinţelor. Pe de altă parte, nu toată activitatea senzorială şi motorie este activitate mentală, la fel cum mintea nu se identifică întregii activităţi cerebrale. Studiile arată că mamiferele nu sunt conştiente de evenimentele din cortexul vizual primar şi că interpretarea explicită a scenelor vizuale se realizează în alte arii cerebrale (15). În acelaşi sens, pacienţii orbi având leziuni corticale vizuale primare au acces la informaţia vizuală dar nu sunt conştienţi de ceea ce văd (16). Conceptul de sine în formare este ilustrat iniţial la individ prin experienţa şi conştientizarea propriului corp. Mai târziu pe parcursul dezvoltării personale, apare capacitatea de reprezentare si autoreflexie. În ultima etapă intervine intelectualizarea eului când sinele îşi dobândeşte natura abstractă eliberându-se de corp. Tot ceea ce va intra in atenţia individului mai apoi va trece prin filtrul subiectiv al sinelui susţinut de corpul de care s-a eliberat. Kinsbourne (17) cercetează dacă imaginea corporală este înnăscuta sau se construieşte prin experienţă şi învăţare. Există cazuri rare de copii ce se nasc fără unul sau mai multe membre (aplazia membrelor). 15% dintre aceşti copii raportează fantome ale membrelor pe care nu le-au avut niciodată. Prin urmare senzaţiile corporale sunt prezente în creier cu mult înainte ca experienţa să le justifice prezenţa. Faptul că majoritatea copiilor născuţi cu aplazie nu raportează membre fantomă, ilustrează importanţa mediului in menţinerea sistemelor neuronale preprogramate precum şi natura flexibilă a structurilor cerebrale (18). Conştientizarea propriului corp devine ast-

fel un proces dinamic ce nu presupune doar extragerea de informaţii preexistente dintr-o anumita arie cerebrală, ci descoperire şi învăţare graduală. Senzaţiile somatice par a fi cele care guvernează descoperirea propriei corporalităţi în detrimentul senzaţiilor vizuale care ocupă un rol secundar. Copilul se va percepe pentru prima dată ca individ pe măsură ce îşi va folosi corpul provocând senzaţii diferenţiate şi în părţile corporale pe care nu le poate vedea. În acelaşi sens, copiii orbi îşi formează o imagine a propriului corp în ciuda deficitului lor iar în cazul membrelor fantomă, pacientul integrează membrul lipsă chiar dacă nu îl poate vedea. Perceperea propriei corporalităţi stă la baza formării iniţiale a conceptului de sine şi continuă a marca semnificativ devenirea persoanei, a relaţiei ei cu lumea şi cu sine însăşi. Perspectiva cognitiv comportamentală propune un model coerent pentru a explica determinismul complex ce funcţionează în constituirea imaginii corporale, precum şi rolul acesteia în alegerea comportamentului individual. Interacţiunea mai multor factori ce acţionează asupra persoanei încă din copilărie, va duce în timp la construirea de convingeri şi credinţe specifice cu privire la parametrii de evaluare ai propriei imagini corporale, precum şi la importanţa acesteia în evaluarea personală globală. Cadrul cultural, experienţele interpersonale timpurii, însuşirile fizice, mediul familial si anturajul precum si trăsăturile de personalitate, fac parte dintre factorii ce vor contribui la conturarea schemelor şi atitudinilor cu privire la imaginea corporală si semnificaţia ei. În funcţie de contextul situaţional, aceste scheme se vor activa determinând gânduri automate ce vor condiţiona emoţii şi comportamente specifice. Dincolo de etichetele şi împărţirile teoretice funcţionarea interactivă a acestor factori se complică in realitatea psihologică intra si interindividuală. La fel cum unele situaţii pot determina activarea anumitor credinţe şi scheme, individul va fi predispus să se concentreze şi să selecteze situaţiile care vin în acord cu propriile credinţe. Noile experienţe trăite se vor adăuga la istoricul personal, întărind atitudinile deja existente sau modificând vag structura sudată de-a lungul mai multor ani. În acest sens Albert Bandura, teoreticianul învăţării sociale, vorbeşte despre „triada cauzală reciprocă" : factorii externi, de mediu, factorii interni personali si comportamentele individuale, funcţionează împreună, determinându-se reciproc (19). Aprofundând relaţiile ce leagă rolurile diferenţiate ale fiecăruia dintre factorii deja amintiţi, considerăm util a-i descrie mai amănunţit. Cadrul cultural impune standarde şi modele estetice, caracteristicile fizice valorizate şi cele dezaprobate, precum şi importanţa imaginii fizice, semnificaţia ei într-o anume societate. Ce este atractiv şi ce înseamnă a fi atractiv. Tot aici se decid caracteristicile de gen, atributele fizice care definesc si diferenţiază conceptele de feminitate şi masculinitate, se stabilesc idealuri plasate şi prezentate în contexte bine conturate (20). Astfel de exemplu, societatea occidentală propune ca ideal de feminitate un trup tânăr, înalt şi foarte slab asociat adesea cu ideea de succes, fericire si dezirabilitate. Femeile vor internaliza acest model investindu-l cu o importanţă semnificativă globală în cadrul evaluării personale, ceea ce le va afecta la nivel perceptual, afectiv, cognitiv si comportamental. Ca urmare a comparării sociale, la nivel perceptual femeile riscă a se considera supraponderale doar pentru că nu corespund standardelor. Distanţa de netrecut dintre imaginea ideală nerealistă şi imaginea percepută duce în plan afectiv la stări distimice, stimă de sine scăzută şi nemulţumire legată de imaginea corporală. Din punct de vedere cognitiv, transformarea aspectului fizic în criteriu central al evaluării globale, va duce la concentrarea excesivă asupra mesajelor legate de acest aspect şi selectarea lor preferenţială pentru construirea imaginii de sine. Comportamental, femeile se vor angaja in activităţi menite să le ajute sa îşi împlinească idealul estetic stabilit. Pentru un sistem complet, tot mediul cultural va stabili şi va crea mijloacele pen-

tru atingerea idealurilor fizice propuse: moda, dietele, operaţiile estetice. În funcţie de acest sistem individul îşi va forma propriile credinţe şi aşteptări cu privire la imaginea sa corporală, va stabili cum se plasează faţă de modelele impuse şi ce importanţă au acestea pentru el, ceea ce va influenţa felul particular de raportare la evenimentele trăite. Chiar dacă societatea stabileşte standarde şi criterii de evaluare ale imaginii corporale, experienţele interpersonale timpurii şi mediul familial au un rol esenţial în influenţarea felului în care individul se va situa în raport cu acestea. Opinii, aşteptări şi feedback verbal sau nonverbal sunt transmise mereu în interacţiunile cu prieteni, colegi, membri ai familiei sau chiar persoane străine. Imaginea corporală este în continuă formare de-a lungul vieţii şi devenirea ei va depinde în parte de compararea socială şi feedbackul primit de la persoanele semnificative din apropierea individului. În copilărie acestea vor fi părinţii, în adolescenţă o importanţă deosebită o vor avea colegii şi prietenii iar la vârsta adultă partenerul romantic va fi cel care va mijloci devenirea imaginii corporale. Cu toate acestea, experienţele timpurii au o importanţă capitală în formarea schemelor cognitive, a credinţelor şi atitudinilor şi prin urmare părinţii şi familia vor avea un rol esenţial. Fisher, Fisher & Stark arată că felul în care părinţii se vor raporta la propriul copil depinde în parte de discrepanţa dintre imaginea ideală, expectanţele lor despre copilul ce se va naşte şi felul în care acesta arată in realitate (21). Criteriile fizice dorite de părinţi pentru copiii lor par a fi asemănarea lor cu membrii familiei şi absenţa malformaţiilor fizice. La baza unei imagini corporale sănătoase şi stabile se află un mediu familial afectuos, tolerant şi atent la nevoile copilului. Fraţii şi surorile au de asemeni un rol important în a stabili importanţa aparentei fizice în evaluarea personală (22). Atât experienţele interpersonale cât şi informaţiile primite din mediul cultural vor fi filtrate şi asumate de indivizi într-o varietate de forme în funcţie de trăsăturile lor de personalitate. O variabilă importantă în acest sens este stima de sine care va influenţa semnificativ evaluarea propriei imagini corporale. Astfel, satisfacţia cu privire la propria aparenţă fizică va corela cu o stimă de sine ridicată şi este un bun predictor pentru stabilitatea acestui raport în faţa diferitelor evenimente exterioare ce pot interveni, în timp ce o stima de sine scăzută predispune persoana la o evaluare preponderent negativă, mai dependentă de factorii externi. Trebuie însa menţionat că şi de această dată interacţiunea este reciprocă. În perioada timpurie, când personalitatea este in formare criticile sistematice cu privire la aspectul fizic pot determina o evoluţie problematică în mai multe planuri ale personalităţii, marcată de neîncredere în sine. Aceasta va deschide cercul vicios permiţând persoanei a se concentra preferenţial asupra mesajelor şi evenimentelor ce vin a confirma ideile sale despre o imagine corporală deficitară, ceea ce îi va întări credinţele şi va perpetua eventual o stimă de sine globală scăzută. Un alt factor de personalitate ce ar putea influenţa formarea imaginii corporale, este conştiinciozitatea. O persoană perfecţionistă îşi va stabili standarde înalte pe care le va urmări cu stricteţe. Aceste criterii se pot referi şi la aspectul fizic. Pentru că adesea aparenţa fizică nu stă doar în puterea individului, eforturile de voinţă se pot dovedi puţin eficiente lăsând persoana frustrată. Dependenţa/independenţa de grup arată în ce măsura persoana îşi este suficientă sieşi, luând decizii şi formându-şi convingeri pe cont propriu. O persoană influenţabilă, dependentă de grup, va fi mai expusă evenimentelor şi părerilor externe astfel încât şi imaginea corporală poate avea o structură mai vulnerabilă (23). Factorii de personalitate nu acţionează izolat, mai importantă fiind interacţiunea dintre ei, precum şi felul în care vor plasa individul în mediul lui cultural, social şi familial. Nu în ultimul rând însuşirile fizice în forma lor absolută vor stabili unde se plasează individul la nivel obiectiv faţă de standardele culturale

estetice ale epocii, precum şi felul în care va fi perceput de ceilalţi. Persoanele cu deficienţe fizice de natură congenitală par a prezenta un risc crescut de a fi nemulţumiţi de propria imagine corporală, prezentând stima de sine scăzută şi întâmpinând dificultăţi interpersonale. Cu toate acestea, toţi ceilalţi factori ce conlucrează complex în formarea individului, fac evoluţia acestor persoane mult mai puţin previzibilă. În timp ce un număr semnificativ dintre ele vor fi afectate de situaţia lor, altele vor avea o evoluţie normală învăţând chiar să profite de pe urma a ceea ce le face diferite sau reducând diferenţa fizică la un rol minor în imaginea lor corporală (24). Obezitatea pare a fi stigmatul social cel mai dificil de suportat. Majoritatea oamenilor consideră că indivizii îşi pot controla corpul spre deosebire de rasă, naţionalitate sau sex, fac atribuiri interne, şi apare tendinţa de a învinui. Persoanele suferind de obezitate vor interioriza aceste opinii şi vor tinde a se culpabiliza şi a se simţi vinovate (25). Ca exemplu suplimentar, în societăţile occidentale în care tinereţea fizică este valorizată, angoasa îmbătrânirii este acutizată de focalizarea indusă asupra aparenţelor corporale (26). Corpul este supus schimbărilor sistematice iar imaginea corporală este un concept dinamic ce trebuie mereu ajustat şi adaptat în consecinţă. Din punct de vedere biologic şi psihologic mintea şi corpul sunt inseparabile. Chiar dacă neuroştiinţele nu au putut încă stabili cu exactitate unde anume în sistemul nervos central se găseşte localizat „spiritul" iar psihiatria şi psihologia încă încearcă a clarifica mecanismele prin care zbuciumul sufletesc determină tulburări somatice semnificative, este însă evident că încercarea de a separa trup şi spirit este una artificială. La fel, încercarea de a contura identitatea unei persoane făcând abstracţie de modul în care aceasta îşi asumă şi înţelege corpul. Persoana umană, ca actor moral, de pildă, se constituie în bună parte şi din felul în care aceasta se raportează la sine ca având un corp şi suportându-i, inevitabil, limitările. Corpul, din perspectivă psihologică, nu reprezintă un accident al personalităţii sau o „închisoare a sufletului" după cum o numeşte Platon, ci un element prin care aceasta se construieşte şi se dezvoltă, după cum, în mod reciproc, structurile mentale şi de personalitate sunt cele care permit constituirea şi apoi raportarea la propria corporalitate.

CONCLUZII

Textul de faţă poate fi citit ca o încercare de a argumenta şi preciza o afirmaţie a lui D. Le Breton din Antropologia corpului şi modernitatea: „Corpul funcţionează ca o frontieră ce delimitează în faţa celorlalţi prezenţa subiectului. El este factor de individuaţie" (27). Ascensiunea individualismului, arată Le Breton, este legată de conştientizarea corpului ca limită, ca barieră a persoanei. Odată cu aceasta, interesul pentru corp ca posesiune a individului, ceea ce trimite, automat, la una dintre sursele fundamentale ale dualismului de la care am pornit: odată cu individualismul, corpul uman nu mai reprezintă, ca în societăţile arhaice, o legătură cu cosmosul, mai bine zis o parte a acestuia. Prin corp, omul nu se mai integrează în cosmos, ci se delimitează de celălalt. Corpul desparte şi, odată cu această funcţie pe care o capătă, el însuşi devine doar un efect de suprafaţă al sufletului, adăugat acestuia, iar nu cosubstanţial cu el. Ceea ce am încercat să arătăm, în textul de mai sus, este că această funcţie nu este dusă până la capăt. Corpul nu doar este semnificat, ci şi semnifică la rândul lui. Este activ, ştie să vorbească, iar tăietura pe care o produce între un sine şi un celălalt ia, de fapt, forma corpului, îi suportă determinaţiile. Identitatea se constituie prin corp, iar interioritatea nu poate fi abstrasă tensiunii în care stă cu cel care o „delimitează", anume corpul. Cele două secţiuni largi ale textului de faţă descriu această tensiune în mod diferit, dar complementar, atât din punctul de vedere al problematicii, dar şi al metodei. Rezultatul este sublinierea relevanţei corpului din punct de vedere etic, dar şi din punct de vedere al constituirii

propriu-zise a imaginii despre sine, a interiorităţii. Asumarea unei probleme de bioetică sau a unei decizii cu privire la întrebările acesteia este susţinută, în fundal, de perspectiva unei persoane „întrupate", diferită de subiectul eticii clasice, aflate în umbra metafizicii. Nu este vorba, altfel spus, despre o subiectivitate autonomă, definită prin atribute „tari" atemporale, supramundane, precum autonomie, substanţialitate sau libertate, ci de un subiect finit, marcat de puterea temporalităţii, a fragilităţii şi a inevitabilelor eşecuri ale lumesculu.

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